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The "protein diet" reduces glomerular diameter and volume density in rats

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Abstract

Objective: This study aimed to evaluate the effect of a protein diet on kidney morphology. *Methods:* Forty adult *Rattus Norvegicus Wistar albino* were divided into four groups (n = 10): Control 1 (C1) Control 2 (C2), High Protein 1 (HP1) and High Protein 2 (HP2). The C2 and HP2 groups were subjected to 30% of food restriction. At the end of the experiment, kidneys were removed, weighed and their length and width, measured. Thereafter, they were cleaved according to the *Orientator* method, fixed in formalin and embedded in paraffin. The blades of the histological sections were stained according to the Hematoxylin & Eosin technique and photomicrographed. Glomerular density was determined by the stereological method and the diameter of renal glomeruli was calculated using Image J software. Serum analyses were performed according to the colorimetric method. Results are expressed as mean and standard deviation. To compare the means between groups, it was used ANOVA and Tukey as post-test, accepting a significance level of 5%. *Results:* The HP groups had higher blood pressure. The kidney size and weight of HP1 was greater than C1 and C2. The HP1 group showed lower glomerular diameter and the HP2 group showed lower glomerular density than C1 and C2 groups. *Conclusion:* The "protein diet" can cause increased systolic blood pressure, abnormal renal morphology, reduced glomerular diameter and glomerular volume density. This study suggests that this diet can lead to changes in kidney tissue, predisposing individuals to development of chronic diseases.

Keywords: Kidney. Diet. Kidney Glomerulus. Dietary Proteins.

Introduction

High protein diets have been the choice made by part of the population to get a lean body in a short period of time. [1,2] This diet is characterized by a high protein and lipid intake and low carbs intake; [2,3] however, the safety of an excessive proteins intake has been questioned and some evidences indicate that a high protein diet may cause damages to the kidney tissue. [4-7]

Kidney alterations caused by a high proteins intake have been accounted to the higher workload received by the organ, secondary to the increased filtration of protein metabolites, especially urea and creatinine.[8] Other related factor is the development of metabolic acidosis caused by a high production of ketonic bodies resulting from protein and lipid metabolism. [6]

These factors are associated with kidney hypertrophy, glomerular hyperfiltration, increased albumin excretion in the urine, increased diuresis, natriuresis, kaliuresis associated to changes in blood pressure, augmented risk for nephrolithiasis, alterations in the kidney morphology, interstitial fibrosis and increased production of oxygen-reactive species, with a consequent increase of oxidative stress. [4-11] These consequences are responses to abnormal renal hemodynamics, a predecessor of renal failure and a factor that contribute to the development of kidney diseases.[11,12]

However, the literature is still controversial regarding the effects of excessive proteins intake while maintaining the organ integrity, given that some researchers did not find an association of high protein intake with kidney damages.[4,5]

Thus, the aim of this study was to assess the effect of "protein diets" on the kidney tissue, aiming to assess alterations that may occur from a diet with this profile.

Materials and Methods

The study was conducted at the Laboratory of Experimental Nutrition, Department of Nutrition and Dietetics, Faculty of Nutrition, Federal Fluminense University, with female albino *Rattus Norvergicus Wistar*, 90 days old, and with approximate weight of 200g. All animals were maintained in experimentation for 60 days housed in individual polypropylene cages duly identified, at constant temperature room (24ºC ± 2ºC) and adequate lighting in 12-hour light/12-hour dark cycle.

The animals were divided into four groups (n=10/group): 1) Control Group 1 (C1) and 2) Control Group 2 (C2)*- who were fed a feed comprised of 69.20% carbohydrates (59.20% starch and 10% sugar); 11.30% protein (casein); 4.80% lipid (soybean oil); 1% vitamin mix (Prag Soluções, São Paulo, Brazil); 3.5% mineral mix (Prag Soluções, São Paulo, Brazil); 5% dietary fibers (cellulose); 0.25% choline bitartrate, and 0.18% L-cysteine, manufactured as recommended by the American

Institute of Nutrition (AIN-93M*;* 3) High Protein Group 1 (HP1) and 4) High Protein Group 2 (HP2)*- which were fed a feed containing 4.73% glucose (lactose); 49.77% protein (47.54% of beef protein and 2.23% milk protein); 15.97% lipid (11.97% meat fat and 4% soybean oil); 1% of vitamins mix (Prag Soluções, São Paulo, Brazil); 3.5% minerals mix (Prag Soluções, São Paulo, Brazil); 7% dietary fibers (5% cellulose and 2% agar); 0.18% L-cysteine and 0.25% choline bitartrate.

Agar was used to thicken and shape the feed. The Groups C2 and HP2 were fed 70% of the amount of feed consumed by the Groups C1 and HP1.

The ingredients used in the formulation of the feed based on "protein diet" were purchased locally. Beef (chuck) was dehydrated, ground, sieved and mixed with the other ingredients.

The care of the animals consisted of providing constant availability of fresh water to all groups. The body weight (g) and water intake (ml) were measured on a weekly basis using a scale (Bioprecisa JY 50001, 0.1g precision) and a test tube, respectively. The remaining water was quantified to determine the daily intake. Control of the feed supply and leftover of the groups C1 and HP1 was performed on a weekly basis. Control of the groups C2 and HP2 was performed on a daily basis because of the 30% food restriction to which they were subjected.

Blood pressure of the animals was measured once a week during the experiment using a tail-cuff plethysmograph (Insight®), expressed in mmHg.

By the end of the experimental period all rats had undergone vaginal lavage for determination of the estrous cycle. After analysis, the rats that were in the estrous phase of the cycle were separated from the group and submitted to fasting for six hours before being euthanized. They were then anesthetized with intraperitoneal injection. Blood was collected via cardiac puncture. Blood was collected in tubes without anticoagulant and centrifuged to obtain serum. The serum samples were frozen at -80ºC for subsequent analyses.

All animals were then subjected to median laparotomy. The kidneys were removed, weighed (g) and their length and width were measured with a caliper (Lee Tools model, 0.05mm precision), and expressed in centimeters.

The kidneys were fixed in 4% buffered formalin. Then, they were cleaved according to the Orientator method, processed for performance of the histological technique of paraffin embedding and cut to a thickness of 5 μm. The slides were stained according to the Hematoxylin & Eosin technique for performance of all histomorphometric analyses. The glomerular volume density (Vv [Glom], %) was determined by the stereological method using the M-42 Test System in 25 random fields per animal.

All tissue cuts used for light microscopy were photographed under the same conditions and with 2.040x1.536 pixels resolution with a digital camera (Olympus DP70, Tokyo, Japan) directly coupled to the microscope (Olympus BX51, Tokyo, Japan).

Determination of the glomerular diameter was performed in 25 random fields per animal using the Image J software, version 1.47, by two linear measurements in two different axes of each glomerulus. The results were expressed in μm.

With respect to the biochemical parameters, the serum concentrations of urea, total proteins and creatinine were determined by the colorimetric method using commercial kits (BioClin, Belo Horizonte, Brazil). Serum concentration of aldosterone and interleucine-6 was determined by the Enzyme Linked ImmunonoSorbent Assay (ELISA) method using commercial kits (UScn, Life Science Inc.).

The results are presented by descriptive statistics as arithmetic mean and standard deviation. Comparative analyses between the means of the studied variables of the four groups were performed by analysis of variance (ANOVA one-way) and Tukey's as post-test. To support the proposed analyses, Kurtosis and Skewness tests were conducted to check for data normality. Pearson's correlation test was used to determine possible associations between the variables studied. A 5% significance level was assumed, using the GraphPad InStat software, version 3.10, 2009.

This study followed the criteria set out by the Brazilian College of Animal Experimentation (COBEA, 2011) and the Brazilian Society for Laboratory Animal Science (SBCAL). The project was submitted to the ethics committee responsible for researches with laboratory animals of the Federal Fluminense University and was approved under protocol number 0027/08.

Results

It was observed during the experiment that the groups that were fed on free-access feeding (C1 and HP1) had a body weight increase while those subjected to feed restriction (C2 and HP2) lost weight ($p<0.0001$). As shown in Table 1, regarding daily feed intake, the C1 and HP1 groups consumed more feed than the groups that were on feed restriction ($p<0.0002$).

The group that was fed high protein diet on a free-access basis (HP1) consumed more water than the other groups. The other groups had similar water intake ($p<0.0001$) (Table 1). It was also observed that the groups that were fed the "protein diet" had a higher diuresis level that the control groups (observational data not shown).

Table 1. Analysis of weight, feed intake and water intake of rats during the treatment. Rio de Janeiro-RJ, 2012.

	C1	HP1	C2	HP2	*p*-value
Initial weight (g)	235.4±13.17	237.2±7.28	237.2±3.03	234.6±17.17	0.05
Final weight (g)	277.4±15.82[a]	272.8±23[a]	192.8±13.33[b]	194.8±3.70[b]	0.03
Weight variation (Δ)	27.33±7.09[a]	30.3±15.00[a]	-16.6±6.10[b]	-15.80±3.03[b]	0.0001
Feed intake (g/day)	10.96±1.38[a]	10.98±0.96[a]	8.35±0.18[b]	8.39±0.20[b]	0.0002
Water intake (mL/day)	22.97±3.99[a]	37.47±4.53[b]	21.65±5.25[a]	26.42±2.44[a]	0.0001

Control group 1 (C1); High protein group 1 (HP1); Control group 2 (C2); High protein group 2 (HP2). Different superscripts indicate statistical difference between the groups (univariate ANOVA, $P<0.05$).

Table 2 shows that the left kidney of the rats of the group HP1 was heavier than the other groups, while the right kidney of the group HP1 was heavier only when compared to the group C2 ($p<0.0001$). Regarding the size of the organ, it was found that the right and left kidneys of the animals of the group HP1 were larger when compared to the rats of the groups C1, C2 and HP2 ($p<0.0001$). When compared to the control group, the right kidney of the group HP1 was 66% larger in size compared to the group C1 while the left kidney was 57% larger when compared to the control group (Table 2).

Table 2. Morphological analysis of the rats' kidneys after 60-day treatment. Rio de Janeiro-RJ, 2012.

	C1	HP1	C2	HP2	*p*-value
Kidneys weight (g)					
Left	0.78±0.04[a]	1.00±0.17[b]	0.61±0.07[a]	0.74±0.09[a]	0.0001
Right	0.85±0.10[a.b]	1.08±0.10[b]	0.64±0.053[a]	0.92±0.20[a.b]	0.0001
Kidneys size (cm)					
Left	1.12±0.44[a]	1.76±0.05[b]	0.64±0.11[c]	0.74±0.13[a]	0.0001
Right	1.080±0.37[a]	1.80±0.10[b]	0.64±0.05[c]	0.84±0.16[a]	0.0001

Control group 1 (C1); High protein group 1 (HP1); Control group 2 (C2); High protein group 2 (HP2). Groups subjected to 30% food restriction (C2 and HP2). Different superscripts indicate statistical difference between the groups (univariate ANOVA, $P<0.05$).

Regarding the glomerular volumetric density (Vv [glom], %), the group HP2 (3.30±1.2%) presented lower Vv when compared to the groups C1 (5.75±1.70%), C2 (6.05±1.17%) and HP1 (4.46±0.70%) ($P<0.0001$).

When assessing the diameter of the glomeruli of the animals' kidneys, it was found that the group HP1 (54.87±4.54μm) had a smaller diameter when compared to the groups C1 (75.25±2.35 μm), C2 (69.05±3.79 μm) and HP2 (68.82±3.09 μm) ($p<0.001$).

The blood biochemical analyses are described in Table 3. It can be seen that the group C1 had higher serum albumin concentrations compared to the groups C2, HP1 and HP2 ($p<0.007$), and no significant differences were found in serum creatinine concentrations between the groups.

With respect to total proteins, the group C1 exhibited higher concentration of proteins only in comparison to the group HP2 ($p<0.01$). When observing the urea concentrations, the group HP1 exhibited higher concentrations compared to the groups C1 and C2, but similar to the group HP2 ($p<0.0002$) (Table 3).

As to aldosterone, the group that was fed "protein diet" with feed restriction (HP2) exhibited a higher concentration of aldosterone compared to the other groups ($p<0.0001$). Concerning interleukin-6, the control groups exhibited lower concentrations when compared to the groups fed high-protein diet ($p<0.0002$) (Table 3).

Table 3. Biochemical analyses of female adult rats after 60 days of treatment. Rio de Janeiro-RJ, 2012.

	C1	HP1	C2	HP2	*p* valor
Albumin (g/dL)	4.10±0.62[a]	3.55±0.35[b]	3.32±0.3[b]	3.48±0.25[b]	0.007
Creatinine (mg/dL)	0.46 ± 0.05	0.54 ± 0.09	0.44 ± 0.05	0.48 ± 0.08	0.7
Total protein (mg/dL)	7.12±0.79[a]	6.48 ± 0.77[a.b]	6.42 ± 0.68[a.b]	5.74 ± 0.73[b]	0.01
Interleukin-6 (pg/mL)	294.22±41.68[a]	457.3±74.32[b]	390.60±57.62[a.b.d]	535.45±97.62[b.c]	0.0002
Urea (mg/dL)	34.36 ± 5.14[b]	48.43 ± 12.8[a]	34.5 ± 6.02[b]	41.75 ± 9.66[a]	0.0002
Aldosterone (pg/mL)	0.26 ± 0.08[a]	0.20 ± 0.06[a]	0.15 ± 0.08[a]	0.62 ± 0.07[b]	0.0001

Control group 1 (C1); High protein group 1 (HP1); Control group 2 (C2); High protein group 2 (HP2). Groups subjected to 30% food restriction (C2 and HP2). Different superscripts indicate statistical difference between the groups (univariate ANOVA, $P<0.05$).

With respect to the animals' systolic pressure, the groups C1 (111.09±18.69) and C2 (92.78±12.48 mmHg) had normal pressure values. But the groups HP1 (144.7±14.59 mmHg) and HP2 (137.85±3.89 mmHg) had high pressure values compared to the controls ($p<0.005$). As to diastolic pressure, there was not significant difference between the groups.

Discussion

In his book, Dr. Atkins[3] claims that the individuals who follow his high protein diet, even though they consume a larger amount of energy/day, they succeed in losing weight. However, there are no scientific evidences that confirm that this diet has metabolic advantages over other traditional weight-loss diets.[13,14]

The present study shows that when a high protein diet is associated with food restriction, weight loss is similar to a balanced low calorie diet, showing that it is not the diet composition that leads to weight loss but, rather, a reduction in daily energy consumption.

It is known that a high protein diet causes the production of ketone bodies derived from lipid oxidation and ketogenic amino acids. This process leads to increased blood osmolality and, consequently, a sensation of thirst and higher water consumption.[15] This fact was observed in this study: the groups that were fed protein diet indicated a higher water intake than the groups fed control diet (Table 1). Additionally, excretion of ketones by the kidneys is accompanied by excretion of sodium, which causes augmented diuresis, a fact that was also observed during the experiment (observational data not shown).[13,14]

Dietary protein has been recognized as a modulator of the kidney function. Some studies demonstrated that high protein intake affects the kidneys size, making them larger, due to the higher workload demanded from this organ, secondary to the increased filtration of protein metabolites, especially urea.[16,17] Other studies suggest that hyperfiltration in response to a higher nitrogen load and greater demand for renal clearance is a normal adaptive mechanism, similar to what happens when women are pregnant. [4,5]

In terms of renal hypertrophy, there are two plausible explanations: the first hypothesis, abovementioned, refers to a compensatory mechanism to allow excretion of a greater renal molar load. The second hypothesis suggests that an increased protein intake increases the growth hormones, probably because of the growth factor similar to type 1 insulin-like (IGF-1), which may account for the increased size of the organ. However, the authors suggest that such hyperplasia may be momentary and once the extra supply of protein ceases, the organ is back to its original size.[17]

In the present work, it was found that the group that was fed free-access high protein diet (HP1) exhibited more weight (in grams) and a greater kidney size (in centimeters) than the control groups (C1 and C2) and the restricted high protein group (HP2), showing that an increase in protein intake has a direct effect on these morphometric parameters (Table 2). **Similar results were found by** Aparicio et al.[3], who reported that the kidneys of animals fed vegetable high-protein diet were heavier than those fed control diet.

In the present study, the animals fed high protein diet with restriction (HP2) exhibited lower glomerular volumetric density (Figure 1), probably due to the renal overload added to the stress caused by food restriction, leading to the loss of nephrons in this group.[12,18]

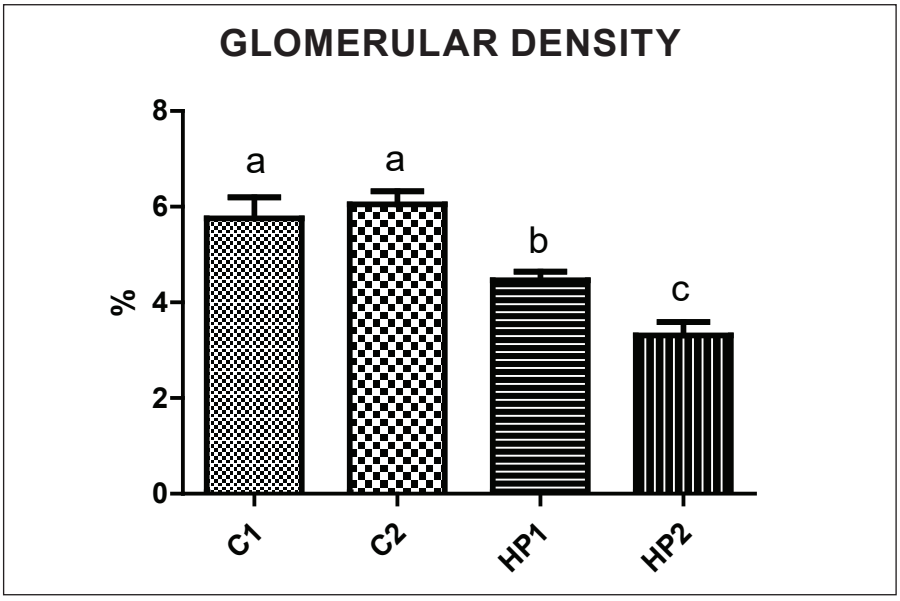

Figure 1. Glomerular density. Control group (C); High protein group (HP). Groups subjected to 30% food restriction (C2 and HP2). Different superscripts indicate statistical difference between the groups (univariate ANOVA, $P<0.05$).

Some authors suggest that a decrease in the number of glomeruli per field is inversely proportional to their size, since a smaller number of glomeruli would lead to the hypertrophy of the remaining ones. In this case, such glomerular hypertrophy is mediated by the endothelial vascular growth.[19,20] However this fact was not demonstrated in the present study because the group with lower glomerular volumetric density (HP2) did not have a size increase in the remaining glomeruli. Instead, the group HP1 exhibited a smaller glomerular diameter compared to the group C1.

Possibly, a lower Vv would reduce the renal filtration surface and diminish the kidneys ability to excrete sodium, causing blood pressure alterations.[19] Given this, morphological alterations of this nature may have severe impacts on health, predisposing the individual to renal diseases and hypertension. [19,20]

This fact was observed in the group HP1, which had a rise in the systolic blood pressure, probably due to a reduction in the glomerular volumetric density and in the glomerulus diameter. So, the elevated systolic blood pressure is probably due to renal malfunction. However, the high systolic blood pressure found in the group HP2 can be explained by the lower glomerular density and higher levels of aldosterone in blood.

With regard to the biochemical parameters, the elevated urea serum is a common finding in literature when animals or humans are subjected to a "protein diet".[3,12,19-21]

In cases of malnutrition and/or elevated catabolism, albumin hepatic synthesis can be reduced, which explains that the groups HP and C2 had lower albumin concentrations than the control group. Regarding total protein, the group HP2 had lower plasmatic concentrations, which shows that the combination of three factors – food restriction, changes in the energy substrate and diminished production of hepatic albumin – has a synergetic effect on these markers (Table 3).[22-24]

According to some authors, high lipid diets are also associated with an increased production of oxygen-reactive species with a consequent increase of oxidative stress and activation of a nuclear proinflammatory transition factor called NF-kβ, responsible for the transcription of proinflammatory proteins, such as interleukin-6.[21,25] Diets rich in saturated fats such as the "protein diet" leads to the synthesis of proinflammatory eicosanoids (prostaglandins and thromboxane series 2 and 4.[21,25,26] In this study, the rats fed "protein diet" had the highest concentrations of interleukin-6, corroborating other studies, which showed that these diets stimulate the generation of a proinflammatory condition, increasing likelihood of developing chronic diseases.[26,27]

Conclusion

The "protein diet" may cause increased systolic blood pressure, abnormal renal morphology, reduced diameter and glomerular volume and an increased production of interleukin-6, a key inflammatory marker. Thus, this study suggests that the "protein diet" leads to changes in the renal tissue, predisposing the individual to the development of chronic diseases.

Further studies with similar methodology and an extended evaluation period are necessary to determine whether other alterations occur or whether adaptations in the parameters studied may occur.

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