Early histological changes in the kidney of people with morbid obesity

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Abstract

Background. Morbid obesity represents a major health problem with increasing incidence worldwide. The clinical manifestation of renal involvement in obesity is proteinuria, and the histological feature is glomerulomegaly with or without focal and segmental glomerulosclerosis (FSGS). In this study, we have investigated the very early histological changes in kidneys of people with morbid obesity and no proteinuria.

Patients and methods. Eighteen patients with body mass index (BMI) > 50 kg/m² who underwent a variant of biliopancreatic diversion with Roux-en-Y reconstruction (BPD-RYGBP) and consented to undergo a renal biopsy during the surgical procedure were included in the study. The estimation of early histological changes was performed on light (n = 18) and electron microscopy (n = 13).

Results. The mean glomerular cross-sectional area was 30 943 ± 10 984 µm² that is higher than that observed in non-obese individuals. In 21% of the examined glomeruli, the glomerular planar surface area (GPSA) was >40 000 µm². Thickening of the glomerular basement membrane (GBM) and scattered paramesangial deposits were identified in 9 of 13 patients (70%) whose renal tissue was examined by electron microscopy. A reduction in the slit pore frequency was observed in obese patients due to extensive foot process effacement. Significant positive correlations between mean GPSA and body weight (r = 0.462, P = 0.05), and between GBM thickness and HbA1c, serum total cholesterol and triglyceride levels (r = 0.60, P = 0.05; r = 0.789, P = 0.004; r = 0.70, P = 0.016, respectively), were observed.

Conclusions. Glomerulomegaly as well as histological lesions resembling those of early diabetic nephropathy are observed in kidney biopsies of patients with morbid obesity even before the appearance of microalbuminuria. The potential regression of these changes after weight loss needs to be clarified.

Keywords: glomerular disease; glomerular volume; obesity; podocytes

Introduction

Obesity represents a major health problem with increasing incidence in the Western world [1,2]. Its prevalence has increased dramatically over the last 15 years taking epidemic proportions [3]. According to most recent data, two-thirds of American adults are overweight or obese [4]. Obesity, specifically central obesity, is the phenotypic hallmark of the metabolic syndrome that is characterized by insulin resistance, hyperinsulinaemia and dyslipidaemia. This syndrome contributes to the development of type 2 diabetes, hypertension, cardiovascular disease and chronic kidney disease (CKD) [5, 6]. A recent meta-analysis showed that obesity is a strong risk factor for the development of kidney disease [7].

The clinical evidence of renal involvement in obesity is manifested by the presence of proteinuria that usually precedes GFR decline by several years [3,8]. Proteinuria is thought to be due to haemodynamic changes that result in glomerular hypertrophy and hyperfiltration. Experimental studies in obese animals show that angiotensin II and insulin are implicated in the development of glomerular hyperfiltration whereas leptin, a hormone produced in adipose tissue, contributes to the development of renal injury via induction of growth factors and cytokines [9]. These findings are consistent with the fact that the adipose tissue itself, and more specifically abdominal fat, may exert systemic effects through secretion of a variety of hormones and cytokines.

The type of kidney disease that has become recognized with increasing incidence over the last decade in kidney biopsies of obese patients with proteinuria is the so-called ‘obesity related glomerulopathy’ characterized by glomerulomegaly with or without FSGS [10].

The purpose of this study is to identify whether patients with no evidence of renal disease (normal renal excretory function and urinary albumin excretion rate) who are undergoing surgery for morbid obesity [body mass index (BMI) > 50 kg/m²] show early histological lesions in the kidney.

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Table 1. Clinical and biochemical features of patients at presentation ($n = 18$)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>7/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39 ± 11</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>59.3 ± 8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>169 ± 25</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>135 ± 18/86 ± 12</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.85 ± 0.15</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>102 ± 10</td>
</tr>
<tr>
<td>Insulin (µIU/ml), median (IQR)</td>
<td>15.2 (12.0–44.9)</td>
</tr>
<tr>
<td>HbA$\text{c}$(%)</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Blood cholesterol (mg/dl)</td>
<td>184 ± 41</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>143 ± 65</td>
</tr>
<tr>
<td>Total protein</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>Urine protein (g/24 h)</td>
<td>0.16 ± 0.1</td>
</tr>
<tr>
<td>Urinary albumin excretion (mg/24 h)</td>
<td>2.5 ± 2.4</td>
</tr>
</tbody>
</table>

Methods and patients

Patients

Eighteen patients (11 females and 7 males) with very extreme obesity who were planned for biliopancreatic diversion with Roux-en-Y reconstruction (BPD-RYGBP) at the Surgical Department of the University Hospital of Patras, Greece, over a 6-month period from the beginning of June 2006 up to the end of October 2006 were included in the study. During the 6-month period of the study, a total of 58 patients underwent surgical intervention for clinically severe obesity at our institution. The BMI was <50 kg/m$^2$ in 25 patients; therefore, a laparoscopic approach was applied according to the protocol of the Department of Surgery and these patients were excluded from the study. Thirty-three patients had a BMI of >50 kg/m$^2$ and were planned for BPD-RYGBP reconstruction by laparotomy. Of these patients, only 18 agreed to undergo an open renal biopsy during the operation and signed an informed consent. The inclusion criteria were severe obesity in need of surgical intervention (BMI > 50 kg/m$^2$), normal renal function (serum creatinine <1.2 mg/dl), absence of proteinuria or microalbuminuria (defined as urinary protein excretion >0.3 g/24 h and albumin excretion >30 mg/24 h), normal blood glucose and HbA1c levels (<110 mg/dl and 6.1%, respectively).

All these parameters (serum creatinine, blood glucose, HbA1c levels, urinary albumin excretion and 24-h urine protein) were measured three times in each patient.

All patients have been suffering from clinically severe obesity for at least a 5-year period prior to surgical intervention.

In 13 of 18 patients, renal tissue was examined with electron microscopy. Biopsies were taken and fixed immediately in 3% phosphate buffered glutaraldehyde post-fixed in 1% aqueous osmium tetroxide, dehydrated in graded alcohols and propylene oxide, impregnated and embedded in TAAB EMIX epoxy resin (www.taab.co.uk). The blocks were sectioned using a Reichart-Jung ultratoc E (www.leica-microsystems.com), initially at 0.6 µm, stained with 1% toluidine blue in 1% sodium tetraborate. Thin sectioning at 90 nm was done using a diamond knife (www.diatome.ch), and the sections were stained with saturated 99% alcoholic uranyl acetate and Reynold’s alkaline lead citrate each for 3 min. The thin sections were examined with a Phillips 400T electron microscope (www.phi.com) and photographed at standard magnifications ($\times 130, \times 800, \times 4600$). The sections were examined as per standard protocol for diagnostic electron microscopy of the renal biopsy. Six different glomerular capillary loops were measured at their thinnest points, from 1–3 glomeruli. Arithmetic mean and range were calculated. The glomerular basement membrane (GBM) thickness and the presence of deposits were estimated on EM. The GBM was considered as thickened if its diameter was >350 nm.

Further EM examination of the biopsies was performed at the Mario Negri Institute for Pharmacological Research, Bergamo, Italy. The frequency of the slit pores along the GBM and the integrity of the slit diaphragm were also estimated by morphometric analysis [14].

The same parameters were examined in the normal part of kidneys excised because of adenocarcinoma in three non-obese patients (two males and one female), 50.7 ± 5.1 years old, used as controls and compared to our subjects. Biopsy specimens of the controls were fixed with 2.5% glutaraldehyde in a 0.1 M cacodylate buffer (pH 7.4) for 4 h at 4 °C and washed in a cacodylate buffer. The kidney fragments were then post-fixed in 1% osmium tetroxide for 1 h, dehydrated through ascending grades of alcohol and embedded in resin.

Statistical analysis

All continuous variables were expressed as mean ± SD after ascertaining the assumption of normality using Kolmogorov–Smirnov and Shapiro–Wilk tests. Only insulin concentration had a significant deviation from normality on the Shapiro–Wilk test and was presented as median and interquartile range (IQR). Comparisons of the means between subjects and controls (electron microscopy), diabetes and non-diabetics, low and high normal levels of albuminuria (<20 mg/24 h and 20–30 mg/24 h) were, therefore, performed using Student’s t-test, except for insulin where the non-parametric Mann–Whitney test was used. Correlations for most variables were performed using Pearson’s method. Correlations between clinical variables and insulin were performed with log-transformation of insulin because of the skewed distribution. Also, as urine albumin the amount of tissue needed, hence minimizing potential complications. There were no complications documented, except minimal bleeding from the site of biopsy that was successfully treated by hand pressure with gauze pads and topical haemostatic agents (SurgiCel®, Johnson & Johnson, Langhorne, PA, USA).
excretion was not normally distributed in the general population, further
tests were done with logarithmically transformed urinary albumin even
though in this cohort albuminuria appeared to be normally distributed.
As there was no difference in the results, only data on the raw variable
(non-logarithmically transformed) were presented.
A P-value < 0.05 was considered significant. Statistical analyses were
conducted using the Statistical Package for the Social Sciences (SPSS
15.0, SPSS Inc, Chicago, IL, USA).

Sensitivity analysis
The statistics were repeated excluding the three diabetic subjects.

Results

Patients
The mean age of the 18 patients recruited was 39 ± 11 years.
Of those, seven were males and three had a formal diagnosis
of diabetes mellitus. The mean BMI was 59.3 ± 8.0 kg/m².
All patients had a normal renal excretory function with
mean creatinine 0.85 ± 0.15 mg/dl (Table 1).

Complications
No complications related to the open renal biopsies were
observed with the exception of minor bleeding that was
easily controlled by localized pressure.

Morphological changes on LM
The early kidney morphological changes were examined on
PAS and Masson’s stained sections containing a total of 115
glomeruli from 18 patients (6.4 glomeruli/patient/biopsy
for LM). The main feature was the presence of enlarged
glomeruli in all biopsies. The mean GPSA was 30 943 ±
10 984 µm². The mean GPSA of the largest glomerular
sections in each patient was 41 219 ± 17 696 µm². The
GPSA was >30 000 µm² in 69 out of the 115 estimated
glomeruli (60%) and >40 000 µm² in 24 glomeruli (21%)
(Figure 1A and B). The mean glomerular area of the exam-
ined glomeruli was significantly higher in male compared
to female obese subjects (37 485 ± 11 232 µm² versus
26 780 ± 8 966 µm², P = 0.04). The mean mesangial area
was 14.7 ± 3.7% of the glomerular tuft.

No global or segmental sclerosis or capsular adhesions
were identified within the examined glomeruli. No signif-
icient changes were observed in the tubular epithelial cells,
interstitium or renal vasculature.

Morphological changes on EM
The early kidney morphological changes observed with
electron microscopy were estimated in sections obtained
from renal biopsies of 13 patients. A thickened GBM was
identified in 9 of 13 patients (70%) (Figure 2A and B). The
mean GBM thickness was 358 ± 36 nm. The GBM thick-
ening was patchy rather than diffuse in most cases. Scattered
or occasional paramesangial deposits were also identified
in 9 of 13 patients (Figure 3).

A significant reduction in the slit pore frequency was ob-
served in obese patients in comparison to the three histori-
cal controls on whom those measurements were previously
done at the Mario Negri Institute (1.110 ± 0.062 versus
2.085 ± 0.066 slits/µm GBM, P < 0.0001). Furthermore,
in comparison to controls, morbidly obese subjects had a
significantly higher proportion of pores with an electron
dense slit diaphragm as opposed to a normal filamentous
appearance (76.1 ± 6.4 versus 62.5 ± 0.5%, P < 0.0001).

Correlation between parameters
A significant positive correlation of mean GPSA with body
weight (r = 0.462, P = 0.05) was found (Figure 4). In
addition, significant positive correlations between the GBM
thickness and HbA1c, cholesterol and triglycerides were
observed (r = 0.60, P = 0.05; r = 0.789, P = 0.004; r =
0.70, P = 0.016, respectively) (Figure 5A–C).

The GBM thickness correlated with the glomerular area
(r = 0.575, P = 0.04) and the percentage mesangial area
with serum creatinine (r = 0.647, P = 0.032). The slit
frequency correlated negatively with triglycerides (r =
−0.650, P = 0.031) and albuminuria with HDL (r =
−0.539, P = 0.038).
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Fig. 2. Increased thickness of the GBM (magnification A: ×800, B: ×2,800).

Fig. 3. Scattered paramesangial deposits (magnification ×10,000).

Fig. 4. Positive correlation of body weight with mean glomerular area ($r = 0.462$, $P = 0.05$).

Sensitivity analysis: exclusion of diabetics

There were no significant differences in the histological findings (glomerular area, GBM thickness, percentage mesangium, pore frequency and proportion of pores with an electron-dense slit diaphragm) between the three patients with diabetes mellitus and the rest of the group. Some of the correlations were attenuated after excluding the three diabetic subjects such as between GBM thickness and HbA1c, but most correlations persisted. The correlations including and excluding diabetic subjects are summarized in Table 2.

Discussion

In this study, early changes in the glomerular structure were demonstrated in kidney biopsies of patients with morbid obesity. Enlarged glomeruli, thickening of the GBM, scattered paramesangial deposits and reduction in the slit pore frequency were the main features identified on LM and EM. Significant correlations between the glomerular area and the body weight and between the GBM thickness and HbA1c, cholesterol and triglycerides blood levels were also observed. These observed changes resembled those described at the early stages of diabetic nephropathy (DN).

Light microscopy findings

The main findings on LM were an increase in the glomerular size and a variable degree of mesangial expansion. The resemblance of those findings to changes seen in early DN suggests a common mechanism that links obesity and diabetes to glomerular damage. Furthermore, there are similarities between our findings and those seen in the syndrome of obesity-related glomerulopathy (ORG), even though none of the patients has developed proteinuria or microalbuminuria. ORG is characterized by obesity, proteinuria and glomerulomegaly with or without FSGS. ORG with FSGS has a better prognosis than idiopathic FSGS, but it is also a serious disease [15]. Although, none of our patients had FSGS, the majority of them had glomerulomegaly. The definition of glomerulomegaly is not uniform in the different studies. For example, Chen et al. [16] defined glomerulomegaly as the glomerular volume of $>3.27 \times 10^6 \, \mu m^3$, whereas Kambham et al. found that the mean glomerular diameter in patients with ORG was $226 \pm 24.6 \, \mu m$ compared to $168 \pm 12 \, \mu m$ in controls [10]. In our cohort, 14/18 patients (78%) had a glomerular volume $>3.27 \times 10^6 \, \mu m^3$ with a mean glomerular volume of $4.28 \times 10^6 \pm 2.17 \times 10^6 \, \mu m^3$ and a mean diameter of $195.2 \pm 37 \, \mu m$. 
Fig. 5. Positive correlation of the GBM thickness with (A) HbA1C ($r = 0.60, P = 0.05$); (B) cholesterol blood levels ($r = 0.789, P = 0.004$) and (C) blood triglyceride levels ($r = 0.70, P = 0.016$).

In a similar study to ours, by Serra et al., in patients undergoing bariatric surgery, the mean glomerular area was $27,425 \pm 7,473 \, \mu m^2$ compared to normal weight controls with a mean glomerular area of $19,086 \pm 4,727 \, \mu m^2$ [17]. The mean glomerular area in our patients was $30,943 \pm 10,983 \, \mu m^2$ that was larger than that in Serra's study and this could be explained by the higher mean BMI of our patients ($59.3 \, kg/m^2$ versus $52 \, kg/m^2$). However, despite the lower mean glomerular area in Serra's study, $40\%$ of their patients had proteinuria and some had already developed glomerular sclerosis. As none of our patients developed glomerulosclerosis despite having larger glomeruli, it is suggested that the development of proteinuria and glomerular sclerosis is not entirely related to reaching a critical glomerular size. Indeed, Serra et al. found no correlation between glomerulomegaly and the presence of FSGS. It is therefore likely that the development of glomerular sclerosis is a ‘multiple hit’ process, whereby glomerulomegaly is the initial event that requires additional insults for the development of the sclerotic changes.

We found no correlation between fractional mesangial volume and glomerular size or BMI. This is consistent with the findings by Chen et al. of increased glomerular volume with normal mesangial volume fraction in obese Chinese patients with proteinuria [16]. However, Serra et al. found that glomerulomegaly was significantly associated with the increased mesangial matrix, though their estimation of mesangial area was semi-quantitative [17].

Electron microscopy findings

Intrinsic changes in the GBM have been demonstrated in this and previous studies. The mean GBM thickness in this cohort is $358 \, nm$. The normal GBM thickness is age dependent and varies from one laboratory to another, but according to our laboratory, this falls towards the higher end of the normal range. It is noteworthy that our standard practice is to take a mean reading of sampling points at the thinnest parts of the GBM. The paramesangial deposits observed in our study cannot be validated because of the lack of immunofluorescence. However, others have described the presence of IgM [10, 17] and IgA deposits [17].

Perhaps the most consistently reported changes are those of podocytes. Studies have shown increased foot process width [16,18], foot process fusion [10,16,18], decreased podocyte number [16,18] and podocyte hypertrophy [10]. In this study, we demonstrate an earlier stage of GBM injury where there is a reduction in the slit diaphragm frequency as a result of foot process effacement. This is accompanied by an increase in the proportion of pores with an electron dense slit diaphragm as opposed to a normal filamentous appearance. The absence of proteinuria at this stage may be explained by the fact that these changes are very early and precede the more prominent changes such as podocyte swelling and fusion. Alternatively, the presence of healthy proximal tubules able to reabsorb and handle excess filtered proteins may explain the absence of microalbuminuria in these individuals. Proximal tubular dysfunction has been suggesting that the development of ORG may be a gradual process that starts with progressive glomerulomegaly before microalbuminuria and proteinuria ensue.

Whilst our results may not be directly comparable to the Chinese population [16], some conclusions may be drawn from comparison with the predominantly Caucasian cohort of Kambham’s study [10]. The mean diameter in our study falls in between those with ORG and the control group.
implicated in the pathogenesis of microalbuminuria in people with diabetes [19].

**Clinical parameters**

There is a significant correlation between body weight and glomerular size in this study. This is presumably an adaptive mechanism to increased filtration. The correlation is with body weight rather than BMI suggesting that the weight per se is the most significant factor supporting a mechanical rather than a metabolic mechanism for glomerulomegaly. This finding is in agreement with Rea et al. who found a correlation between weight and GPSA [13].

A significant positive correlation was observed between GBM thickness and HbA1C. It is well known that strict control of blood glucose by intensive insulin treatment delays the onset and slows the progression of DN [20,21]. The blood HbA1c levels have been closely related to the glomerular structural changes of diabetic patients, whereas the baseline GBM thickness has been proved to be a predictor of increased urine albumin excretion rate [22,23]. The main effect may be on podocytes, as the podocyte number and density are inversely related to fasting blood glucose levels and proteinuria [18].

Furthermore, GBM thickness correlated with serum cholesterol and triglycerides. Serum lipids that are a manifestation of the metabolic syndrome may have a direct pathogenic effect on the GBM. Unfortunately, we could not verify the independence of the correlation between serum lipids and GBM thickness in multivariate analyses due to the small sample size. Nonetheless, an independent relationship is plausible. Clinically, it has been shown that increased baseline serum cholesterol, triglycerides, haemoglobin A1c and mean arterial blood pressure are independent predictors of the development of microalbuminuria in diabetic patients [24]. Thus, blood glucose and lipids possibly have a synergistic effect on the development of glomerular injury in diabetes and severe obesity. It is known from experimental studies that obese Zucker rats develop obesity due to hyperphagia resulting from missense mutation of brain leptin receptors and show features observed in obese human individuals [25]. Those animals develop hypertension and glomerular hypertrophy and finally die of kidney disease. However, early food restriction can prevent glomerular injury [25]. Furthermore, in our cohort, HDL cholesterol correlated negatively with albuminuria.

No correlation between the degree of albumin excretion and other clinical parameters, glomerular size, GBM thickness or slit pore frequency was observed. Chen et al. also found no correlation between 24-h protein excretion and podocyte hypertrophy [18]. However, in advanced glomerular injury where there is a reduction in the podocyte number, proteinuria was related inversely to the podocyte number and density and correlated directly to foot process width on the peripheral GBM [18].

Glomerular and mesangial areas correlated with GBM thickness and the mesangial area negatively with pore frequency, suggesting a simultaneous process of glomerular enlargement, mesangial expansion and change in GBM thickness and structure. Of the histological parameters, the only one that correlated with creatinine was mesangial volume fraction. Therefore, it is possible that mesangial expansion is an advanced stage that is associated with a reduction in renal excretory function. In this study, we chose to represent the renal excretory function as serum creatinine due to the fact that none of the GFR estimation equations has been validated in the morbidly obese population.

**Exclusion of diabetics**

Most of the correlations of GBM thickness remained significant even after the exclusion of diabetics. The attenuated correlations may be due to the loss of statistical power rather than a genuine difference between diabetics and non-diabetics. We chose to present the discussion including all subjects, as diabetes in these patients is a complication of obesity and should be seen as part of a continuum of severity.

**Limitations**

The main limitation is the small number of subjects, and therefore, some potentially significant correlations may be missed. This may explain the lack of correlations between...
proteinuria(albuminuria) and the histological parameters. Another explanation is the lack of accurate data on the use of angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB).

Furthermore, it is not feasible to perform multivariate analysis due to the small number of subjects. Therefore, where more than one clinical variable correlates with a histological parameter, it is not clear which of those associations is independent and potentially causative.

**Conclusion**

Histological changes resembling those of early DN and ORG are observed in patients with morbid obesity and no clinical evidence of kidney disease. Further research is required in order to identify the potential reversal of histological changes after surgical treatment of clinically severe obesity and substantial weight loss.

Conflict of interest statement. None declared.

References


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