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Effect of Obesity on Albino Rat Kidney

Waleed S. Mohamed[1,2]* and Ahamed S. Ashour[3t]
[1]Internal Medicine Department, Tanta Faculty of Medicine, Tanta University, Egypt
[2]Internal Medicine Department, Taif College of Medicine, Taif University, KSA
[3]Anatomy and Embryology Department, Tanta Faculty of Medicine, Tanta University, Egypt

Abstract

Background and study aim: Obesity and concomitant co-morbidities have emerged as public health problems of the first order. Obese individuals have an increased risk for Chronic Kidney Disease (CKD). The aim of this study is to study the metabolic and early renal histopathologic changes that are associated with obesity in experimental animals.

Materials and Methods: This study was conducted on sixty adult male albino rats; thirty with body weight ranging between 180-200 gm (control) beside thirty rates with body weight more than 250 gm. Control animals were fed a standard rat chow while obese rats were fed a semisynthetic diet enriched in sucrose. After 4 weeks, blood samples were collected to assess: Fasting Blood Glucose (FBG), Serum Insulin (SI), serum Total Lipid (TL), serum Triglyceride (TG), Total Cholesterol (TC), serum High Density Lipoprotein (HDL) and serum Low Density Lipoprotein (LDL). Kidney tissue samples were stained with Hematoxylin and Eosin, Anti-Collagen IV antibody then examined by light and electron Microscope.

Results: There was a significant increased Body Weight (BW) and kidneys weight of obese group. There was a significant increased of FBS (p 0.0001), SI (p 0.0001), TL (p 0.0001), TC (p 0.0001), TG (p 0.0001), and LDL (p 0.0043) with significant decreased of HDL (p 0.0133) in obese group. Serum creatinine was significantly increased in obese group with a significant positive correlation between it and BW, FBS, SI, and TG. Histological examination revealed moderately expanded Bowman's capsule, wide renal tubules, a positive reaction for collagen IV, increased thickness of glomerular basement membrane, foot processes fusion and many vacuolation in the cells lining of proximal convoluted tubules of obese rats kidneys.

Conclusions: Obesity is associated with many metabolic abnormalities like insulin resistance, impaired glucose tolerance, dyslipidemia, morphological and structural renal changes which may proceed to Glomerulosclrosis (GS) and CKD.

Keywords: Chronic kidney disease; Metabolic abnormalities; Obesity; Renal histological changes

Introduction

Obesity has become a serious global health issue affecting both adults and children. The prevalence of overweight and obesity is approximately 66% in the United States [1]. Over the last two decades, a worldwide rise in obesity has resulted in 1.46 billion overweight (Body Mass Index (BMI] >25) and 502 million obese (BMI >30) adults [2]. Obesity potentially leads to a decrease in overall life expectancy [3]. The metabolic syndrome a major consequence of obesity [4]. The prevalence of the metabolic syndrome in the United States is 23% in those who are 20 year or older and 40% in those who are 60 year or older [5]. The metabolic syndrome is defined as the presence of at least three of the following criteria, with or without diabetes: central obesity (waist circumference in men 102 cm and in women 88 cm), hypertriglyceridemia (150 mg/dl), low HDL cholesterol (men 40 mg/dl, women 50 mg/dl), elevated fasting glucose (110 mg/dl), and hypertension (130/85 mmHg) [6]. A central feature of the metabolic syndrome is insulin resistance, which results in hyperglycemia and hyper-insulinemia, and eventually leads to the development of diabetes [7]. Chronic inflammation is another feature of the metabolic syndrome, which, together with insulin resistance, results in complex metabolic derangements [8].

Obesity has deleterious effects on metabolic homeostasis, and affects numerous body organs. Co-morbidities for obesity include type 2 diabetes mellitus, cardiovascular diseases, hepatic steatosis, Alzheimer's disease, CKD and certain cancers [9,10]. Obesity was shown to affect independently the progression of preexisting renal diseases, such as IgA nephropathy, [11] patients with unilateral renal agenesis, [12] or after unilateral nephrectomy [13]. Kidneys obtained from obese donors were more likely to exhibit a lower Glomerular

Filtration Rate (GFR] and a higher rate of allograft dysfunction over several years than kidneys obtained from lean individuals [14]. These data suggest that obesity contributes to and perhaps even initiates CKD. Published data quantifying the real impact of obesity on renal function are deficient. Some studies revealed a link between variable degrees of proteinuria and obesity [15]. Clinically, patients may present with nephrotic syndrome [13].

Praga et al. [16] reported the absence of features of nephrotic syndrome despite heavy proteinuria in 15 obese patients. The histopathologic changes founded in a proteinuric obese patient consist of glomerulomegaly with or without Focal Segmental Glomerulosclerosis (FSGS). These patients tend to have less podocyte injury and a more indolent progression than patients with idiopathic FSGS [15]. The changes are thought to be related to altered renal hemodynamics, namely increased renal blood flow, hyper-filtration, and increased filtration fraction [17]. However, it is likely that these observations are somewhat biased as renal biopsies are usually obtained only in patients with proteinuria. Therefore, obesity-related glomerulopathy may not be the only histopathologic feature of obesity-related renal disease, particularly in non-proteinuric obese patients. Hence, the aim of this

*Corresponding author: Waleed S. Mohamed, Internal Medicine Department, Tanta Faculty of Medicine, Tanta University, Egypt, Internal Medicine Department, Taif College of Medicine, Taif University, KSA, Tel: +966/0553420886; Fax: +966/27256900; E-mail: wsmohamed1@yahoo.com

study is an attempt to examine the histopathological renal changes that are associated with obesity in experimental animals.

Materials and Methods

Sixty adult male albino rats of 10-12 weeks of age, thirty with body weight ranging between 180-200 gm (control) beside thirty rates with body weight more than 250 gm [14] were used in the current work. They were classified into two groups each contain thirty rats:

- Group I (control group): Control animals were fed a standard rat chow (Ralston Purina, St. Louis, MO).

- Group II (obese group): Obese rats were fed a semisynthetic diet enriched in sucrose (63 g sucrose/100 g) containing 20% (wt/wt) vitamin-free casein, 60% sucrose, and 5% lard [15] with an unlimited supply of drinking water to both groups.

All experiments were taken place in the research laboratories, Faculty of Medicine, Tanta University. Animals were housed individually in clean rodent cages, in a room at relative humidity not less than 30% and not exceeding 70%, at room temperature 22-30°C, with artificial lighting with a sequence being 12 hours light and 12 hours dark. Animals were randomly selected, marked to permit group identification. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health as well as the guidelines of the Animal Welfare Act. Animal death rate was about 1%. After 4 weeks, all animals fasted overnight, and then weighted, blood samples were obtained. The animal is made comfortable in a restrainer while maintaining the temperature around at 24 to 27°C. If the vein is not visible, the tail is dipped into warm water (40°C). Local aesthetic cream applied on the surface of the tail 30 min before the experiment. A 23G needle is inserted into the blood vessel and blood is collected using a capillary tube or a syringe with a needle. After completed blood collection, pressure/silver nitrate ointment/solution is applied to stop the bleeding. Restrainer is washed frequently to avoid/prevent pheromonally induced stress or cross infection. Blood samples centrifuged at 4000 xg for 10 min at 4°C and supernatant kept on -70°C for further biochemical measurements.

Biochemical assays

Biochemical studies were done to assess the following biochemical parameters:

- FBG according to glucose-oxidase method using kits from Diamond Diagnostic.

- SI level was estimated according to Angel [17] using a commercially available ELISA kit which was modified for use in microtiter plates. The adapted assay is based on the binding of porcine anti-guinea pig insulin antibodies to microtiter plates and uses insulin-peroxidase conjugate as displacer.

- Serum TL concentration were analyzed using Sulfo-phospho-vainilline colorimetric method, kits were obtained from Spinreact Company.

- Serum TG and TC level was estimated according to (GPO-POD) method using kits from Spinreact.

Serum HDL Level was estimated by "enzymatic colorimetric method using kits from Spinreact. Serum LDL was calculated using Friedewald's formula if the triglycerides were less than 4.5 mmol/l, as following: LDL=TC – HDL – TG/5.

Rats were anesthetized by intra-peritoneal injection with pentobarbital sodium (50 mg/kg body weight). The kidneys were collected from each rat by carefully removal of the skin of the rat to expose the abdominal muscles. Gently peel the skin from the muscles, using scissors and a probe to tease away muscles that stick to the skin, cut abdominal muscles in the midline. The kidney is removed and weighted. Finally sacrificed rats safely collected in a special package according to safety and health precaution measures to be incinerated later.

Histological studies

The samples of kidneys were immediately fixed at 10% neutral buffered formal saline at pH 7.0 for 24 hours. Dehydrated in ascending grades of alcohol and cleared in in xylol as routine. Samples from each group were impregnated in pure soft paraffin for two hours at 55°C was followed by embedding in hard paraffin for two hours and then paraffin blocks were prepared. Paraffin sections were made at 5 μm thick by microtome and stained with Hematoxylin and Eosin for demonstrating any histological changes. Then sections were examined under the light microscope [18].

Electron microscopy study

Specimens were photographed under the EM JOEL E/M 100 SX at the electron microscopic unit in Faculty of Medicine, Tanta University

Immunohistochemistry study

Immunohistochemical stains were imported from ABCAM company (Address: 1 Kendall Square, Suite B2304, Cambridge, MA 02139-1517, USA). The kidney samples were stained with Anti-Collagen IV antibody. The kidney samples were fixed in paraformaldehyde then embedded in paraffin and sectioned 2 micrometer. Samples were subjected to immunohistochemistry, anti-bovine type IV collagen rabbit serum was used as first antibody and biotinylated anti-rabbit Iggy goat serum was used as secondary antibody. Collagen IV immunohistochemistry appeared as brownish coloration in the mesangial matrix [19,20].

Statistical analysis

Data were analyzed using SPSS version 20. Quantitative variables were expressed in means ± SDs. Fisher's exact tests were applied to observe an association between qualitative variables. The comparison of quantitative data was performed by independent t-test or Mann Whitney test according to the normality of distribution for independent variables consisting of two groups. Statistical significance was set at 0.05 levels [18]. A p-value of < 0.05 was considered as statistically significant.

Results

Table 1 revealed comparison between control and obese rats group. There was a significant difference between the two groups as regard body weight (p 0.0001) with marked increase body weight of obese rats. There was a significant increase in kidneys weight of obese group compared to control group (p 0.02). Metabolic profile revealed a significant increase of FBS (p 0.0001), SI (p 0.0001), TL (p 0.0001), TC (p 0.0001), TG (p 0.0001), and LDL (p 0.0043) with significant decreased of HDL (p 0.0133) in obese group compared to the control group. Serum creatinine was significantly increased in obese group compared to control group despite within the normal range.

Table 2 revealed a significant positive correlation between serum creatinine and BW, FBS, SI, and TG with insignificant positive correlation between serum creatinine, TC and LDL. There was an insignificant negative correlation between serum creatinine and kidney

Parameters	Control group (N=25) mean ± SD	Obese group (N=25) mean ± SD	P
BW/g	226.88 ± 27.27	457.56 ± 56.28	0.0001
Kidney weight/g	4.11 ± 0.67	4.48 ± 0.38	0.0200
FBG mg/dl	92.12 ± 8.7	118.8 ± 7.77	0.0001
SI ng/ml	51.24 ± 5.93	95.64 ± 10.02	0.0001
TL mg/dl	4.44 ± 0.47	5.94 ± 0.45	0.0001
TC mg/dl	142.64 ± 5.61	173.8 ± 15.37	0.0001
TG mg/dl	129.92 ± 9.13	154.28 ± 13.9	0.0001
LDL-C mg/dl	67.28 ± 8.44	74.8 ± 7.58	0.0043
HDL-C mg/dl	46.36 ± 3.09	44.0 ± 3.39	0.0133
Creatinine mg/dl	0.65 ± 0.07	0.73 ± 0.08	0.0017

Table 1: Comparison between control and obese rats group.

weight and HDL. Light microscopic study of haematoxylin and Eosin stained sections control group rats revealed rounded renal corpuscles with a centrally located glomerulus formed of a coiled mass of capillaries surrounded peripherally with Bowman's capsule. Parietal layer of Bowman's capsule was formed of simple squamous epithelium while the visceral layer was formed of podocytes with a prominent nucleus. The proximal convoluted tubules were lined with high cuboidal epithelium with a rounded euchromatic nucleus. Distal convoluted tubules were lined with cuboidal epithelium with a rounded euchromatic nucleus. The collecting ducts were lined with a low cuboidal epithelium with a round euchromatic nucleus (Figures 1 and 2). Stained sections of rats of group II revealed moderately expanded Bowman's capsule. The lumen of the tubules appeared wide with degenerated shaded luminal border (Figure 3).

Light microscopic study of immunostained renal samples sections stained with collagen IV immune histochemistry appeared as brownish coloration in the mesangial matrix. Examination of control rats revealed no brownish coloration in the mesangial matrix indicated negative reaction for collagen IV (Figure 2) while examination of obese rats revealed weak positive reaction for collagen IV (Figure 3). Transmission electron microscopic examination of ultrathin sections of control rats revealed the glomerular basement membrane separated between capillary and urinary spaces. It is formed of a central lamina densa and lamina rara on either side. Foot processes of podocytes were separated from one another by a regular narrow space called filtration slit . The lining epithelium of proximal convoluted tubules showed rounded and regular nucleus with finely dispersed chromatin. The apical surface had numerous microvilli forming the brush border while obese rats revealed increase in the thickness of glomerular basement membrane and also foot processes fusion. The cells of the lining epithelium of proximal convoluted tubules revealed many vacuolation scattered in the rarefied cytoplasm.

Discussion

There is an epidemic of obesity across the world. The epidemic of obesity has been paralleled by an increase in the incidence of Chronic Kidney Disease (CKD). Several epidemiologic studies have shown that obesity is an independent predictor of CKD. In addition to diabetes and hypertension, several other mechanisms have been postulated to initiate and maintain kidney injury in patients with obesity. This work try to study the potential mechanisms involved in renal injury in obese rats. Our study revealed a significant difference between the two studied groups as regard body weight (p=0.0001) with marked increase body weight of obese rats. There was a significant positive

|		BW	KW	FBS	SI	TC	TG	LDL	HDL
Cr	r	0.4577*	-0.0299	0.4291*	0.4381*	0.2479	0.4562*	0.2715	-0.0517
	p	0.0008	0.8370	0.0019	0.0015	0.0826	0.0009	0.0565	0.7215

Table 2: Correlation between serum creatinine and other parameter in obese rats group. *Fisher's Exact. *A *p*-value <0.05 was considered as statistically significant.
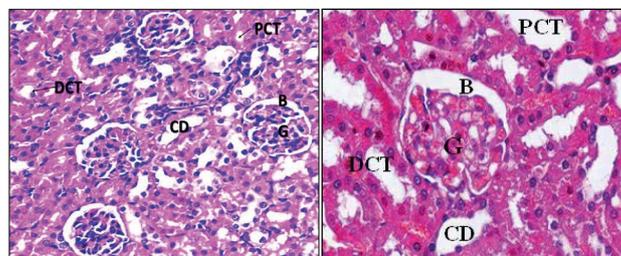
Figure 1: A Photomicrograph of a section in kidney of a control rat revealed: A: Normal renal structure with rounded renal corpuscles formed of the Glomerulus (G) and the Bowman's capsule (B) surrounded with Proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT) and Collecting Duct (CD). B: Dilated PCT, DCT and CD with degenerated irregular luminal borders and vacuolated glomerulus surrounded by expanded Bowman's capsule (H&E 1000x).
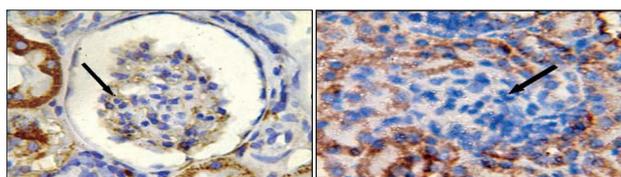
Figure 2: Photomicrograph of a section in kidney. A: Normal control rat kidney showed negative reaction for collagen IV (no brownish coloration in the mesangial matrix). B: an obese rat kidney showed positive reaction for collagen IV (arrow). (Collagen IV immunostaining 1000x).
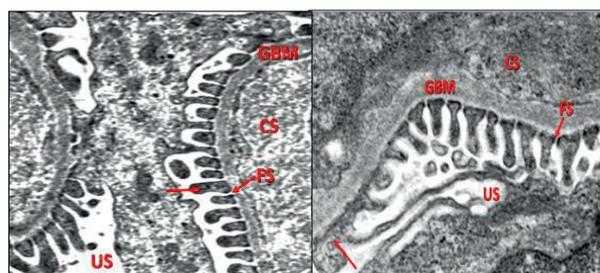
Figure 3: An electron micrograph of an ultrathin section in the kidney: (A) A control rat showed Glomerular Basement Membrane (GBM) separating between Capillary Space (Cs) and Urinary Space (Us). Foot processes of podocytes (arrow) are separated from one another by a regular narrow space called Filtration Slit (FS). B: obese rat showed thickened GBM. Some foot processes (arrow) are fused together (EM 8000x)

correlation between serum creatinine level and BW. There was a significant increase in kidneys weight of obese group compared to control group (p=0.02). This is in agreement with Eknoyan [21] who stated that glomerulomegaly is one of pathological effects of overweight and obesity on the kidney. Our study revealed insignificant negative correlation between serum creatinine and kidney weight. In force-fed dog study, obesity was associated with an increase in kidney weight of about 40% accompanied by an increase of glomerular size together with podocyte injury and expansion of the mesangium, and in sustained obesity resulted in mesangial sclerosis [22]. Henegan et

al. [17] stated that these structural changes were prevented by dietary restriction in hyperphagic Zucker rats. Rea et al. [23] compared kidney biopsies from obese to lean living kidney donors and found that the glomerular planar surface area was significantly greater in those who were obese but no evidence of GS. In humans, despite the occurrence of glomerulomegaly, hyperfiltration, and albuminuria, most obese individuals do not develop GS [24].

Metabolic profile revealed a significant increase of FBS (p=0.0001), SI (p=0.0001), TL (p= 0.0001), TC (p=0.0001), TG (p=0.0001), and LDL (p=0.0043) with significant decreased of HDL (p=0.0133) in obese group compared to control group with a significant positive correlation between serum creatinine and FBS, SI, and TG. This increased SI level and FBG indicate a state of insulin resistance. This in agreement with Sarafadis and Ruilope [25] who stated that insulin resistance characterizes the obese and type 2 diabetics is among the commonly implicated mechanisms of organ damage, and may account for the obesity-related hyper-filtration, glomerulomegaly, and albuminuria that are also the characteristics of early diabetic nephropathy. Our study revealed that obese rats had high TL, TC, TG and LDL with low level of HDL compared to control group. This is in agreement with Howard et al. [26] who clarified that the primary dyslipidemia related to obesity is characterized by increased triglycerides, decreased HDL levels, and abnormal LDL composition. The pathogenesis of the dyslipidemia of obesity seems to be closely related to insulin resistance. All of the components of the dyslipidemia, including higher TG, decreased HDL levels, and increased small, dense LDL particles, have been shown to be atherogenic. It was observed that weight loss and exercise, even if they do not result in normalization of body weight, can improve this dyslipidemia.

Experimental and clinical studies have suggested a correlation between the progression of renal disease and dyslipidemia. High cholesterol and triglyceride plasma levels have been demonstrated to be independent risk factors for progression of renal disease in humans. The underlying pathophysiologic mechanisms for the relationship between lipid levels and progression of renal disease are not yet fully understood, although there are data that oxidative stress and insulin resistance may mediate the lipid-induced renal damage. In the animal model, lipid-lowering agents seem to ameliorate glomerular damage, preventing GS and interstitial fibrosis [27]. Serum creatinine was significantaly increased in obese group compared to control group despite within the normal range. This in agreement with Li et al. [28] who demonstrated that obesity is an independent risk factor associated with increasing serum creatinine levels in children aged more than 10 years and weight control is important in the protection of renal function.

Histological study of haematoxylin and eosin stained sections of obese rats revealed moderately expanded Bowman's capsule. The lumen of the tubules appeared wide with degenerated shaded luminal border, while immunostained stained sections revealed weak positive reaction for collagen IV. Electron microscopic examination of obese rats sections revealed increase in the thickness of glomerular basement membrane and foot processes fusion. The cells of the lining epithelium of proximal convoluted tubules revealed many vacuolation scattered in the rarefied cytoplasm. These findings were in agreement with Bagby [29] who confirmed that the kidney undergoes many significant structural and metabolic changes with obesity, including glomeruli hyperfiltration, thickening of the glomerular basement membrane, proliferation of mesangial cells, thickening of the mesangial matrix, and expansion of Bowman's capsule. Jiang et al. [30] added that, the mesangial matrix proliferation was confirmed by previous researchers who confirmed that, the high-fat-fed mice also exhibited signs of GS and proteinuria,

including mesangial expansion with increased extracellular matrix protein content and a significant increase of urinary albumin excretion, that were not present in low-fat-fed mice.

In the present work, examination of obese rats kidneys showed cytoplasmic vacuolation of tubular lining epithelium, thickened glomerular basement membrane and fused podocytes foot processes. These findings were in agreement with Sharma et al. [31] who stated that, clinical evidence suggests that obesity-induced reductions in adiponectin may be involved in renal dysfunction by affecting podocyte biology. Also, he revealed that mice deficient in adiponectin have increased albuminuria and fusion of podocyte foot processes, perturbations that were normalized with adiponectin treatment. Also, there was increased expression of collagen IV in obese rats kidney. This finding was in accordance with Abrass et al. [32] who found glomerular over expression of type IV collagen which is one of the main components of sclerotic glomerulus. Also, Kambham et al. [33] added that, in humans, obesity-related kidney damage and diabetic nephropathy characterized by glomerulosclrosis.

Conclusions

Obesity is associated with many metabolic abnormalities like insulin resistance, impaired glucose tolerance and dyslipidemia which may affect renal function. Also, obesity is associated with morphological and structural renal changes as glomerulomegaly, increased expression of collagen IV and hyper-filtering kidney which may proceed to GS and CKD. So obesity may be considered as an independent risk factor for the development of chronic kidney disease.

Limitation of the Work

Some factors may be considered as study of some factors like adiponectin and its relation to the kidney changes, the effect of weight loss on reversal of these changes beside, small sample numbers of experimental animals involved in the study.

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