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High Protein Diet that Cause Weight Loss and Lower Blood Glucose Level Have a Serious Impact on the Kidney Functions of Male Diabetic Obese Albino Rats

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Abstract

Background: High protein (HP) diets are increasingly being recommended as one of the management strategies for weight control in overweight and obese individuals. The health benefits of high protein diets are well-established, but the mechanisms of action on body systems responsible for the changes in body weight and glycaemic control are not well-clear. **Objective**: The present study aimed to examine the effect of HP diets on the kidney functions of diabetic obese albino rats. **Material and Methods:** Eighty male adult male albino rats were used in this study. The animals were divided into eight equal groups (10 rats for each). Type 2 DM and obesity were induced. At the end of the 12 weeks, samples were collected for biochemical analysis. **Results**: The high protein diet led to significant decrease in BW, FI, BG, TC, LDL, TG, Lactate dehydrogenase, albumin, urine pH and urine citrate; while serum insulin, HDL, urea, creatinine, total protein, urine volume and urinary excretion of Ca were significantly higher in high protein diet groups. **Conclusion**: A high protein intake in diabetic obese albino rats for 12 weeks led to changes in the serum and urine levels of markers of renal function which indicated abnormalities in the functions of the kidney.

Keywords

Obesity, Diabetes, High Protein Diet, Kidney Functions

1. Introduction

According to the World Health Organization, the prevalence of obesity world-

wide has nearly has nearly tripled since 1975 [1]. Despite the high rates of morbidity and mortality caused by this epidemic, safe and effective obesity treatments remain elusive [2]. The use of high-protein (HP) diets is gaining in popularity among the general population [3]. Indeed, HP diets are increasingly being recommended as one of the management strategies for weight control in overweight and obese individuals [1]. Human research has demonstrated that high protein diets lead to weight loss, which may be due to an increased dietary thermogenesis, increased satiety and a decreased subsequent energy intake [4]. Additionally, a high-protein diet has been shown to conserve fat-free mass and to contribute to fat mass loss [5]. The health benefits of high protein diets are well-established [4] [6] [7] [8] [9] [10] but the mechanisms of action that are responsible for these changes in body are still unclear [11]. There is a scientific controversy on the effects of long-term consumption of these diets, and it has recently been reported that high protein intake is related with a higher risk of weight gain and with increased risk of fatal and non-fatal outcomes [12]. Although there are many human and animal studies focused on the physiological, biochemical and/or pathological effects induced by specific nutrients and dietary factors, there have been relatively few studies investigating safety and potential adverse effects of HP diets but diets with high protein content are generally considered safe and healthy in subjects without any pathology [5].

Because 90% of people with type 2 Diabetes Meletus (DM) are obese [4]. It is important to understand the effects of high levels of protein intake on health of diabetic individuals.

The present study aimed to examine the effect of HP diets on the kidney functions of diabetic obese albino rats.

2. Material and Methods

2.1. Animals

Eighty male adult male albino rats (8 weeks of age) of a local strain, weighing from 150 to 200 g used in this study were obtained from The Nile Co. For Pharmaceuticals and Chemical Industries (Cairo), and kept in suitable tainless-steel cages (20 × 32 × 20 cm for every 3 rats) at room temperature, with the natural light/dark cycle in the animal laboratory of Physiology Department, Al-Azhar Faculty of Medicine (Assiut). They were fed on the standard food prepared from commercial rat food formula (El-Nasr-Pharmaceutical Co.) in addition to bread and green vegetables, with free water supply. They were kept for ten days to adapt to the new conditions before starting the experiment.

The animals were divided into eight equal groups:

1) Normal control received normal protein diet.

2) Normal received high protein diet.

3) Obese received normal protein diet.

4) Obese received high protein diet.

5) Diabetic control received normal protein diet

6) Diabetic received high protein diet.

7) Diabetic obese received normal protein diet.

8) Diabetic obese received high protein diet.

2.2. Chemicals

1) Blood glucose (BG) kit (Egyptian Company for Biotechnology-Egypt) [13].

2) Insulin kit (Sigma-Aldrich Co. LLC-USA) [14].

3) Serum cholesterol kit (Egyptian company for biotechnology-Egypt) [15].

4) Serum triglycerides kit (Egyptian Company for Biotechnology-Egypt) [16].

5) Serum high density lipoprotein (HDL) kit (Egyptian Company for Biotechnology-Egypt) [16].

6) Serum urea kit (Egyptian Company for Biotechnology [17].

7) Serum creatinine kit (Biolabo reagents kits - France) [18].

8) Urine Cacontent was determined by using a PerkinElmer Analyst 300 spectrophotometer (PerkinElmer, Wellesley, MA, USA) [19].

9) Urinary pH was analysed with a bench pH-meter (Crison, Barcelona, Spain) [20].

10) Urinary citrate with a commercial kit (Spinreact, S.A. Gerona, España) [20].

11) Total proteins (TP), albumin and lactate dehydrogenase were measured with a Hitachi-Roche p 800 autoanalyzer [20].

12) Urine collection using clear plastic wrap [21].

2.3. Induction of Diabetes

Type 2 DM was experimentally induced by feeding a high fat diet (HFD) for an initial period of 2 weeks followed by an intraperitoneal injection of 35 mg/kg bwt streptozotocin dissolved in citrate buffer pH 4.5 [22]. After injection of streptozotocin, the rats were kept for next 48 hours on oral 10% glucose solution on top of their chew. Administration of glucose was to prevent hypoglycaemia as STZ is capable of producing fatal hypoglycaemia due to destruction of β cells which in turn results in to massive pancreatic insulin release [23].

Seven days after the injection, rats were screened for serum glucose levels in blood samples taken from tail, using Accu-Chek glucometer (Roche, Germany). Rats having serum glucose ≥ 200 mg/dl, after 2 hours of glucose intake, were considered diabetic [22].

Induction of obesity was by feeding a high fat diet (HFD) (45% energy from fat) and glucose in water for a period of 2 weeks [24]. Obesity was diagnosed by using body mass index (BMI) = 1/4 body weight (g)/length^2 (cm^2) [25].

2.4. Experimental Diet

A 45% high protein diet was formed as in (**Table 1**) to meet the nutritional requirements of adult rats as recommended by the American Institute of Nutrition (AIN-93M). [19] [26].

Table 1. Nutritional composition of the experimental diets.

Nutritional Composition (g/l00 g BW)	Normal-protein diet	high-protein diet
Soy protein supplement	13.1	57.4
Mineral mix (AIN-93M-MX)	3.5	3.5
Vitamin mix (AIN-93-VX)	1	1
Fat (olive oil)	4	4
Choline chloride	0.25	0.25
Cellulose	5	5
Starch	62.4	28.6
Methionine	0.5	-
Sucrose	10	-

Body weight (BW) was measured weekly for all animals and the amount of food intake (FI) rat were registered daily.

At the end of 6^{th}, 9^{th}, 12^{th} weeks, a 12-hour urine samples were collected and urine volumes (UV) were recorded, samples were transferred into graduated centrifuge tubes for measurement of pH, Ca, and citrate.

At the end of the 12^{th} week, food was withdrawn from the rats and they were fasted for 8 h, but had free access to water, and then anesthetized with CO_2. Blood specimens were collected from orbital venous plexus in non-heparinized tubes. Blood specimens were centrifuged at 2500 rpm for 15 min, and the clear samples of blood serum were separated and stored at −80˚C until used for the determination of the levelsof blood glucose (BG) level (mg/dl), insulin level (pmol/L), total cholesterol (TC) (mg/dl), LDL-cholesterol (mg/dl), HDL-cholesterol (mg/dl), triglycerides(TG) (mg/dl), urea (mg/dl), creatinine (mg/dl), total proteins (g/dl), albumin (mg/dl), and lactate dehydrogenase (u/L).

2.5. Statistical Analysis

The obtained data were subjected to analysis of variance according to the procedures out lined by Snedecor and Cochran. The mean values were compared according to Duncan's multiple range test (DMRT) [27]. The data were analysed using CoStat software for windows (version 6.3), (CoHort Software, Monterey, Calif).

3. Results

There were significant decreases in BW and FI ($p \geq 0.01$) between HP diet groups. The BG was significantly lower and insulin was significantly higher in high protein diet groups (($p \geq 0.01$) when we compared the (G1 and G2), (G2 and G4), (G5 and G6) and (G7 and G8). There is also significant increase in insulin level in obese groups when compared with non-obese rats (**Table 2**).

Means followed by a common letter are not significantly different at the 1% level by DMRT. Where G1: Normal control received normal protein diet; G2: Normal received high protein diet; G3: Obese received normal protein diet; G4:

Table 2. (Means ± Standard deviations (SD)) of body weight, food intake, blood glucose and insulin for all groups.

Groups	BW (mg)	FI (gm)	BG (mg/dl)	Insulin (ng/dl)
G1	320.8d ± 4.4	14.84d ± 1.2	107f ± 4.2	32.8d ± 2.3
G2	232.6f ± 15.5	13.62e ± 1.0	75.2g ± 6.9	38.6c ± 1.4
G3	509.6a ± 16.8	20.04b ± 1.5	120.3e ± 4.0	43.4b ± 1.6
G4	351.8c ± 13.7	15.72d ± 0.7	81.3g ± 7.1	47.9a ± 1.3
G5	272.3e ± 14.9	23.45a ± 1.7	359.6b ± 27.0	6.03h ± 0.8
G6	204.3g ± 4.6	20.1b ± 0.8	223.6d ± 16.7	15.8g ± 1.2
G7	507.6a ± 14.0	19.78b ± 1.2	447.4a ± 21.6	20.41f ± 1.2
G8	385.7b ± 12.1	17.74c ± 1.0	270.5c ± 11.4	24.97e ± 1.1
P.	0.00**	0.00**	0.00**	0.00**

**indicate $P < 0.01$. Means followed by a common letter are not significantly different at the 1% level by DMRT.

Obese received high protein diet; G5: Diabetic control received normal protein diet; G6: Diabetic received high protein diet; G7: Diabetic Obese received normal protein diet; G8: Diabetic obese received high protein diet.

Data in **Figure 1** showed that maximum reduction percent of BW for diabetic control rat's groups (15.12%), BW was increased relative to normal control by (58.85%). FI and BG was maximally increased in diabetic obese rats by (58.2%) and (318.3%) respectively, Maximum reduction in insulin level in diabetic control group by (−81.6%) compare to normal control group (**Figure 2**).

As shown in **Figure 2**, BW were increased at least 51.25% and 65.82 %, of control received high protein diet in obese and diabetic obese groups, respectively. The diabetic control received high protein diet treatment decreased BW by 12.17 % compare to normal control group.

3.1. Effect of High Protein Diet on Lipid Profile (Table 3)

Total cholesterol (TC) was significantly ($P < 0.01$) affected by protein diet treatments and was significantly decreased in G2, G4, G6, and G8 when compared with G1, G3, G5 and G7 respectively, G2 gave the lowest value (56.3 mg/dl). LDL was significantly affected by protein diet and varied from 22.7 to 92 (**Table 2**) the highest value of LDL was recorded in G7, there was insignificant difference in LDL between G1 and G 6. There was significant increase in (HDL) by high protein diet in G2, G4, G6 and G8 when compared with G1, G3, G5 and G7 respectively, TG level was significantly decreased by protein diet treatment in G2, G4, G6, and G8 when compared with G1, G3, G5 and G7 respectively.

Data in **Figure 3** showed that TC was increased in diabetic obese rat received normal diet relative to normal control by (246.57%). LDL and TG were maximally increased in diabetic obese rats by (111.49%) and (69.6%) respectively,

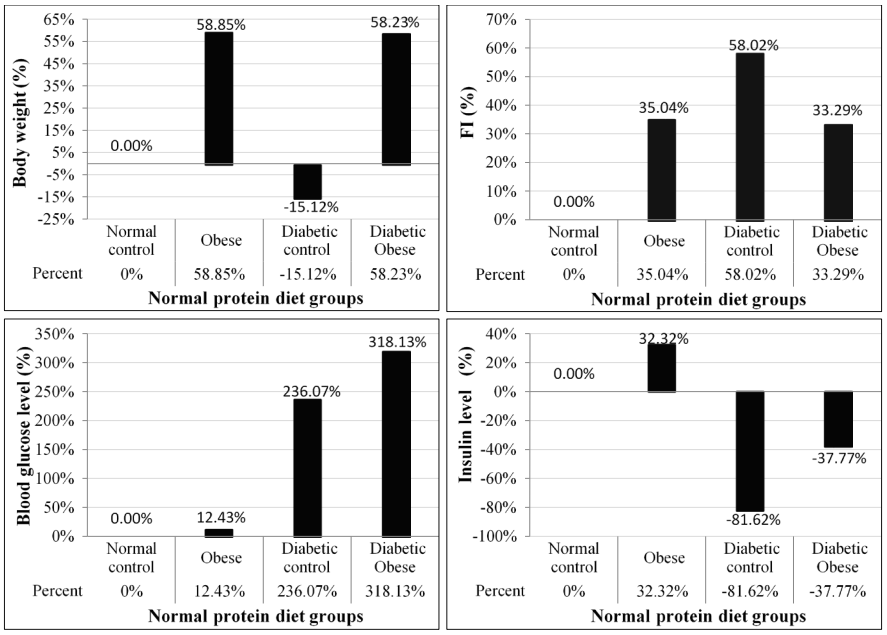

Figure 1. Percentage of body weight, FI, blood glucose level and insulin level for different groups received normal protein diet compare to normal control.

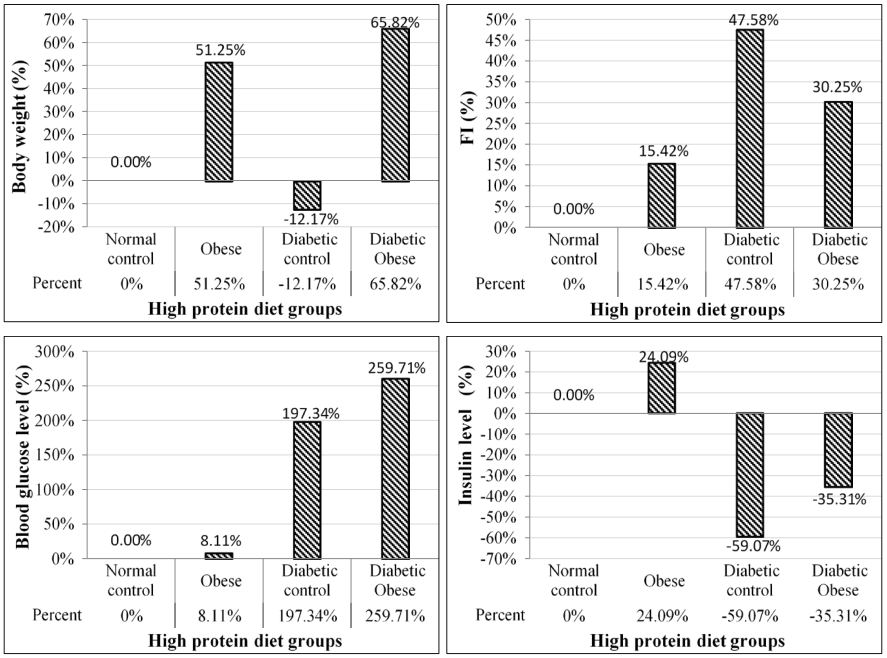

Figure 2. Percentage of body weight, FI, blood glucose level and insulin level for different groups received high protein diet compare to normal control.

Maximum reduction in HDL level in diabetic obese group by (−58.2%) compare to normal control group.

Data in **Figure 4** showed that TC was increased in diabetic obese rat received HP diet relative to normal control by (245.7%). LDL and TG were maximally increased in diabetic obese rats by (129.9%) and (120.8%) respectively,

Table 3. Means ± (SD) of total cholesterol, low density lipoprotein, high density lipoproteinand triglyceride for all groups.

Groups	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TG (mg/dl)
G1	78.8g ± 4.3	43.5e ± 2.5	42.4b ± 4.1	93.2e ± 5.7
G2	56.3h ± 4.6	22.7g ± 1.9	64.1a ± 3.2	55.3f ± 6.4
G3	222.3b ± 8.2	65c ± 4.0	30.4cd ± 2.1	132.9b ± 7.8
G4	125.9e ± 11.2	34.4f ± 3.6	41.1b ± 2.6	111.1d ± 7.5
G5	152.4d ± 8.8	79.1b ± 4.8	18.4e ± 2.7	131.7b ± 7.8
G6	107.2f ± 4.1	44.1e ± 5.5	32c ± 3.7	106.3d ± 5.2
G7	273.1a ± 13.5	92a ± 4.0	17.7e ± 3.1	158.1a ± 8.5
G8	199.7c ± 9.8	52.2d ± 6.7	29.1d ± 2.6	122.1c ± 5.6
P.	0.00**	0.00**	0.00**	0.00**

**indicate P < 0.01. Means followed by a common letter are not significantly different at the 1% level by DMRT.

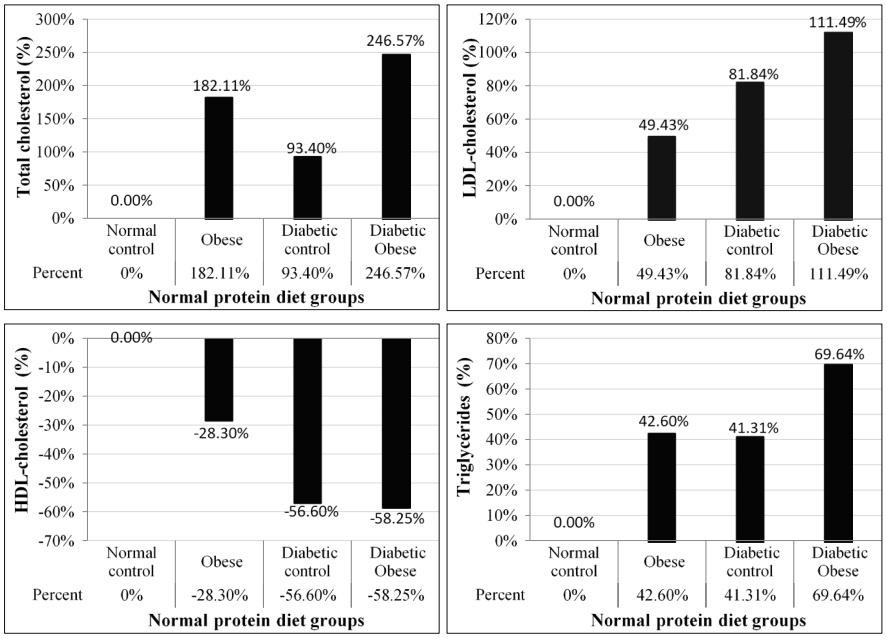

Figure 3. Percentage of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides for different groups received normal protein diet compare to normal control.

Maximum reduction in HDL level in diabetic obese group by (−54.2%) compare to normal control group.

3.2. Effect of High Protein Diet on Urea, Creatinine, TP, Albumin and Lactate Dehydrogenase

As shown in (Table 4), the serum urea, creatinine and total protein in the HP diet groups (G2, G4, G6, G8) animals was observed to be significantly higher (P < 0.01) than those in the normal diet groups (G1, G3, G5, G7) as shown in (Table 4).

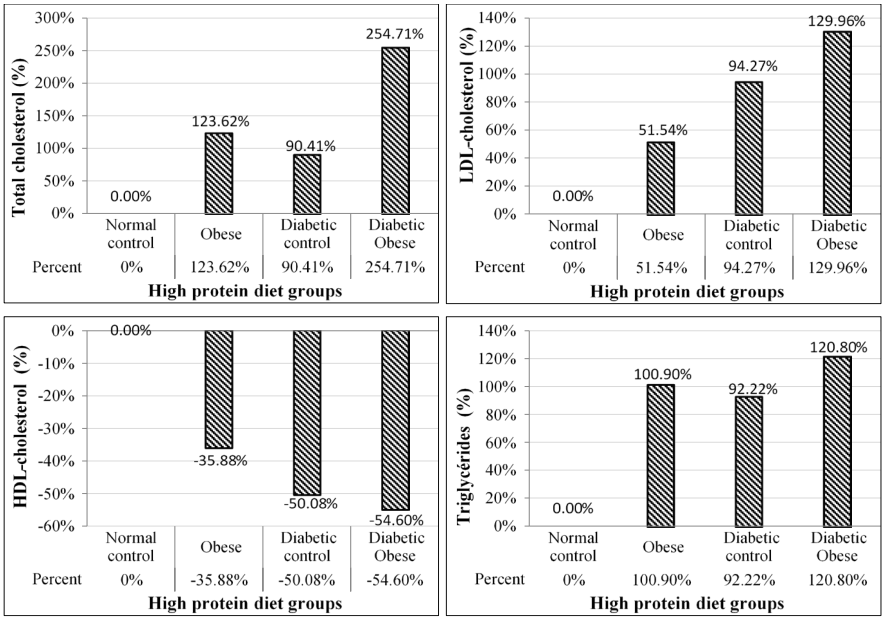

Figure 4. Percentage of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides for different groups received high protein diet compare to normal control.

Table 4. Means ± (SD) of urea, creatinine, TP, albumin and Lactate Dehydrogenase for all groups.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	TP (g/dl)	Albumin (mg/dl)	Lactate (u/L) Dehydrogenase
G1	18.9e ± 1.9	0.474d ± 0.08	4.71cd ± 0.35	2.56b ± 0.35	640.8c ± 40.7
G2	28.8d ± 1.3	1.26b ± 0.1	5.69a ± 0.36	2.91a ± 0.27	514.6e ± 18.0
G3	19.4e ± 2.0	0.352e ± 0.1	4.21e ± 0.28	2.54b ± 0.27	762.1b ± 44.4
G4	32.4d ± 3.6	1.179b ± 0.12	4.95bc ± 0.38	2.58b ± 0.19	593.5d ± 14.5
G5	109.1c ± 8.1	1.076c ± 0.09	4.39 de ± 0.32	2.41b ± 0.32	832.4a ± 30.0
G6	212a± 6.8	1.51a ± 0.19	5.12bc ± 0.61	2.6b ± 0.31	743.1b ± 21.8
G7	114.3b ± 9.5	1.001c ± 0.08	4.19e ± 0.22	2.12c ± 0.14	848.9a ± 33.8
G8	214.6a ± 6.4	1.41a ± 0.08	5.25b ± 0.73	2.53b ± 0.33	765.1b ± 19.4
P.	0.00**	0.00**	0.00**	0.00**	0.00**

**indicate P < 0.01. Means followed by a common letter are not significantly different at the 1% level by DMRT.

The levels of serum Lactate dehydrogenase and albumin was significantly (P < 0.01) decreased by protein diet treatments in G2, G4, G6 and G8 when compared with G1, G3, G5 and G7 respectively.

Data in **Figure 5** showed that urea was increased in diabetic obese rat received normal diet relative to normal control by (504.7%). Creatinine was maximally increased in diabetic by rats by (127%). Albumin and TP were maximally reduced in diabetic obese group by (−11.04%) and (−17.19%) respectively when compare to normal control group.

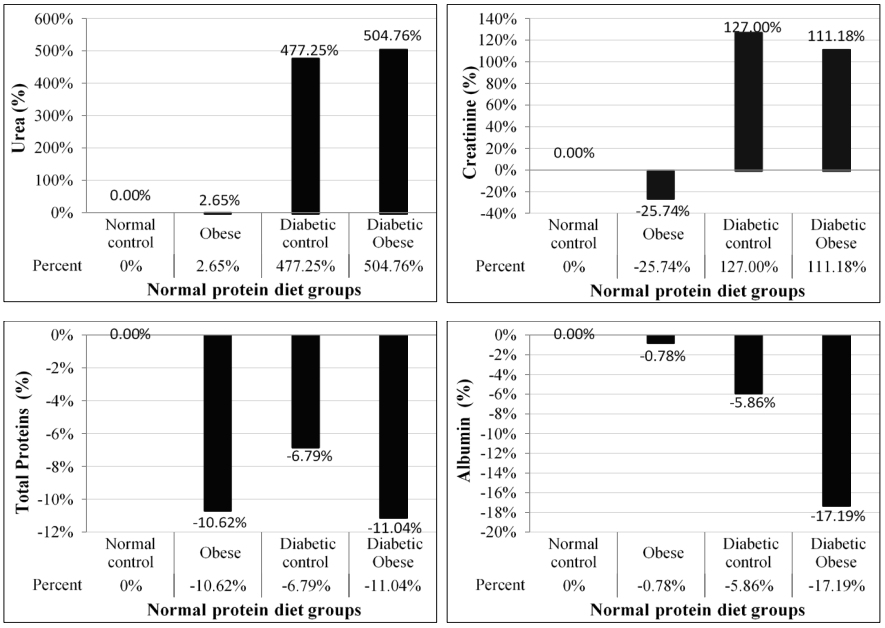

Figure 5. Percentage of urea, creatinine, total proteins and albumin for different groups received normal protein diet compare to normal control.

Data in **Figure 6** showed that urea was increased in diabetic obese rat received HP diet relative to normal control by (645.1%). Creatinine was maximally increased in diabetic by rats by (19.84%). Albumin and TP were maximally reduced in diabetic obese group by (−54.60%) and (−13.06%) respectively when compare to normal control group.

Data in **Figure 7** showed that LD was increased in diabetic obese rat received normal diet relative to normal control by (32.4%) and in diabetic obese rat received HP diet by (48.68%).

3.3. Effect of High Protein Diet Onurine

In our study, there were increased urine volume and urinary excretion of Ca, with decreased urinary citrate in non-diabetic rats. There was also significant decrease in urine pH in obese rats received high protein diet as shown in **Table 5**.

Data in **Figure 8** showed that UV was increased in diabetic obese rat received normal diet relative to normal control by (455%). Urine pH was maximally decreased in diabetic obese rats by (v24.8%). Urine Ca was maximally increased in diabetic control rats by (24.56%). Urine Citrate was maximally reduced in obese rats by (−23%) when compare to normal control group.

Data in **Figure 9** showed that UV was increased in diabetic obese rat received HP diet relative to normal control by (190%). Urine pH was maximally reduced in diabetic obese rats by (−15.6%). Urine Ca was maximally increased in obese control rats by (2.47%). Urine Citrate was maximally increased in obese rats by (397.56%) when compare to normal control group.

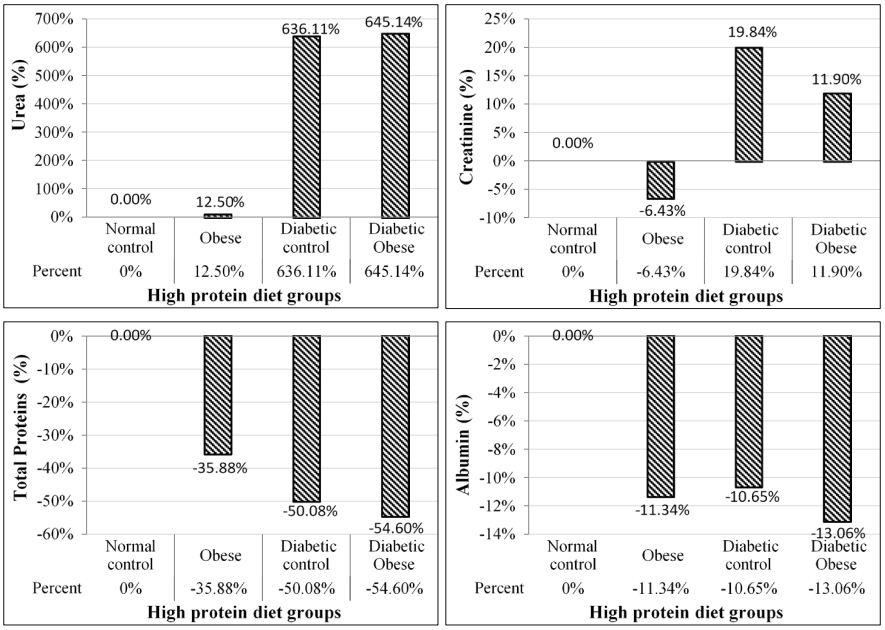

Figure 6. Percentage of urea, creatinine, total proteins and albumin for different groups received high protein diet compare to normal control.

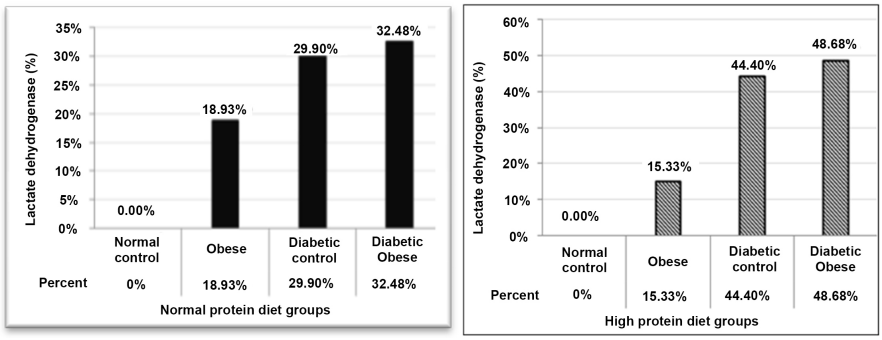

Figure 7. Percentage Lactate dehydrogenase for different groups received normal or high protein diet compare to normal control.

4. Discussion

For many years, researches were concerned for management of DM and its predisposing factors. Obesity is the major risk factor for developing T2DM and pare-diabetes [4] [28] [29]. There are many reports in the literature on short-term beneficial effects of high protein diet on BW and BG [4] [30]; However, the risks of using a high-protein diet with carbohydrate limitation for the long term are still being studied especially in high risk groups.

The results of the present study showed that the diet contained 45% of food as protein received for 12 weeks for normal, obese, diabetic, and diabetic-obese rats lead to significant decrease in BW and FI. The reduction in BW could be a direct result of decreased food intake. Although the differences in food intake could be due to taste and less sucrose content than normal diet. The HP diet reduces hunger and increases feelings of fullness, which result in decreased food intake.

Table 5. Means ± (SD) of urine volume, urine pH, urine ca and urine citrate for all groups.

Groups	UV (ml)	U. pH	U. Ca (mg/day)	U. Citrate (g/L)
G1	2.66f ± 0.35	6.73b ± 1.55	0.509d ± 0.03	2.895b ± 0.1
G2	4.01e ± 0.34	6.28b ± 0.10	0.85a ± 0.06	0.369g ± 0.07
G3	2.37f ± 0.28	7.64a ± 0.17	0.585c ± 0.03	2.227d ± 0.07
G4	3.63e ± 0.26	6.26b ± 0.10	0.871a ± 0.04	1.836e ± 0.14
G5	12.47b ± 1.06	5.23cd ± 0.07	0.634b ± 0.04	3.515a ± 0.08
G6	8.44d ± 0.65	5.74c ± 0.15	0.86a ± 0.04	1.802e ± 0.11
G7	14.78a ± 0.64	5.06d ± 0.04	0.6bc ± 0.07	2.37c ± 0.11
G8	11.63c ± 0.85	5.3cd ± 0.31	0.869a ± 0.05	1.705f ±0.012
P.	0.00**	0.00**	0.00**	0.00**

**indicate P < 0.01. Means followed by a common letter are not significantly different at the 1% level by DMRT.

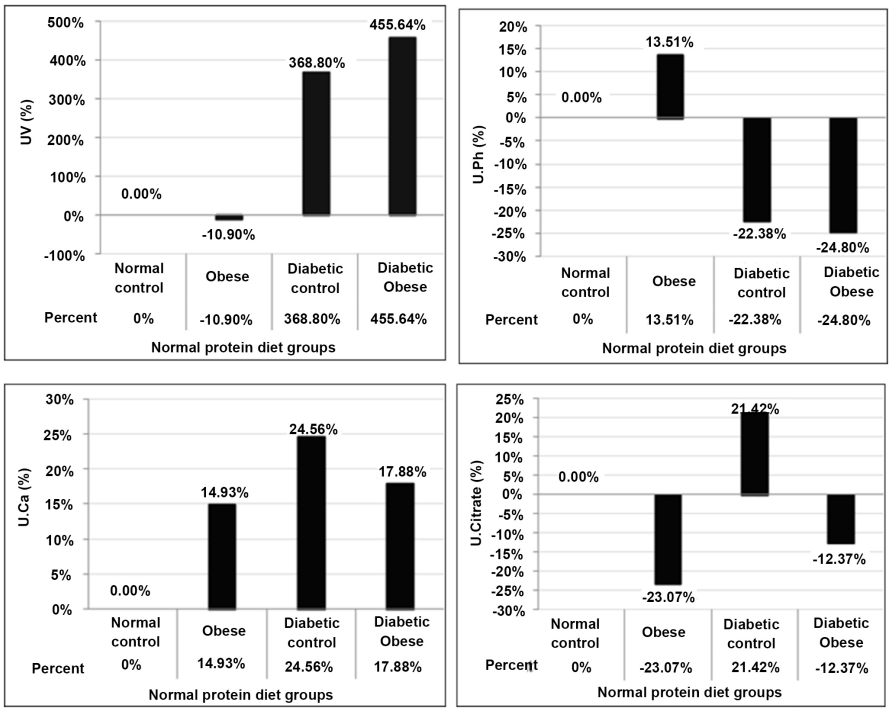

Figure 8. Percentage of UV, U. pH, U. Ca and U. Citrate for different groups received normal protein diet compare to normal control.

Rats fed an HP diet seemed to develop less leptin resistance than rats fed an NP diet, which is in line with their marked reduction of adipose tissue [31] [32]. Our finding was in agreement with previous studies of Farnsworth *et al.* [5], Layman *et al.* [33], Lynch and Adams [34], Pesta and Samuel, [10], and French *et al.* [5].

In the present study, the blood glucose level decreased significantly in high protein diet groups and insulin significantly increased. This decrease in glucose concentrations observed can be explained by decrease FI and the smaller amount

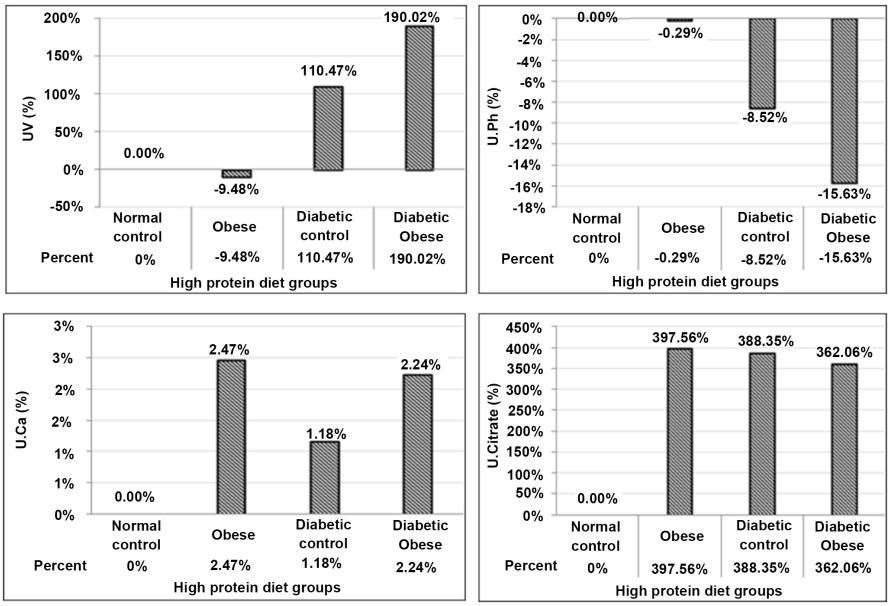

Figure 9. Percentage of UV, U. pH, U. Ca and U. Citrate for different groups received high protein diet compare to normal control.

of carbohydrate in the diet. Thus, the smaller amount of glucose absorbed after meals and also due to a reduced store of glycogen and thus a decrease in glycogenolysis rate. Insulin concentration increased as dietary protein strongly stimulates insulin secretion [35] [36].

The results of the present study showed that there was significant decrease in TC, LDL, TG, while serum HDL was significantly higher in high protein diet groups compared to normal control group. Several studies have reported an improvement in lipid profile levels with high protein diet. However, the findings of the Aparicio *et al.*, [19] showed that HP diet significantly reduced body weight but without clearly improving plasma lipid profile [8] [37] [38] [39].

The results of the present study showed that there was significant decrease in Lactate dehydrogenase, albumin, urine pH and urine citrate while serum urea, creatinine, total protein, urine volume and urinary excretion of Ca was significantly higher in high protein diet groups. These results were similar to what was found by, Li. [40] & bin Zaraah *et al.* [41] & Hoy. [42]. The levels of serum Lactate dehydrogenase and albumin was significantly decreased by protein diet treatments in cases of malnutrition and/or elevated catabolism and albumin hepatic synthesis can be reduced [43], which explains that the groups HP had lower albumin concentrations than the control group. In our study, there were increased urine volume and urinary excretion of Ca, with decreased urinary citrate in non-diabetic rats. There was also significant decrease in urine pH in obese rats received high protein diet. Our results is coordinated with the finding of Amanzadeh *et al.* They found that rats on the high protein diet had much higher urinary volumes and increase in urinary excretion of calcium. They also found that Urinary citrate excretion and urinary pH was significantly lower were both markedly reduced [44]. In study of Aparicio *et al.* And bin Zaraah, *et al.* The HP

diet increased urinary excretion of Ca and strongly decreased urinary pH and citrate and there was increasing in 24-hour urine output in the experimental rats [19] [41]. Hostetter. Stated that dietary protein has an effect to increase the level of GFR of the kidneys and this explain the increase in urine volume [45]. GFR has also been shown to increase by 20% - 30% within 1 hour of consuming a high protein meal and that if this were to stimulate urine output [46].

Our result indicated that the high protein induced diminutions in renal function. Despite the metabolic advantages of HP diet, the long-term HP consumption may lead to the development of end stage renal disease (ESRD) said by Malhotra *et al.* [47]. This finding were supported by the studies of Martin *et al* [48], Kurpińska and Skrzypczak. & Hammond and Janes. [49].

On the other hand, Lacroix *et al.* studied the effects of a diet containing 50% protein on renal function in Wistar rats and did not observed abnormalities in renal function or pathology [7] [50]. Similarly, Collins *et al.* 1990, also reported no adverse effects of long-term consumption of high protein diets on renal function in rats [50]. However, some recent studies have shown a positive correlation between high protein intake and ESRD [51] [52]. A protective role for low protein diet has been suggested in patients with diabetic kidney disease; but no nutritional recommendations are made for individuals who are obese, or pre-diabetic [5].

Malhotra *et al.* observed that, with HP diet there was decline in an estimated glomerular filtration rate (eGFR) among those with diabetes and preserved baseline kidney function, while there was no association was observed between amount of protein intake and eGFR decline among participants without diabetes [47].

The study of Malhotra *et al.* among black men and women with a high burden of risk factors for kidney disease, they have demonstrated that higher protein intake was associated with ESRD, a finding consistent with metabolic studies suggesting that diabetes and obesity (which are common among blacks) alter protein metabolism [53].

Our finding can be explaned by hyperfiltration that caused by HP leading to subsequent renal damage, as postulated by Brenner *et al.*, [47] [54]. It may also be possible that by-products of excess protein metabolism could cause injury to podocytes and other kidney cells resulting in impaired kidney function and subsequent ESRD [53].

5. Conclusion

In conclusion, a high protein intake in albino rats fed for 12 weeks showed changes in the serum and urine levels of markers of renal function which indicate abnormalities in the function of the kidney especially in diabetic and pare-diabetic groups.

Conflicts of Interest

The authors declare that they have no competing interest.

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Author's Contributions

Nour El-Deen A. and Mansour A. involved in the study concept , design and recruitment of animal, induction of diabetes, obesity and follow up, and contributed to data acquisition; Taha A performed the biochemical tests; Nour El-Deen A. and Taha A. performed statistical analysis and designed the figures; Nour El-Deen A. and Mansour A. performed data interpretation; Mansour A., Nour El-Deen A., and Taha A. wrote the manuscript; all the authors reviewed the manuscript and finally approved it for submission.

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