The Influence of Sex on Early Stage Markers of Kidney Dysfunction in Response to Juvenile Obesity

Ian D. Bloor, Sylvain P. Sebert, Ravi P. Mahajan, Michael E. Symonds

Abstract—Changes within the kidney in response to obesity are critical in determining the magnitude of later dysfunction. However, the cause of this process in response to juvenile onset obesity and how it can be determined by sex is poorly understood. We therefore examined the effect of juvenile obesity induced by exposure to a restricted activity environment from weaning until early adulthood on the molecular responses within the kidney together with glomerular area and nucleated cell number. This was stratified by sex and was undertaken in a sheep model of early obesity. Despite a similar magnitude of increase in fat mass with obesity onset between sexes, adverse effects on glomerular area and cell number together with raised gene expression within the kidney only occurred in males. Irrespective of obesity, gene expression of C-C motif receptor 2 was higher, and interleukin-6 lower, in male kidneys compared with female kidneys. The effects of sex on molecular differences within the kidney were amplified with obesity, which had no effect on any gene studied in females but had an enhanced response in males. Obese males therefore showed increased gene expression of a range of markers relating to the glucocorticoid axis, inflammation, and lipid sensing. In conclusion, young females were protected from adverse renal effects of obesity, which results in very little inflammatory or related responses. Our findings emphasize the critical importance of sex specificity in disease pathogenesis. An increased understanding of the specific mechanisms will have important implications for therapeutic strategies aimed at preventing adverse consequences of obesity. *(Hypertension. 2012;60:991-997.)* ◼ Online Data Supplement

Key Words: kidney inflammation ■ sex differences ■ obesity
contributing to structural renal impairment, dysfunction, and damage.

Materials and Methods

Animal Model
Immediately after weaning, that is, at 3 months of age, nonidentical twin sheep born to Bluefaced Swaledale mothers were separated into 2 different physical activity groups and were either allocated into a restricted activity area, that is, an obesogenic environment with a stocking rate of 6 sheep per 19 m², or an unrestrained activity area, that is, a lean environment with a stocking rate of 6 sheep per 1125 m². The animal numbers per group were lean females n=7, obese females n=9, lean males (LM) n=11, and obese males (OM) n=9. All animals at each stage were fed to fully meet their metabolic requirements with a mix of hay and concentrated pellets (Manor Farm Feeds, Oakham, United Kingdom). Both diastolic and systolic blood pressure measurements were determined at 16 months with a standard blood pressure measuring device under sedation when scanned using dual x-ray absorptiometry. This gave blood pressure values that are in accordance with those recorded in conscious adult sheep. Then at 17 months, 5-ml blood samples were withdrawn after an overnight fast into lithium heparin tubes and centrifuged at 2500 g for 10 minutes at 4°C to obtain plasma. Finally, when all of the animals were 17 months of age they were humanely euthanized by electrical stunning and exsanguination. This age was adopted as we have previously found no effect of sex in the kidney of obese animals aged 12 months, and 17 months is the age when mature adult body weight is attained. Kidneys were weighed and partially dissected before being fixed in 10% formalin and embedded in paraffin wax for subsequent histological analysis. The remaining tissue was snap frozen in liquid nitrogen and stored at −80°C. All experimentation was conducted in accordance with the UK Home Office and the UK Animals (Scientific Procedures) Act (1986).

Gene Expression Analysis
Total RNA was extracted from 0.15 g of cortical renal tissue with an RNeasy Plus Mini extraction kit (Qiagen, West Sussex, United Kingdom) using an adapted version of the single-step acidified phenol-chloroform homogenization method. Sample integrity was confirmed by Nanodrop ND-1000 spectroscopy (Nanodrop Technologies, UK). RNA samples were normalized by dilution to 1 ng/μL, which were then reverse transcribed using a Superscript Reverse Transcriptase kit (Invitrogen Ltd, Paisley, United Kingdom) using an adapted version of the single-step acidified phenol-chloroform homogenization method. Sample integrity was confirmed by Nanodrop ND-1000 spectroscopy (Nanodrop Technologies, Wilmingtom, DE). RNA samples were normalized by dilution to 1 ng/μL which were then reverse transcribed using a Superscript Reverse Transcriptase kit (Invitrogen Ltd, Paisley, United Kingdom) and cDNA amplified on a Touchgene Gradient thermocycler (Techne Inc, Stone, Staffordshire, UK). Quantitative-polymerase chain reaction was performed using SYBR Green Taq polymerase master mix (Thermo Scientific, Leicester, United Kingdom) and oligonucleotide primers against a cDNA gene standard curve to verify the efficiency of the reaction and appropriate negative controls. The mRNA abundance for the following genes were determined: 11β-hydroxysteroid dehydrogenase type 2, glucocorticoid receptor (type 2), chemokine C-C motif receptor 2 (CCR2), interferon-γ, IL-6, IL-10, IL-18, monocyte chemoattractant protein-1, tumor necrosis factor-α, inducible nitric oxide synthase, peroxisome proliferator-activated receptor-γ, cluster of differentiation 14, toll-like receptor 4, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, erythropoietin receptor, and renin (Table S1 in the online-only Data Supplement). Gene expression was determined against ribosomal 18s and acidic ribosomal protein PO (RPLO) using the 2−ΔΔCt calculation.

Glomerular Area, Cellularity, and Lipid Peroxidation
Between 10 and 20 sections (5 μm) were taken at random from the center of each kidney, mounted on Superfrost Plus slides (Menzel-GläserInc, Braunschweig, Germany) and stained using hematoxylin-eosin. These were visualized through a Leica digital resolution microscope (Leica Microsystems, Wetzlar, Germany) at ×10 magnification and photographed for analysis (Hamamatsu digital camera, Hertfordshire, United Kingdom) (Figure 1A through 1D). A similar method was used to quantify collagen deposition based on Masson trichrome staining. Glomerular cross-sectional areas (in μm²) (excluding Bowman space) and glomerular cell counts were assessed using Velocity v5.2.0 image software (Perkin Elmer, MA) in 40 randomly selected glomerular sections. There was no evidence of differential shrinkage between groups, so it was not necessary to make any correction for this potential effect. Lipid peroxidation was determined using thiobarbituric acid reactive substances analysis.

Immunohistochemistry
As described above, 10 to 20 kidney sections, including >5 cortical, 2 juxtamedullary, and 3 medullary regions, were immunostained to detect protein abundance for proliferating cell nuclear antigen (Abcam 18912, Cambridge, United Kingdom) and Caspase-3 (Abcam 4051) using a Leica BondMax immunohistochemistry slide processor (Vision Biosystems Bond v3.4A, Mount Waverley, Australia) using Bond polymer detection reagents. Samples were initially treated with epitope retrieval solution and peroxide block before exposure to the diluted primary antibody (proliferating cell nuclear antigen 1:4000; or Caspase-3 1:50) and negative control. Sections were then treated with a conjugated secondary antibody and exposed to 3,3-diaminobenzidine until a brown precipitate developed. Finally slides were treated with hematoxylin-eosin staining for nuclear orientation and visualized.

Ten random blinded areas at ×10 magnification were analyzed for total and glomerular positive cell staining using Velocity.

Plasma Creatinine, Metabolite and Hormone Measurements
Plasma creatinine analysis was performed using the Abcam assay kit (ab65340). Plasma glucose, nonesterified fatty acids, and triglyceride concentrations were determined colorometrically and insulin and cortisol by ELISA. Because urine samples were not collected and an estimate for estimated glomerular filtration rate is not available for sheep, plasma creatinine was corrected against lean muscle mass to provide an estimate of potential creatinine clearance and thus renal function.

Statistical Analysis
All data are expressed as means±SEM. Data were subjected to the Kolmogorov-Smirnov test to determine normal distribution followed by either ANOVA or Kruskal-Wallis statistical tests, respectively, for parametric and nonparametric data, with applied multiple testing post hoc corrections made. These analyses were performed using PASW v17.12 statistics software (IBM). Statistical significance was accepted with a CI of 95% (P<0.05).
Development of Juvenile Obesity, Metabolic Homeostasis, and Effects on Glomerular Morphology, Cellular Proliferation, and Cardiovascular Physiology

After exposure to an obesogenic environment, all of the animals exhibited increased adipose tissue accumulation in all depots, irrespective of sex (Table 1). This was accompanied by an increase in total lean mass, again irrespective of sex, but relative lean mass was unaffected. Kidney weight was, however, only raised with obesity in males. After obesity, all of the animals remained normoglycemic with normal plasma nonesterified fatty acid and triglyceride concentrations (data not shown). Plasma insulin was raised with obesity irrespective of sex (eg, LM, 0.09±0.01 nmol/L; OM, 0.14±0.01 nmol/L; **P<0.01), whereas cortisol was only increased with obesity in females (lean females, 32±2 nmol/L; obese females, 69±16 nmol/L; *P<0.05).

To determine the potential impact of juvenile obesity on the kidney, examination of glomerular area and glomerular nucleated cell number was conducted. This demonstrated a significant increase in glomerular size and cellularity with obesity in males but not in females (Figure 2A and 2B). Consequently, both of these measurements were raised in OM compared with obese females. Across all animals studied, glomerular area and nucleated cell were positively correlated (Figure 2C). Then to determine the nature of the observed glomerular hypercellularity with obesity, protein expression of markers involved in proliferation and apoptosis was measured. OM showed downregulation of Caspase-3 glomerular staining compared with their lean counterparts (Table 2), a difference not observed in females. There were no differences in either glomerulosclerotic collagen deposition or lipid-derived oxidative stress (thiobarbituric acid reactive substances) between groups.

Basal blood pressure or heart rate was not different between groups (eg, systolic LM, 133±4 mm Hg; OM, 139±5 mm Hg and mean blood pressure LM, 110±4 mm Hg; OM, 114±4 mm Hg). Plasma creatinine corrected for lean body mass was decreased with obesity in males only (LM, 2.51×10^{-3}±0.2×10^{-3} nmol/μL per kilogram; OM, 1.7×10^{-3}±0.2×10^{-3} nmol/μL per kilogram; *P<0.05) suggesting creatinine hyperfiltration, a response not observed in females.

Expression of Genes Involved in Renal Inflammation

An investigation into the renal inflammatory and related molecular responses was performed on a number of important genes involved in obesity-mediated glucocorticoid action and renal function. In lean animals the only difference with sex was higher CCR2 and lower IL-6 in males (Table 3). With obesity the majority of genes examined were greater in males compared with females and related to pathways involving glucocorticoid action, inflammation, lipid sensing, cellular adhesion, and renal function. This difference was primarily attributed to an increase in gene expression with obesity for which mRNA abundance for the following was raised in males only, that is, 11β-hydroxysteroid dehydrogenase type 2, glucocorticoid receptor (type 2), CCR2, cluster of differentiation 14, toll-like receptor 4 vascular cell adhesion molecule-1, and erythropoietin receptor.

Discussion

We have made a unique observation that, despite similar metabolic and cardiovascular outcomes related to juvenile obesity, adverse cellular responses within the kidney were shown only in males. Obesity in males was therefore characterized with an increase in kidney mass in conjunction with enlarged glomerular area and nucleated cell number together with raised Caspase-3 abundance. These possible signs of hypercellularity and reduced apoptosis have been linked to the development of glomerular proliferative nephritis in humans. We have thus found an early response that is compatible with the development of either membranoproliferative glomerulonephritis or focal segmental glomerulosclerosis in normotensive obese individuals. Coincidently, these adaptations were accompanied by a pronounced effect on markers of metabolic and cellular stress and components of the inflammatory cascade in the cortex of male kidneys only. This could thus contribute to the established sex disparity in the progression of renal disease.

Several studies have shown that glomerular hypertrophy and increased glomerular volume are key factors in the pathogenesis of focal segmental glomerulosclerosis and glomerular scarring. However, in the present study the presence of juvenile onset obesity or sex did not influence renal collagen deposition or basal blood pressure. This suggests that the adaptations with obesity in males, only, are early markers of renal responses that will become enhanced with prolonged

Table 1. Body Mass, Composition, and Kidney Weights of Lean and Obese Young Adult Sheep

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Lean Female (n=7)</th>
<th>Obese Female (n=9)</th>
<th>Lean Male (n=11)</th>
<th>Obese Male (n=9)</th>
<th>Effect of Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>49.5±1.0</td>
<td>61.6±2.1*</td>
<td>56.4±2.5</td>
<td>83.8±1.8*</td>
<td>0</td>
</tr>
<tr>
<td>Total fat mass, kg</td>
<td>3.85±0.48</td>
<td>8.11±0.74*</td>
<td>3.61±0.44</td>
<td>9.67±0.88*</td>
<td>NS</td>
</tr>
<tr>
<td>Relative fat mass, %</td>
<td>8.4±1.1</td>
<td>14.3±1.3*</td>
<td>6.8±0.7</td>
<td>12.9±1.1*</td>
<td>NS</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>39.27±0.87</td>
<td>44.03±1.22</td>
<td>49.09±1.89</td>
<td>62.41±1.30</td>
<td>0</td>
</tr>
<tr>
<td>Ratio of lean: fat mass, kg</td>
<td>11.40±1.59</td>
<td>5.83±0.57†</td>
<td>15.31±1.62</td>
<td>6.93±0.67</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>118±9</td>
<td>117±5</td>
<td>111±5</td>
<td>144±5*</td>
<td>0</td>
</tr>
<tr>
<td>Relative kidney weight, g/kg</td>
<td>2.4±0.2</td>
<td>1.9±0.1</td>
<td>2.0±0.1</td>
<td>1.7±0.1†</td>
<td>NS</td>
</tr>
</tbody>
</table>

*O indicates effect of sex between obese groups where P<0.005; NS, no significant difference. Values are mean±SEM. Effect of obesity: *P<0.01; †P<0.05.
obesity. One adaptive response in this group that may act to initially limit the adverse cardiovascular outcomes is the increase in renal erythropoietin receptor gene expression.28 The exact mechanism behind the displayed glomerular hyper trophy is unknown, but glomerular expansion could occur through increased glomerular cell infiltration. Conversely, glomerular hypercellularity may be an effect of glomerular hypertrophy, a mechanism attributed to development of proliferative glomerulonephritis in humans29 that can be driven by a reduction in functioning nephrons30; a problem exacerbated with obesity and mediated in part by increased vascular cell adhesion molecule-1.31 Additionally, compensatory glomerular hypertrophy is associated with higher numbers of endothelial and mesangial cells, at least in genetically prehypertensive rats.32

With regard to renal function, normalization of plasma creatinine concentrations with total muscle mass in OM demonstrates an increase in plasma creatinine clearance, a finding that may indicate glomerular hyperfiltration or reduced muscle accretion. It is possible that raised synthesis and secretion of creatinine causes adaptive hyperfiltration by enlargement of the glomeruli and accompanying glomerular hypercellularity that are associated with an increased risk in the development of glomerulosclerosis and eventual end-stage renal disease.33 During our study, no sign of actual kidney damage or hemodynamic impairment was apparent with obesity in males, suggesting that the renal adaptations observed were not maladaptive but represent a set of coordinated and regulated alterations, which are proportional to metabolic and excretory needs.34 Prolonged exposure to obesity will ultimately lead to hypertension and related kidney injury as we have observed previously in sheep with more severe obesity.15 Such a hypothesis suggests that males show an increased risk to the development of obesity-mediated kidney disease, a finding in agreement with human epidemiological data.11 Our study emphasizes that the sex disparity observed in the progression of human renal disease may not be simply attributed to differences in diet, glomerular hemodynamics, or kidney size.11 It has also been suggested that oestrogens and their receptors can slow the progression rate of kidney disease.11 Additional studies are therefore needed to establish whether this process differs between sexes and ultimately results in hypertension. We have seen previously in sheep made obese by a combination of inactivity and increased food intake that they are hypertensive by 1 year of age, irrespective of sex,15 suggesting that the effect of sex can be overridden with more rapid obesity onset. Consequently, the use of a large animal model in which physical activity is simply reduced from the time of weaning, rather than an acute change of diet in adulthood as adopted in many

Table 2. Glomerular Staining (%) of Caspase-3, PCNA, TBARS, and Collagen Index Measurements in the Kidneys of Young Adult Sheep

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Lean Female (n=7)</th>
<th>Obese Female (n=9)</th>
<th>Lean Male (n=11)</th>
<th>Obese Male (n=9)</th>
<th>Effect of Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular Caspase-3, %</td>
<td>9.07±1.64</td>
<td>8.55±3.06</td>
<td>18.21±3.82*</td>
<td>5.72±1.58*</td>
<td>NS</td>
</tr>
<tr>
<td>Glomerular PCNA, %</td>
<td>20.21±2.96</td>
<td>14.21±1.86</td>
<td>16.41±2.34</td>
<td>17.23±2.41</td>
<td>NS</td>
</tr>
<tr>
<td>TBARS (MDA [µmol/L]/protein [µg/mL])</td>
<td>1.13±0.06</td>
<td>0.99±0.08</td>
<td>1.02±0.09</td>
<td>1.17±0.13</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen index</td>
<td>2.98±0.26</td>
<td>2.61±0.25</td>
<td>2.92±0.13</td>
<td>2.45±0.13</td>
<td>NS</td>
</tr>
</tbody>
</table>

PCNA indicates proliferating cell nuclear antigen; TBARS, thiobarbituric acid reactive substances; NS, no significant difference; MDA, malondialdehyde.

Values are mean±SEM.

Effect of obesity: *P<0.05.
Table 3. Effect of Sex and Juvenile Obesity on Gene Expression Markers of Glucocorticoid Action, Inflammation, Lipid Sensing, Cell Adhesion and Renal Function Apoptosis, Cell Proliferation, and Glucose Metabolism in the Kidneys of Young Adult Sheep

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene</th>
<th>Lean Female (n=7)</th>
<th>Obese Female (n=9)</th>
<th>Lean Male (n=11)</th>
<th>Obese Male (n=9)</th>
<th>Effect of Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticoid action</td>
<td>11βHSD2</td>
<td>0.88±0.19</td>
<td>0.81±0.11</td>
<td>0.76±0.12</td>
<td>1.85±0.25*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>0.86±0.16</td>
<td>1.29±0.23</td>
<td>2.27±0.25</td>
<td>15.46±1.56†</td>
<td>0</td>
</tr>
<tr>
<td>Proinflammation</td>
<td>CCR2</td>
<td>0.78±0.09</td>
<td>1.11±0.17</td>
<td>3.98±0.59</td>
<td>7.42±0.82†</td>
<td>LO</td>
</tr>
<tr>
<td></td>
<td>IFNγ</td>
<td>1.35±0.37</td>
<td>1.27±0.26</td>
<td>15.98±1.87</td>
<td>21.43±7.48</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>2.07±0.37</td>
<td>1.98±0.23</td>
<td>1.07±0.14</td>
<td>1.13±0.14</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>IL-18</td>
<td>1.60±0.44</td>
<td>1.12±0.20</td>
<td>1.02±0.04</td>
<td>1.20±0.24</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>MCP-1</td>
<td>2.53±0.54</td>
<td>1.98±0.23</td>
<td>1.81±0.17</td>
<td>2.77±0.31</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TNFα</td>
<td>0.12±0.03</td>
<td>0.07±0.01</td>
<td>0.12±0.02</td>
<td>0.14±0.01</td>
<td>0</td>
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<tr>
<td>Anti-inflammation</td>
<td>IL-10</td>
<td>0.85±0.25</td>
<td>0.86±0.02</td>
<td>2.71±0.06</td>
<td>3.07±0.04</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>INOS</td>
<td>0.85±0.02</td>
<td>0.09±0.02</td>
<td>0.16±0.03</td>
<td>0.23±0.03</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PPARγ</td>
<td>0.16±0.04</td>
<td>0.16±0.20</td>
<td>0.29±0.02</td>
<td>0.37±0.08</td>
<td>0</td>
</tr>
<tr>
<td>Lipid sensing receptors</td>
<td>CD14</td>
<td>3.98±1.12</td>
<td>5.21±1.09</td>
<td>11.06±2.45</td>
<td>26.96±7.81†</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TLR4</td>
<td>4.41±0.64</td>
<td>5.34±0.91</td>
<td>17.24±2.27</td>
<td>33.11±5.89*</td>
<td>0</td>
</tr>
<tr>
<td>Cell adhesion molecules</td>
<td>ICAM-1</td>
<td>1.69±0.60</td>
<td>1.23±0.15</td>
<td>2.56±0.34</td>
<td>4.20±0.72</td>
<td>0</td>
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<tr>
<td></td>
<td>VCAM-1</td>
<td>0.10±0.04</td>
<td>0.11±0.02</td>
<td>0.20±0.02</td>
<td>0.35±0.05*</td>
<td>0</td>
</tr>
<tr>
<td>Renal function</td>
<td>EPOR</td>
<td>0.31±0.09</td>
<td>0.43±0.08</td>
<td>0.54±0.10</td>
<td>1.09±0.13*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Renin</td>
<td>0.39±0.19</td>
<td>0.26±0.03</td>
<td>0.29±0.05</td>
<td>0.40±0.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

11βHSD2 indicates 11β-hydroxysteroid dehydrogenase type 2; GR, glucocorticoid receptor (type 2); CCR2, C-C motif receptor 2; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; INOS, inducible nitric oxide synthase; PPARγ, peroxisome proliferator activated receptor γ; CD14, cluster of differentiation 14; TLR4, toll-like receptor 4; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; EPOR, erythropoietin receptor; L, effect of sex between lean groups where \( P<0.005 \); O effect of sex between obese groups where \( P<0.005 \); NS, no significant difference. Values are mean±SEM.

One prominent response to obesity in males was an increase in genes related to glucocorticoid action that are likely to be accompanied by increased local bioactivity of cortisol, together with enhanced lipid sensing, as indicated by raised cluster of differentiation 14 and toll-like receptor 4 gene expression. It is intriguing that these responses were only seen in males, being absent in females. Males did not, however, exhibit any change in plasma cortisol in response to obesity, whereas in females plasma cortisol was doubled, corroborating the differential sensitivity of the hypothalamic-pituitary-adrenal axis between sexes. Furthermore, a higher rate of apoptosis in the kidneys of lean males is in accordance with similar findings in the human heart. One possible explanation for the sex-specific outcomes is that such a widespread increase in mRNA transcription is mediated in part by epigenetic differences, which can differ greatly between sexes. For example, methylation patterns of 2 inflammatory genes from leukocytes show differential patterns between lean and obese adolescents and are proposed to impact on immune function and subsequent pathogenesis of obesity. Alternatively, persistent downregulation of renal inflammatory gene expression may occur in females because of epigenetic effects causing gene silencing. Clearly, further investigation into complex epigenetic alterations, together with gene expression, could elucidate the mechanisms involved in the dimorphic stress/inflammatory gene expression profiles in response to obesity exposure between sexes.

Monocyte chemoattractant protein-1, acting through its receptor CCR2, can contribute to maintaining optimal kidney health and function, and renal gene expression for CCR2 is observed to decline with age in the sheep, a process that could be delayed in OMs. Despite the structural and molecular changes within the kidneys of OMs, we found no evidence of impaired function with respect to oxidative stress or cardiovascular control under basal conditions. It should be noted that our study was not designed to study the roll of blood pressure differences between sexes. However, renal gene expression of renin showed no differences, reflecting the absence of hemodynamic adaptations and indicating that the renin-angiotensin system was not overstimulated with juvenile obesity. Whether this would persist in the longer term remains to be determined, because prolonged obesity would be expected to increase renin-angiotensin system activity and the associated glomerulopathy. It is also possible that other factors including nitric oxide and prostaglandins may be affected by sex and may further influence kidney function with renal expression of inducible nitric oxide synthase, decreasing with obesity in females but not males.

**Perspectives**

We have shown that the renal pathways controlling metabolic and oxidative stress together with inflammation are unchanged in females after early onset obesity. In contrast to obesity, whereas in females plasma cortisol was doubled, however, exhibit any change in plasma cortisol in response to obesity, whereas in females plasma cortisol was doubled, corroborating the differential sensitivity of the hypothalamic-pituitary-adrenal axis between sexes. Furthermore, a higher rate of apoptosis in the kidneys of lean males is in accordance with similar findings in the human heart. One possible explanation for the sex-specific outcomes is that such a widespread increase in mRNA transcription is mediated in part by epigenetic differences, which can differ greatly between sexes. For example, methylation patterns of 2 inflammatory genes from leukocytes show differential patterns between lean and obese adolescents and are proposed to impact on immune function and subsequent pathogenesis of obesity. Alternatively, persistent downregulation of renal inflammatory gene expression may occur in females because of epigenetic effects causing gene silencing. Clearly, further investigation into complex epigenetic alterations, together with gene expression, could elucidate the mechanisms involved in the dimorphic stress/inflammatory gene expression profiles in response to obesity exposure between sexes.

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age-matched males displayed augmented renal malfunctioning with an indication of renal hyperfiltration, glomerular hypertrophy, and hypercellularity, conditions that eventually lead to eventual renal damage and failure. In comparison with previous observations where the sex specificity was not examined or in more advanced cases of severe obesity, our results emphasize some of the primary adaptations with the early onset of obesity. This study indicates the importance of complementary studies designed to enhance our understanding of the sex-specific mechanisms involved. Ultimately these could have significant implications for the future of sex-based therapeutic strategies aimed at preventing adverse consequences of obesity in early life.

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We would like to thank D.S. Gardner for his work on the animal husbandry and dissection of tissues used in this article.

Disclosures

None.

References


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**Novelty and Significance**

**What Is New?**

- Females are protected from the adverse effects of juvenile obesity on the kidney.
- Despite males showing the same increase in fat mass around the kidneys as females, only males show enlarged glomerular area and nucleated cell number.

**What Is Relevant?**

- The signs of renal hypercellularity and reduced apoptosis in the kidneys of obese males are compatible with the development of either membranoproliferative glomerulonephritis or focal segmental glomerulosclerosis.
- These outcomes have previously been considered to show different pathogeneses.

**Summary**

Our findings emphasize the critical importance of sex specificity in the progression of kidney disease with juvenile obesity.
The Influence of Sex on Early Stage Markers of Kidney Dysfunction in Response to Juvenile Obesity
Ian D. Bloor, Sylvain P. Sebert, Ravi P. Mahajan and Michael E. Symonds

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The influence of gender on early stage markers of kidney dysfunction in response to juvenile obesity

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Running title: Gender and kidney dysfunction after obesity

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Detailed methodology of the ovine specific primers used for gene expressions analysis

Ovine specific forward (F) and reverse (R) primers used are detailed in Table 1.

References


**Supplement Table S1:** Gene primer sequences used for mRNA quantification.

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Oligonucleotide sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forward</strong></td>
<td><strong>Reverse</strong></td>
</tr>
<tr>
<td>11β hydroxysteroid dehydrogenase 2 (11βHSD2)¹</td>
<td>AGCAGGAGACATGCCGTTC</td>
</tr>
<tr>
<td>Glucocorticoid receptor (GR)</td>
<td>ACTGCCCCAAGTGAAAACAGA</td>
</tr>
<tr>
<td>Chemokine C-C motif receptor 2 (CCR2)²</td>
<td>TGTCCATGCTGTTGTGGCTT</td>
</tr>
<tr>
<td>Interferon-γ (IFN-γ)</td>
<td>TGCAGATCCACGCAGAAAGCCA</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)³</td>
<td>ACCACTCAGCCACACAC</td>
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<tr>
<td>Interleukin-18 (IL-18)</td>
<td>AACGACCAAGTTCTCTCTCATT</td>
</tr>
<tr>
<td>Monocyte chemoattractant protein-1 (MCP-1)⁴</td>
<td>GCTGTGATTTTCAAGACCATTCC</td>
</tr>
<tr>
<td>Tumour necrosis factor α (TNFα)</td>
<td>CCGAGTCTGGGCAGGGTCTAC</td>
</tr>
<tr>
<td>Interleukin-10 (IL-10)</td>
<td>GTGCTCTGGTGGCTGGTCTTC</td>
</tr>
<tr>
<td>Inducible nitric oxide synthase</td>
<td>TTGAGCAGGTGGTGAGGTC</td>
</tr>
<tr>
<td>Gene/Receptor</td>
<td>Promoter 1</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Peroxisome proliferator activated receptor-γ (PPAR-γ)</td>
<td>CGCATGCCACAGGCCGAGAA</td>
</tr>
<tr>
<td>Cluster of differentiation -14 (CD-14)</td>
<td>TCAAGGCTCTGCGCTTCGG</td>
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<tr>
<td>Toll-like receptor 4 (TLR4)³</td>
<td>TGCTGGCTGCAAAAAAGTCTG</td>
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<tr>
<td>Intracellular adhesion molecule-1 (ICAM-1)</td>
<td>ATGGACTACTGTGACCGTGGA</td>
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<tr>
<td>Vascular cell adhesion molecule-1 (VCAM-1)</td>
<td>TGCTGCTCAGGTTGCGACTC</td>
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<tr>
<td>Erythropoietin receptor (EPOR)</td>
<td>CCAGGGAGGCCGAAAATG</td>
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<tr>
<td>Renin</td>
<td>GCAGACACCGCGCCTTCAG</td>
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