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Caloric Restriction and Litter Size Do Not Compromise Renal Function of Male Wistar Rats

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Abstract: This work had the purpose of comparing some renal parameters of Wistar rats raised in small litters either freely-fed (G3L) or subjected to 30% caloric restriction (G3R) with those of rats from normal litters (G9) at the age of 90 days. The results showed that the relative weight of the kidneys and urinary flow were not significantly different. All the groups had GFR (glomerular filtration rate) and proteinuria within normal values, despite group G3R having values statistically lower. However, group G3R had diminished glomerular area and increased numbers of renal corpuscles. Group G3L did not exhibit differences compared with controls (G9). The effects of caloric restriction did not compromise kidney structure and function in 90-day-old rats.

Key words: Small litter, caloric restriction, rat, renal morphology, renal physiology.

1. Introduction

The difficulty in adopting a healthy lifestyle, together with the growing availability of foods of fast preparation, low nutritional value and high palatability, have increased the incidence of obesity and its related health issues [1-4]. Obesity is a chronic disease having genetic and environmental components, but mostly triggered by a positive energy balance, and it is the starting point for several metabolic disorders [5].

Chronic renal disease is one of the possible adverse effects of obesity. A pioneering study, carried out in 1974 by Weisigner et al. [6], identified glomerulosclerosis in obese patients. Other research showed a relationship between high BMI (body mass index) and chronic renal disease, increased kidney weight and glomerular area [7], increased glomerular filtration rate [8, 9] and increased renal blood flow [9, 10]. The adaptive changes by the kidney glomeruli take place because of the decreased kidney functional mass and these alterations are followed by glomerular sclerosis [11].

Caloric restriction is characterized as a caloric ingestion below the energy demands of an individual. In 1935, one of the first and most remarkable studies on caloric restriction was carried out by McCay [6], demonstrating that a caloric ingestion decreased in 40% since weaning could extend the lifespan of rats by two times. Recently, it has been claimed that caloric restriction, at controlled and moderate (10-30% of the free-will ingestion) levels, is an excellent prophylactic measure against metabolic and cardiovascular disturbances, excess weight, diabetes and cancer. It has also been linked to greater longevity and higher expectations of a healthy life [12-19]. At these levels, caloric restriction does not lead to malnutrition and its associated diseases [20].

In the kidneys, caloric restriction was shown to delay aging-related processes, such as glomerulosclerosis [21], tubulointerstitial fibrosis, decreased GFR (glomerular filtration rate) and renal blood flow [22]

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and alterations of tubular function [23-25]. In addition, it prevents oxidative stress [19, 21], which tends to be increased by aging [26, 27].

In rats, litter size reduction is a classical model of overweight/obesity and increased adiposity [28-31] which is based on the modification of the post-natal nutritional environment (that is, reduction in the number of pups per litter during lactation) as a mean of "programming" the central controls of appetite and energy balance and leading to the obese phenotype of the adult animal [32-37]. Using this model, this work analyzed the effect of a 30% caloric restriction on some structural and functional parameters of the kidneys of adult male Wistar rats raised in small litters. The purpose was to determine whether litter size or caloric restriction would cause alterations in the adult kidney that would impair its functional role.

2. Method and Materials

2.1 Study Design

Rattus norvegicus from the Wistar strain were used. The dams and their litters were kept at the local animal house under regular light/dark cycles (12 h light/12 h dark) and controlled temperature (22 ± 2 ºC). The protocols of handling, treatment and experimentation were carried out after approval by the Ethics Commission on the Use of Animals (CEUA 088/2014).

On the second day after birth, the litters were organized so that each dam had either nine (control group, G9) or three (reduced-litter group, G3) pups, preferably males. The dams had free access to water and rodent chow during gestation and lactation.

At weaning, when they were 21 days old, the male pups were put in plastic boxes in groups of three per box. Euthanasia of the dams and female pups was carried out by excess anesthetic [thiopental, 120 mg/kg body weight (b.w.), intraperitoneal]. At this point, the following experimental groups were established and followed until 90 days of age:

G9 (n = 8): male rats from nine-pup litters that had free supply of chow and water during the whole experimental period.

G3L (n = 8): male rats from three-pup litters that had free supply of chow and water during the whole experimental period.

G3R (n = 8): male rats from three-pup litters that had their supply of chow reduced by 30% relative to the ingestion of G9 animals of the same age, during the whole experimental period.

Body weight and chow ingestion were periodically recorded. The mean chow ingestion of group G3L (per 10 g b.w.) was used to calculate the caloric restriction of group G3R.

At the age of 90 days, the animals were placed in individual metabolic cages for 12 h to record urine volume and water ingestion. Urine samples were used for further analytical determinations, as described below.

The euthanasia of the animals (thiopental 120 mg/kg b.w., intraperitoneal) of each group (G9, G3L and G3R) was carried out at 90 days of age after overnight fasting.

Blood samples were collected, centrifuged and the plasma stored for biochemical analyses. Both kidneys were collected and processed as described below.

2.2 Evaluation of the GFR (Glomerular Filtration Rate)

Plasma and urine creatinine were assayed by kinetic-colorimetric method (commercial kit Labtest®; GoldAnalisa, Belo Horizonte, Brazil). The GFR was calculated by the creatinine clearance and the results were expressed as mL/min/100 g b.w.

2.3 Proteinuria

Urine proteins were assayed by the colorimetric method of pyrogallol red (commercial kit Labtest®) and the values were expressed as mg/12 h/100 g b.w.

2.4 Morphologic Analysis of the Kidney

The preparations of the material followed conventional histotechnical methods [38]. The kidneys

were removed, fixed in Metacarn for 24 h in a refrigerated room, dehydrated in increasing series of alcohols (70%-100%), cleared in xylene and blocked in paraffin at 58 ºC. The 5-µm sections were mounted in glass slides and stained with Masson's Trichrome for analysis of the glomerular and tubulointerstitial regions.

Images of the histological sections were captured in optical microscope (Olympus U-TV0.5XC-3, Tokyo, Japan) coupled to digital camera. The number of glomeruli per field was visually determined under 10X objective [39] and the morphometric parameters were analyzed under 40X objective.

The glomerular areas were obtained by manually drawing the glomerular outlines; the area was then calculated by the image analyzer ImagePro Plus® (Media Cybernetics, Rockville, MD, USA).

The analysis and interpretation of the data were carried out by two observers without previous knowledge of the experimental groups.

2.5 Statistical Analysis

The data sets were expressed as mean ±SD (standard deviation) and subjected to Shapiro-Wilk normality test. Comparisons were made through one-way ANOVA followed by Tukey *post-hoc* test. Significance level was set at 5%. Statistical analyses and graphs were made in Prism® 5.0 (GraphPad, San Diego, CA, USA).

3. Results

3.1 Biometric Characterization

At the age of 90 days, groups G9 and G3L had similar ($p > 0.05$) values of body weight, but visceral fat relative weights were higher in the latter (Table 1). In contrast, these parameters were lower in group G3R (Table 1) compared to groups G9 and/or G3L. In percentage terms, caloric restriction (group G3R) decreased body weight by 36% and visceral fat by 40% compared to group G9. The relative weight of the kidneys was higher in group G3R than in groups G9 and G3L (Table 1).

3.2 Functional Parameters

The analysis of the urinary flow did not show any significant differences ($p > 0.05$, Fig. 1A). The GFR of the rats from reduced litters and subjected to caloric restriction (G3R) was decreased as compared to the other groups, as shown in Fig. 1B. Also, groups G9 and G3L were similar in their levels of proteinuria ($p > 0.05$, Fig. 1C), while those of group G3R had a significant decrease of this parameter when compared to G9 and G3L.

3.3 Morphological Parameters of the Kidneys

The morphological analysis of the kidneys did not reveal inflammatory infiltrates either in the glomerular or the tubulointerstitial regions in any of the groups. Signs of glomerular sclerosis or tubulointerstitial fibrosis were not observed.

There was a significant decrease in glomerular area in the animals of group G3R compared with the other groups (Fig. 2A), but not between groups G9 and G3L. As for the number of renal corpuscles, the difference between the control animals (G9) and those from reduced litters (G3L) was not significant ($p > 0.05$, Fig. 2B). However, this number was significantly increased in those rats from group G3R compared with the other groups.

Table 1 Biometric parameters of groups G9, G3L and G3R at the age of 90 days.

	G9 (n = 8)	G3L (n = 8)	G3R (n = 8)
Body weight (g)	344.1 ± 9.95a	351.9 ± 22.27a	221.2 ± 32.13b
Visceral fat (g/100 g body weight)	2.82 ± 0.28a	3.24 ± 0.28b	1.66 ± 0.28c
Kidneys (g/100 g body weight)	0.75 ± 0.05a	0.71 ± 0.03a	0.80 ± 0.10b

Data shown as mean ± SD. Numbers followed by different letters are significantly different ($p < 0.05$, one-way ANOVA).

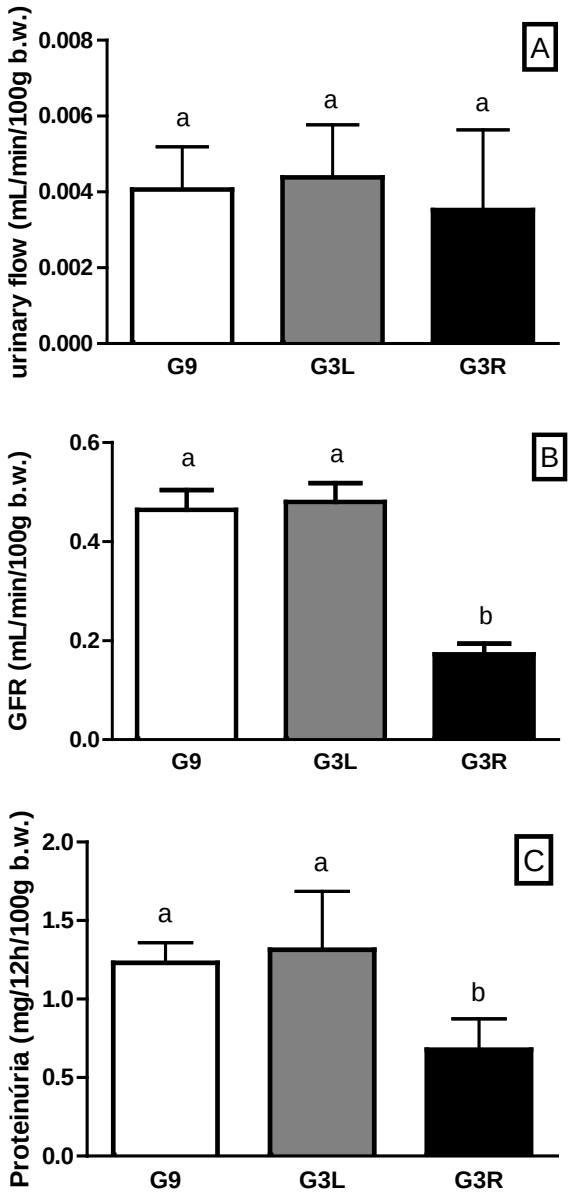

Fig. 1 Urinary flow (A), glomerular filtration rate (B) and proteinuria (C) of groups G9, G3L and G3R at the age of 90 days.

Data shown as mean ± SD; n = 8 per group. Columns with different letters are significantly different ($p < 0.05$, one-way ANOVA).

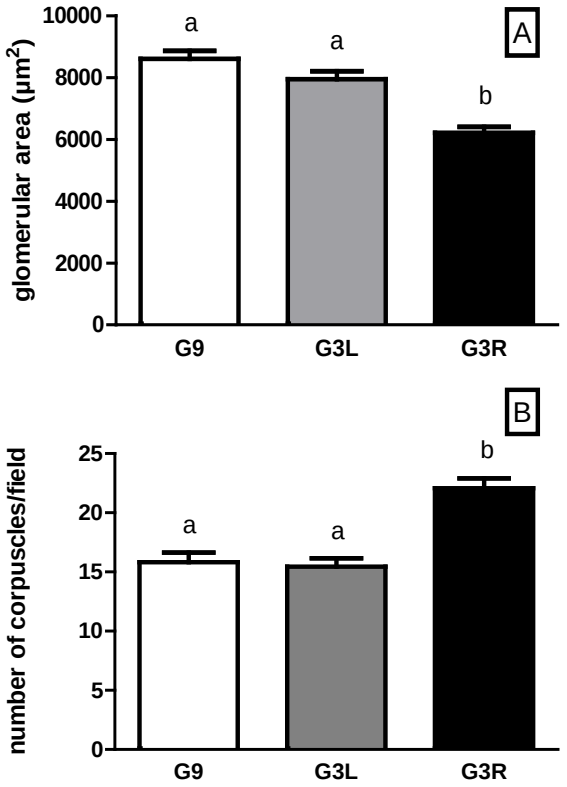

Fig. 2 Glomerular area (A) and number of renal corpuscles (B) of groups G9, G3L and G3R at the age of 90 days.

Data shown as mean ± SD. Columns with different letters are significantly different ($p < 0.05$, one-way ANOVA).

4. Discussion

As previously shown [40], body weight did not differ significantly between rats raised in normal (nine pups) or reduced litters (three pups) when they were 90 days old. This was observed in other investigations, and it was argued that body weight differences between control and reduced litters are more pronounced after weaning but tend to decrease with aging [26, 33, 40-45]. On the other hand, the higher adiposity of reduced-litter rats indicates that it may have been programmed by the lactational overfeeding and remained until adult life. As expected and already observed [40], the 30% caloric restriction since weaning (group G3R) decreased both body weight and visceral fat.

The purpose of this investigation was to assess some morphologic and functional aspects of the kidneys of these rats from control or reduced litters and the impact of caloric restriction on these aspects. In summary, litter size did not change kidney morphology or functional parameters, while caloric restriction had some relevant effects.

Litter size did not change GFR (groups G9 and G3L),

but caloric restriction (G3R) decreased it. This finding could be linked to the decreased body weight of these animals, because plasma creatinine, despite being within the normal range, was significantly lower in group G3R than in the other (freely-fed) groups. A similar result was found by Wiggins et al. [46] with Fischer rats under 60% caloric restriction for 24 months, which showed decreased creatinine levels when compared to freely-fed animals. In a study of obese patients aging 23 to 46 years, the GFR was increased. After weight loss, GFR and renal plasmatic flux were reduced by 24% and 13%, respectively [22].

The number of renal corpuscles was higher in the G3R but did not differ between G9 and G3L animals. In contrast, glomerular area was lower in group G3R. It seems that caloric restriction, possibly by decreasing interstitial tissue of the renal cortex, could have brought corpuscles closer, thus yielding larger numbers of these structures per microscopic field. In addition, caloric restriction possibly decreased the corpuscular size, as assessed by the glomerular area. In the adult human being, each kidney has 800,000-1,000,000 nephrons and is incapable of forming new ones [47]. With aging, there is a natural decline of this number [46-48], diminishment of the number of glomeruli [49] and of epithelial cells of the convoluted tubules, increased number of interstitial cells and interstitial fibrosis [48]. Chagnac et al. [22] emphasize that nephrons do not increase in number with increased adiposity, but that there is instead an increased GFR per nephron.

During aging, metabolic resistance and oxidative stress may occur in different tissues [50]. Caloric restriction has been shown to decrease acute pro-inflammatory cytokine release in response to endotoxins and other stimuli, thus preventing the inflammatory response [51]. Obesity is linked to an inflammatory process that may cause glomerular inflammation and chronic renal disease. Aged Fischer rats not having diabetes or hypertension develop glomerulosclerosis when fed at will [52, 21]. Glomerulosclerosis, tubulointerstitial fibrosis and oxidative damage were prevented by caloric restriction for 24 months [53-57]. Inflammatory infiltrates were not observed in any of the groups of this study, either in the glomerular or in the tubulointerstitial region.

Chronic renal disease may result from pathophysiological mechanisms affecting the glomerular, tubular, vascular and/or interstitial compartments of the kidney. As it progresses, chronic renal disease causes glomerular and tubulointerstitial lesions, with presence of inflammatory infiltrates that evolve to glomerular sclerosis and tubulointerstitial fibrosis [58-62]. Proteinuria is an important factor in this process. The leakage of proteins through the filtration membrane of the renal corpuscle and the ensuing increase in protein resorption by the epithelial cells of the proximal tubule may trigger the production of pro-inflammatory factors, an inflammatory reaction that may end up in tubulointerstitial fibrosis [63-65].

Our results showed that rats from reduced litters and fed at will (G3L) had normal proteinuria at the age of 90 days, while those under caloric restriction (G3R) had decreased proteinuria, which is consistent with their decreased body weight and adiposity. At later ages and under more severe caloric restriction [46], proteinuria is reported to increase, suggesting that aging and diet are highly influential on this parameter.

5. Conclusions

Litter size did not alter any of the morphological or functional renal parameters analyzed, while reduced-litter, calorically restricted rats had decreased, although within the normal range, GFR and proteinuria. In addition, this group (G3R) had significantly lower glomerular area and higher numbers of renal glomeruli. Altogether, these changes may be important for the preservation of the renal function in these animals. Investigations on aspects such as macrophages, fibronectin and type I collagen might reveal more detailed features of kidney structure and function upon caloric restriction.

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