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Calorie Restriction: The Elixir of Youth for Kidneys and Testicles?

Kalori Kısıtlaması: Böbrekler ve Testisler için Gençlik İksiri mi?

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ABSTRACT

Objective: Reactive oxygen species that accumulate during aging can cause oxidative stress in kidney and testicular tissues, contributing to the progression of chronic kidney disease and to male infertility. Caloric restriction (CR) is considered the most effective non-pharmacological method to promote healthy aging through multiple mechanisms, including modulation of oxidative stress. This study aimed to investigate the effects of short-term CR on kidney and testicular tissues during aging.

Methods: Twenty-eight rats were divided into four groups: young control, young CR, old control, and old CR. Control groups were fed ad libitum, whereas CR groups received approximately one-third of their daily caloric intake. After 10 weeks, blood parameters [creatinine (Cre), blood urea nitrogen, uric acid (UA), testosterone levels], oxidative stress markers [malondialdehyde (MDA) and glutathione (GSH)], and histological changes were analyzed.

Results: Aging was associated with increased body and kidney weights and a reduced testicular index $p < 0.005$. CR reduced body and kidney weights and increased kidney and testicular indices ($p < 0.005$). Renal function markers (Cre and UA) increased with age but were significantly reduced by CR ($p < 0.005$). Aging increased MDA levels and decreased GSH levels in both renal and testicular tissues. Aging increased inflammatory cell infiltration and fibrosis in the kidney and testicular tissues, whereas CR significantly reduced the fibrosis percentage in aged rats.

ÖZ

Amaç: Yaşlanma sırasında biriken reaktif oksijen türleri, böbrek ve testis dokularında oksidatif strese neden olarak kronik böbrek hastalığının ve erkek kısırlığının ilerlemesine katkıda bulunabilir. Kalori kısıtlaması (CR), oksidatif stresin modülasyonu da dahil olmak üzere çeşitli mekanizmalar aracılığıyla sağlıklı yaşlanmayı destekleyen en etkili farmakolojik olmayan yöntem olarak kabul edilir. Bu çalışma, kısa süreli CR'nin yaşlanma sırasında böbrek ve testis dokuları üzerindeki etkilerini araştırmayı amaçlamıştır.

Yöntemler: Yirmi sekiz sıçan, dört gruba ayrıldı: genç kontrol, genç CR, yaşlı kontrol ve yaşlı CR. Kontrol grupları al-libitum olarak beslenirken, CR grupları günlük kalori alımlarının yaklaşık üçte birini tüketti. On hafta sonra, kan parametreleri [kreatinin (Cre), kan üre azotu, ürik asit (UA), testosteron düzeyleri], oksidatif stres belirteçleri [malondialdehit (MDA) ve glutatyon (GSH)] ve histolojik değişiklikler analiz edildi.

Bulgular: Yaşlanma, vücut ve böbrek ağırlıklarında artış ve testis indeksinde azalma ile ilişkiliydi $p < 0,005$. CR, vücut ve böbrek ağırlıklarında azalma ve böbrek ve testis indekslerinde artış gösterdi ($p < 0.005$). Böbrek fonksiyon belirteçleri (Cre ve UA) yaşla birlikte artmış, ancak CR ile anlamlı olarak azalmıştı ($p < 0,005$). Yaşlanma, hem böbrek hem de testis dokularında MDA düzeylerini artırdı ve GSH düzeylerini düşürdü. Yaşlanma, böbrek ve testis dokularında inflamatuar hücre infiltrasyonunu ve fibrozisi artırırken, CR yaşlı sıçanlarda fibrozis yüzdesini anlamlı şekilde azalttı.

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ABSTRACT

Conclusion: Short-term CR may alleviate structural and functional impairments in the kidney and testis during aging by reducing oxidative stress.

Keywords: Caloric restriction, aging, kidney, testis, oxidative stress, infertility

ÖZ

Sonuç: Kısa süreli CR, oksidatif stresi azaltarak yaşlanma sırasında böbrek ve testisteki yapısal ve fonksiyonel bozuklukları hafifletebilir.

Anahtar Sözcükler: Kalori kısıtlaması, yaşlanma, böbrek, testis, oksidatif stres, infertilite

INTRODUCTION

Aging causes morphological and functional changes in various organs, increasing the risk of many important diseases, including neurodegenerative and cardiovascular diseases, as well as renal failure (1). Advancing age is associated with a significant decline in kidney function and in male fertility. The gradual decline in kidney glomerular filtration rate (GFR) and blood flow contributes to impaired renal function (2). In addition, the age-related loss of nephrons-the essential functional and structural units of the kidney-contributes to reduced renal capacity (3). This condition elevates the risk of developing chronic kidney disease (CKD) in older adults (4). CKD is 3–13 times more common in older individuals (5) and is anticipated to become the fifth most prevalent cause of death worldwide by 2040 (6).

Infertility is a significant health issue affecting 10–15% of couples worldwide. The etiology of this problem, which is male-related in 25–50% of cases, is largely unknown (7). Different levels of kidney disease in men have been associated with azoospermia, oligospermia, and low testosterone levels (8). Therefore, assessing reproductive and renal functions during aging is essential for preventing age-related disorders and promoting healthy aging (2).

Age-related dysfunction in organs such as the kidneys and testes results from an imbalance between reactive oxygen species (ROS) and the antioxidant defense system, leading to increased oxidative stress (9). Renal tissue is susceptible to oxidative damage due to age-related decreases in renal blood flow and GFR (10). Similarly, testicular tissue is vulnerable to oxidative stress due to its high metabolic activity, low antioxidant defenses, and abundant unsaturated fatty acids (11).

Caloric restriction (CR), in which total caloric intake is reduced while adequate intake of essential nutrients is maintained, is the most effective non-pharmacological strategy to support metabolic health (12). CR has been reported to improve kidney function by lowering levels of blood urea nitrogen (BUN), creatinine (Cre), and urinary protein. Thus, it delays the onset and progression of CKD, the risk of which increases with age (13). In addition, short-term CR has been shown to reduce glomerulosclerosis, urinary protein excretion, and renal fibrosis (14,15). It also reduces oxidative stress and supports testosterone production in the testes (16). In obese mice, CR increases the testicular organ index and serum testosterone levels by reducing oxidative stress levels and also regulates sperm morphology (17).

Various interventions that slow the aging process may help prevent organ damage. Our research examines the potential protective effects of CR against age-related changes in renal and testicular tissues, using markers of organ function, oxidative stress parameters, and histological assessments.

MATERIALS AND METHODS

Animals and the Experimental Group

The experimental procedures were approved by the Gazi University Animal Experiments Ethics Committee (approval number: #G.Ü.ET-25.068, date: 18.07.2025). All procedures involving rats were conducted in accordance with the European Convention (ETS 123) guidelines. Twenty-eight male Wistar albino rats obtained from the Gazi University Experimental Animal Research and Application Center were included in the study. The rats were kept under standardized laboratory conditions at 23 °C, with a 12-hour light-12-hour dark cycle from 08:00 to 20:00. A total of 28 rats were divided into two age groups: young (3 months old) and old (20 months old). They were further divided into four subgroups: young control (YC) (YC, n = 6); young experimental [young calorie restriction (YCR), n = 6); old control (OC), n = 8]; and old experimental [old calorie restriction (OCR), n = 8].

The control group was fed standard chow ad libitum for 10 weeks. To ensure that the effects of short-term CR were achieved, the Altromin C-1012 diet (Altromin Spezialfutter GmbH, Germany) was administered to the CR group for the same period [30% of daily caloric intake (~1,303 kcal/kg)] (18). Rats' weights were monitored weekly throughout the experiment. At the end of the 10-week period, after a 12-hour fast, all rats were anesthetized with 10% ketamine and 2% xylazine. Subsequently, cardiac blood was collected, and the rats were decapitated. The right testis and right kidney were removed, and wet organ weights were measured.

The organ index for the kidney and testis was calculated as (organ weight/body weight) × 100. For histological evaluation, tissue samples were placed in 10% formaldehyde, whereas the remaining tissue samples were washed with PBS and immediately frozen in liquid nitrogen. Then they were stored at -80 °C until further analysis.

Evaluation of MDA and GSH Levels in Tissue Samples

Testis and kidney tissues were homogenized using rotor-stator tissue homogenizer in trichloroacetic acid (TCA) [200 mg tissue and 1.8 mL TCA (10% w/v)]. Supernatants obtained by centrifugation at 4,000 rpm for 15 minutes were analyzed for malondialdehyde (MDA, an indicator of lipid peroxidation, using the thiobarbituric acid-reactive substances assay (19). The supernatants were mixed with butylated hydroxytoluene [1% (w/v)] and thiobarbituric acid [(TBA, 0.67% (w/v)] in glass tubes and then boiled at 100 °C for 15 minutes. The resulting mixtures were transferred to plates and analyzed spectrophotometrically at 532 nm.

To assess antioxidant defense capacity in tissue samples, glutathione (GSH) levels were measured using the modified Ellman method (20). Supernatants from tissues homogenized at room temperature were reacted with 0.3 M disodium hydrogen phosphate (Na_2HPO_4)

and 5,5'-dithiobis-(2-nitrobenzoic acid) (0.4 mg/mL in 1% sodium citrate). The mixtures were then transferred to plates and analyzed spectrophotometrically at 412 nm.

Evaluation of Kidney Parameters and Plasma Testosterone Levels

Prior to sacrifice, blood samples were collected from the rats via cardiac puncture and then centrifuged at 4,000 rpm for 15 minutes. Plasma BUN, Cre, and uric acid (UA) levels were measured in the supernatants using colorimetric methods on a Siemens Advia Chemistry XPT (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), and plasma testosterone levels were measured in the supernatants using chemiluminescent immunoassay on a Siemens Advia Centaur XPT at the Gazi University Hospital Biochemistry Central Laboratory.

Histological Analysis

Morphometric Measurements and Histological Staining

Tissue sections (5 µm thick) were prepared from renal and testicular samples using a microtome (Slee, CUT 5062, Germany). Histological analyses of kidney tissue were performed using hematoxylin-Eosin (H&E) and Masson's Trichrome (M&T) stains. Stained sections were evaluated under a ZEISS Axiolab 5 computer-assisted light microscope (Germany) using ZEN Blue 3.4 software. After M&T staining, the fibrotic area ratio was quantified in randomLy selected kidney sections. The fibrotic area ratio (%), glomerular diameters (µm) in kidney sections, and seminiferous tubule diameters (µm) in testicular sections were evaluated using ImageJ software.

Additionally, testicular tissue was analyzed histologically using H&E, following the same protocol. After staining, the samples were examined microscopically, and histological evaluations of the seminiferous tubules and interstitial space were performed.

Johnsen Testis Score Evaluation

Histopathological assessment of spermatogenesis was performed using the Johnsen testicular biopsy score. Scores were calculated from 100 randomLy selected seminiferous tubules per group (21). Only scores in the range of 7–10 were observed in testicular sections from the evaluated groups. The scoring system for testicular biopsies was as follows: 10 (complete spermatogenesis); 9 (numerous late spermatids, irregular epithelial cells); 8 (fewer than five spermatocytes per tubule, few late spermatids); and 7 (no spermatocytes, no late spermatids, numerous early-stage spermatids).

Statistical Analysis

Study data were analyzed using SPSS 22. Data are presented as group mean ± standard deviation. One-way ANOVA (post-hoc LSD) was used for group comparisons, and a paired-sample t-test was used to compare weight changes. Pearson's r was also calculated to examine the relationship between variables. Statistical significance was set at $p < 0.05$.

RESULTS

Effects of CR on Weight Change, Right Testis/Body Weight Ratio, and Right Kidney/Body Weight Ratio

Initially, the body weights of older rats were greater than those of younger rats YC: 179.50 ± 6.60, YCR: 185.33 ± 7.94, OC: 360.50 ± 30.18, OCR: 351.38 ± 37.05; $p < 0.001$). While weight gain was observed in the OC group at the end of 10 weeks, significant weight loss was observed in young and old rats due to CR (YCR: 167.33 ± 16.40, $p < 0.001$; OCR: 258.88 ± 42.82, $p < 0.001$) (Figure 1A). Kidney weight was significantly higher in the OC group compared to the YC group (1.27 ± 0.11, 0.87 ± 0.1, $p < 0.001$, respectively), while CR reduced kidney weight in both young and elderly rats (0.70 ± 0.06, 0.97 ± 0.08, $p < 0.001$, $p < 0.001$, respectively) (Figure 1B). There was no difference in the kidney index between the control groups, whereas CR increased the kidney index in both young and old rats (0.41 ± 0.04, $p = 0.001$; 0.39 ± 0.04, $p = 0.024$, respectively) (Figure 1C). There was no significant difference in testis weight between the YC and OC groups. Testicular weight was lower in the OCR group than in the OC group (1.35 ± 0.28 and 1.61 ± 0.17, respectively; $p = 0.013$) (Figure 1D). The testicular index was lower in the OC group than in the YC group (OC: 0.44 ± 0.03; YC: 0.56 ± 0.06; $p < 0.001$). CR significantly increased the testicular index in both young and aged rats (0.84 ± 0.06, $p < 0.001$; 0.52 ± 0.04, $p = 0.004$, respectively) (Figure 1E).

The Effect of CR on Kidney Function

Serum Cre was significantly elevated in the OC group (0.44 ± 0.06; $p = 0.019$); CR decreased Cre in both the YCR and OCR groups (0.29 ± 0.07 and 0.26 ± 0.03; $p = 0.009$ and $p < 0.001$, respectively) (Figure 2A). BUN levels were significantly higher in aged rats (YC: 16.67 ± 3.61, YCR: 16.83 ± 2.48, OC: 25.13 ± 1.95, OCR: 24.25 ± 0.49, $p < 0.001$), whereas CR had no significant effect in either young or aged rats (Figure 2B). Serum UA levels were significantly elevated in aged rats (YC: 0.44 ± 0.05; OC: 15.63 ± 0.20; $p < 0.001$), and CR decreased UA levels in aged rats (OCR: 0.39 ± 0.07; $p < 0.001$) (Figure 2C).

Effect of CR on Blood Testosterone Levels

Plasma testosterone levels were significantly decreased in the OC group (63.75 ± 14.62, $p < 0.001$). CR reduced testosterone levels in both young and old rats [YCR: 26.29 ± 14.35 ($p < 0.001$]); OCR: 33.89 ± 15.55 ($p = 0.032$)] (Figure 3).

Effect of CR on MDA and GSH Status in Kidney and Testicular Tissue

Aging significantly increased kidney MDA levels (YC: 12.44 ± 1.25, OC: 19.15 ± 3.95, $p < 0.001$), whereas CR significantly decreased MDA levels in aged rats (12.41 ± 3.24, $p < 0.001$; Figure 4A). Kidney GSH levels declined with age in control rats (YC: 2.69 ± 0.29, OC: 2.31 ± 0.15, $p = 0.006$), and CR increased GSH levels in aged rats (2.62 ± 0.29, $p = 0.016$) (Figure 4B). Kidney MDA levels were negatively correlated with GSH and positively correlated with Cre, BUN, and UA ($r = -0.38$, $p = 0.046$; $r = 0.435$, $p = 0.021$; $r = 0.452$, $p = 0.016$; $r = 0.788$, $p < 0.001$, respectively). Testicular MDA levels were significantly higher and GSH levels were lower in aged rats compared to young rats (MDA; YC: 2.05 ± 0.33, OC: 3.50 ± 1.15, $p = 0.002$; GSH; YC: 2.40 ± 0.17, OC: 2.11 ± 0.05, $p = 0.005$). CR significantly decreased testicular MDA levels in aged rats (2.44 ± 0.52; $p = 0.024$;

Figure 4C), whereas GSH levels remained unchanged (Figure 4D). Furthermore, tissue MDA levels were negatively correlated with the organ index (r = -0.455, p = 0.029).

Histological Findings

H&E staining of kidney tissue showed a normal histological architecture in young rats, whereas aged rats showed inflammatory cell infiltration, tubular dilatation, and vacuolar degeneration in some areas. No statistically significant differences were observed between the YC and YCR groups (p = 1.000). M&T staining revealed an increase in the fibrotic area in both the OC and OCR groups compared with the YC group (p = 1.000). M&T staining revealed an increase in the fibrotic area in both the OC and OCR groups compared with the YC group (p < 0.001 and p = 0.006, respectively). The fibrotic area in OCR kidneys was lower than in OC kidneys (p

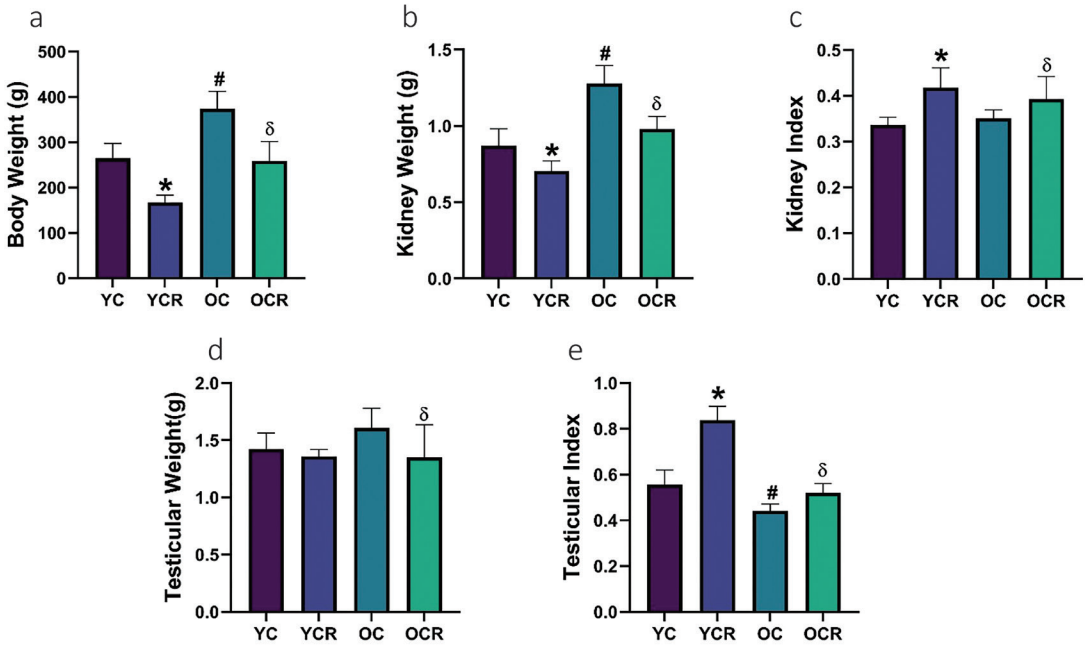

Figure 1. Effect of caloric restriction on body weight (a), kidney weight (b), kidney organ indices (c), testicular weight (d) and testicular indices (e) in young (YC, YCR) and old (OC, OCR) rats.

*p < 0.05 compared with the YC group; #p < 0.05 compared with the YC and YCR groups; δp < 0.05 compared with the OC group.

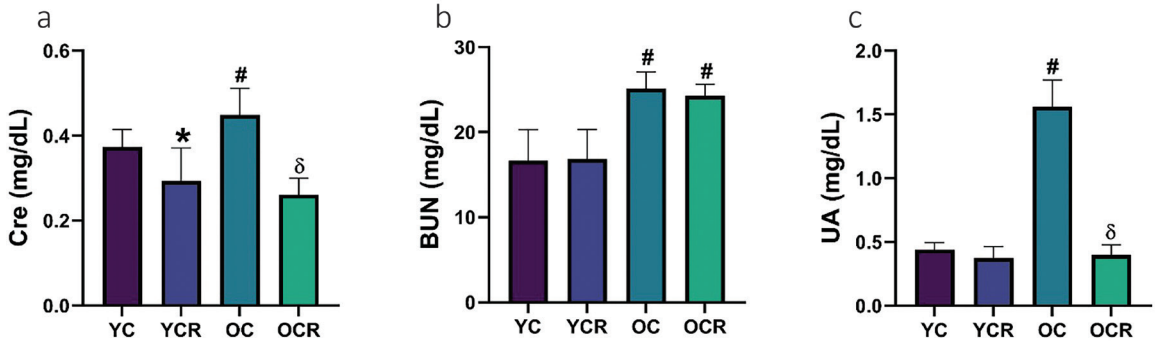

Figure 2. Effect of CR on serum Cre (a), BUN (b) and UA (c) levels in young (YC, YCR) and old (OC, OCR) rats.

*p < 0.05 compared with the YC group; #p < 0.05 compared with the YC and YCR groups; δp < 0.05 compared with the OC group.

< 0.020), whereas the fibrotic area in YCR kidneys was lower than in OCR kidneys ($p < 0.001$). CR reduced fibrosis in aged rats ($p < 0.005$). Glomerular diameter in the YCR, OC, and OCR groups decreased compared with the YC group ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively).

Testicular histology in YC rats revealed a normal interstitium and normal seminiferous tubules. In OC rats, seminiferous tubules were dilated, spermatogenic cells showed atrophic changes, interstitial edema increased, Leydig cell degeneration was observed. The seminiferous tubule diameter was significantly lower in the YC group compared with the OC and OCR groups ($p < 0.001$ for both comparisons). The Johnsen score was significantly higher in the YC compared with the OC ($p < 0.001$). CR significantly decreased the Johnsen score in young rats and increased it in aged rats ($p < 0.001$ and $p = 0.011$, respectively). The Johnsen score was positively correlated with plasma testosterone levels and inversely correlated with BUN and UA ($p = 0.013$, $p < 0.001$, and $p < 0.001$, respectively) (Table 1).

DISCUSSION

Aging, a gradual and pathological process, affects many organs and systems (22). Our findings suggest that aging decreases testicular index while increasing kidney weight. Imaging studies in healthy individuals without kidney disease report age-related reductions in kidney volume (23,24). Findings from experimental animal models support these results (25,26).

Gonadal aging involves morphological, hormonal, and metabolic changes that impair reproductive function and quality of life. It is also associated with an increased risk of age-related diseases, including diabetes, renal dysfunction, cardiovascular failure, and cancer (27). Aging is commonly associated with decreased testicular volume in men (28). Studies show that men aged over 75 years have a lower average testicular volume than men aged 18–40 years (29). Similarly, our study observed a reduction in testicular weight in aged rats.

The literature indicates that aging impairs renal and testicular function. It increases Cre, BUN, and UA and decreases testosterone levels (26,30–32). It has been reported that testosterone levels begin to decline in the third decade of life and continue to decrease gradually throughout life (33). Consistent with these data, our study found higher levels of kidney function markers (Cre, UA) and lower levels of serum testosterone with aging.

Because of their high oxygen consumption, the kidneys are particularly vulnerable to ROS-induced damage (34). ROS-induced oxidative stress, along with tissue damage and structural deterioration, is a key factor in premature aging associated with CKD (35). Moreover, increased oxidative stress, influenced by endogenous and environmental factors, leads to irreversible changes in the kidneys and testes (36,37). These changes predispose them to various pathologies (38).

ROS contribute to several physiological processes, including spermatogenesis, sperm capacitation, and acrosomal activity. However, controlled ROS levels are essential for healthy sperm function and, consequently, successful fertilization (39). Elevated ROS levels impair sperm function and increase DNA fragmentation in the sperm nucleus (40), both of which contribute to male infertility (41). Previous studies have reported elevated ROS concentrations in the semen of 25–40% of infertile men (42). Furthermore, lower levels of antioxidant enzymes are detected in the seminal plasma of these individuals compared than in controls (43). ROS found in seminal plasma have been shown to be primarily the superoxide anion, the hydroxyl radical, and hydrogen peroxide (44). Our earlier research demonstrated that aging in male Wistar rats increases oxidative stress in testicular tissue, leading to a decline in testosterone and GSH levels (36). Moreover, a study of 66 men at

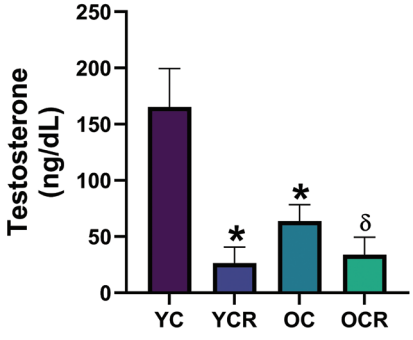

Figure 3. Effect of CR on plasma testosterone levels in young (YC, YCR) and old (OC, OCR) rats.

*p < 0.05 compared with the YC group; δp < 0.05 compared with the OC group.

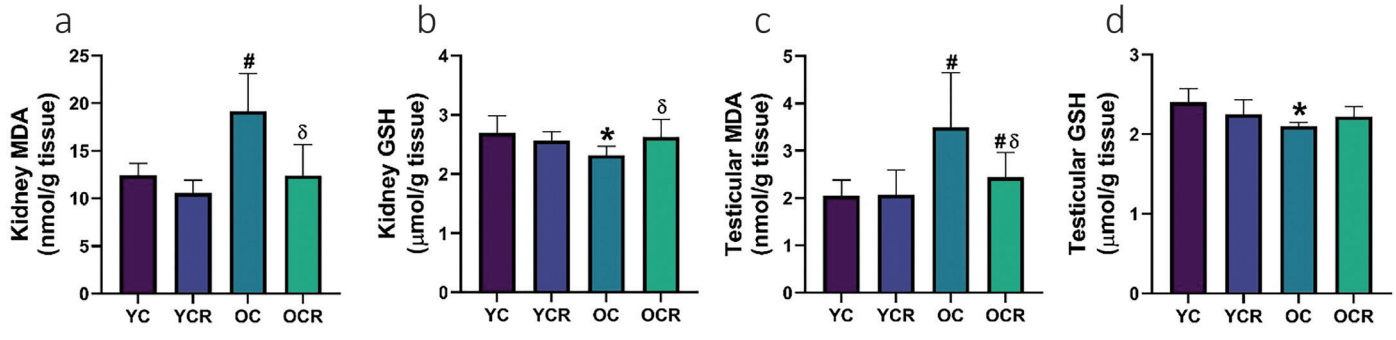

Figure 4. Effect of CR on MDA (a-c) and GSH (b-d) levels in kidney and testicular tissue in young (YC, YCR)and old (OC, OCR) rats.

*p < 0.05 compared with the YC group; #p < 0.05 compared with the YC and YCR groups; δp < 0.05 compared with the OC group.

Table 1. Histological evaluation results.

	Young		Old	
	Control	**CR**	**Control**	**CR**
Renal fibrotic area (%)	1.68 ± 0.22	1.23 ± 0.12	9.89 ± 0.66*	4.86 ± 0.63*#δ
Glomerular diameter (μm)	64.25 ± 0.79	57.51 ± 0.47*	58.44 ± 1.07*	60.51 ± 1.04*
Seminiferous tubule diameter (μm)	216.11 ± 1.71	222.54 ± 1.79	273.49 ± 1.60*	249.62 ± 1.47*#
Johnsen score (0–10)	9.63 ± 0.12	9.17 ± 0.13*	8.28 ± 0.07*	8.51 ± 0.26*#

*p < 0.05, compared with the YC group; #p < 0.05, compared with the OC group; δp < 0.05, compared with the YCR group.

various stages of kidney disease revealed a significant association between reduced testosterone levels and progressive loss of kidney function (45).

Changes associated with aging are not limited to biochemical and inflammatory processes; they also encompass morphological alterations. Morphological changes with aging include a reduced number of glomeruli in the kidneys as well as an increase in glomerular volume and fibrosis (14). Other changes observed in the testis include reduced tubular diameter, increased basement membrane thickness, fibrosis, and decreased numbers of sertoli and spermatogenic cells (2). These structural deteriorations reduce the Johnsen score, a marker of male fertility (36).

CR is an effective dietary strategy to mitigate age-related degenerative changes. It functions by reducing oxidative stress, inflammation, and metabolic disorders through the limitation of energy intake (46). In our study, CR resulted in decreased organ weight and body weight regardless of age, whereas renal and testicular indices increased significantly. Evidence from other studies suggests that CR may increase the organ index, leading to a compensatory mechanism against mass reduction (47,48). CR has also been shown to slow the progression of CKD in rodents. It lowers blood markers, such as Cre, BUN, and UA, and delays disease onset, thereby increasing survival (49,50). CR suppresses oxidative damage to lipids, proteins, and DNA that is associated with aging, while enhancing cellular defense mechanisms against oxidative stress (51). CR also reduces mitochondrial ROS formation in the cardiac and skeletal muscles and the liver (52). In the kidney, CR decreases levels of MDA, a marker of oxidative stress, and increases levels of GSH, an important antioxidant (53). In mice, 60% CR for 2 months increased GSH levels and decreased levels of oxidized GSH and GSH peroxidase in the kidneys (54). In rats, short-term 60% CR decreased body weight, triacylglycerol levels, glomerular volume, fibrosis, and cellular senescence (14,15). Furthermore, CR decreases the incidence of histopathological nephropathy and improves renal function (14,15). Although CR prolongs lifespan (55), it can have adverse effects on reproduction (56). Studies in adult rats have demonstrated that CR can decrease body, testicular, epididymal, and prostate weights (57). Short-term CR decreases blood testosterone levels in rats, whereas long-term CR increases testosterone levels compared with rats fed ad libitum (58). While these results are consistent with our findings, the observed differences may be attributable to the duration of CR application, age, and hormonal status.

Study Limitations

Although our study is preliminary and suggests that CR may protect kidney and testicular tissues by reducing oxidative stress during ageing, it is limited by the lack of measurements of oxidative stress parameters (such as superoxide dismutase and GSH oxidase) and hormone levels (such as FSH and LH). However, renal and reproductive dysfunctions may coexist and should be considered during follow-up.

CONCLUSION

In conclusion, our findings show that CR during aging helps preserve the structural and functional integrity of the kidney and testis. These effects are demonstrated by reduced oxidative stress, increased antioxidant defenses, and reduced fibrosis on histology. However, further histological and molecular analyses are needed to clarify the underlying mechanisms and their interactions.

Ethics

Ethics Committee Approval: The experimental procedures were approved by the Gazi University Animal Experiments Ethics Committee (approval number: #G.Ü.ET-25.068, date: 18.07.2025).

Informed Consent: Not applicable.

Footnotes

Authorship Contributions

Concept: A.C., K.G.A., Design: A.C., K.G.A., Data Collection or Processing: A.C., E.T., Analysis or Interpretation: A.K.A, S.Ö.A.D., Literature Search: A.C., K.G.A., Writing: A.C., K.G.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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