Pre- and post-glomerular basal diameter changes and reactivity to angiotensin II in obese rats

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Abstract

Obesity in humans is associated with proteinuria and an increased glomerular filtration, possibly related to an increase in glomerular capillary pressure. We investigated in obese and lean Zucker rats whether this might be related to alterations in the diameter of pre- and postglomerular microvessels and their reactivity to the resistance regulator angiotensin II (AngII), using the hydrenephrotic kidney model.

The obese rats exhibited a hyperinsulinemic but still euglycemic state and tended to have higher blood pressures. Urinary protein concentration and fluid intake were both increased three-fold. Basal diameters of distal interlobular arteries (ILA) and afferent arterioles (AA) were larger in the obese rat than in the lean rat (ILA: 25.7±0.3 vs. 23.0±0.4 µm and AA: 18.8±0.3 vs. 16.7±0.5 µm, respectively; P≤0.01), while diameters of efferent arterioles (EA) were smaller in obese animals (14.2±1.1 vs. 18.2±1.2 µm; P≤0.05).

AngII induced a concentration-dependent constriction in ILA, AA and EA with an augmented response in the obese as compared to the lean rats. Thus, at higher concentrations, AngII abolished the diameter difference between obese and lean animals in preglomerular microvessels, while exaggerating that in postglomerular arterioles.

Our data indicate for the first time that in obese rats, a vasodilated state in small preglomerular microvessels and a vasoconstricted state in the postglomerular arteriole exists. Although AngII cancelled the former, the latter remained. Therefore, these data reveal periglomerular vascular changes that in obesity may play a role in the possibly increased glomerular capillary pressure, which likely contributes to the increased glomerular filtration rate and proteinuria.
Introduction

The prevalence of obesity is increasing worldwide, not only in adults but in children as well. The last 25 years it has risen by more than 75% (8). Excess body weight is associated with renal changes, among others, by an increase in glomerular filtration rate (GFR), increased renal plasma flow (RPF) and an increased urinary albumin excretion (7, 11). The latter is considered a first sign of endothelial and/or podocyte dysfunction in the glomerulus (4). In addition, glomerular hyperfiltration is a well-known pathophysiological link to glomerulosclerosis (11). It has been suggested that the pathogenesis of hyperfiltration in obese, non-diabetic subjects differs from that in patients with diabetes mellitus, and that in the former it is mainly due to an increased transcapillary pressure difference in the glomeruli (7). The latter is regulated by the diameters of the pre- and postglomerular arterioles. However, so far no direct observations have been reported regarding these microvessels and possible changes in their reactivity in obese, non-diabetic animals.

A well-known prediabetic model of obesity is the genetically obese Zucker rat (16). These rats develop obesity because of leptin resistance due to a missense mutation in its hypothalamic receptor (24, 33). Lean control rats are genetically identical except for this mutation. The obese rat displays many of the health problems seen in human obesity such as hyperlipidemia, hypercholesterolaemia and hyperinsulinaemia (5, 14, 18, 26, 31). Moreover, with increasing age the obese Zucker rat develops proteinuria and focal segmental glomerulosclerosis, ultimately leading to kidney failure (14, 18).

In the present study, we employed these obese and lean animals to directly visualize that part of the renal microvasculature predominantly important for regulation of glomerular perfusion and capillary pressure, i.e. distal interlobular arteries (ILA), afferent (AA) and efferent arterioles (EA). This was done ex vivo with the isolated perfused hydronephrotic kidney model. We determined basal diameters at 80 mm Hg as well as the reactivity of these microvessels to angiotensin II (AngII), an important regulator of preglomerular and postglomerular vascular resistance (34).
Materials and/or Methods

Male obese Zucker (fa/fa) and lean Zucker rats (+/+ or +/fa) were obtained from Harlan (Horst, The Netherlands). They are a crossbreed between Merck Stock M and Sherman rats (13M). The animals were housed and handled according to the guidelines of the Institutional Animal Care and Use Committee. Between the age of 5 to 10 weeks, weight, food and water intake were determined on a weekly basis. Water and rat chow (AM2, Hope farms, Woerden, The Netherlands) were both available ad libitum.

Hydronephrotic kidney model and microvascular diameters

To induce unilateral hydronephrosis, the rats were anesthetized at 4 weeks of age with isoflurane (3%), O₂ (0.76 L/min) en N₂O (1.2 L/min). Subsequently, their left ureter was exposed through a small mid-abdominal incision and tied off with a suture. For pain relief following surgery, the animals were injected subcutaneously with Temgesic (0.03 mg/kg; Buprenorfine base; Schering-Plough B.V. Amstelveen, The Netherlands). Six to 8 weeks later, tubular atrophy had advanced to a stage that allowed direct visualization of the renal microvasculature (32).

Isolation and in vitro perfusion of hydronephrotic kidneys have been described in detail elsewhere (36). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.; Sanofi Sante, Maassluis, The Netherlands) and ketamine (25 mg/kg i.m.; Kombivet, Etten-Leur, The Netherlands). First, blood was taken from the tail-tip to assess their non-fasting glucose levels with a haemo-glukotest (Accutrend Alpha, Boehringer Mannheim, Germany) and insulin levels with a radio-immuno-assay (Diasorin, Saluggia, Italy). Second, mean arterial blood pressure was determined with a catheter (PE50) placed in the right carotid artery. Third, the hydronephrotic kidney was exposed through a wide abdominal incision. Its renal artery was cannulated via the abdominal aorta and perfusion was started in vivo. In the obese rats cannulation was difficult because of the presence of a large amount of abdominal and perivascular fat. Perfusion medium consisted of Dulbecco’s Modified Eagle’s Medium Base (DMEM) supplemented with (in mmol/L) 23.8 bicarbonate, 5.5 D-glucose, 1 sodium pyruvate and 5.6 HEPES (Sigma). The medium was equilibrated with 95% air - 5% CO₂ at 37°C. Under continuous single-pass perfusion, the hydronephrotic kidney was excised and moved to the stage of an inverted microscope (Axiovert 100; Zeiss, Weesp, The Netherlands) that was equipped with a thin glass viewing port on the bottom surface. Renal perfusion pressure was monitored at the level of the renal artery and kept constant at 80 mm Hg unless otherwise stated. After
the kidney had been excised a urine sample was taken from the bladder to
determine urinary protein concentration. Total urinary protein concentration was
determined with an U/CSF protein assay (Roche diagnostics, Mannheim,
Germany). The, length of the hydronephrotic kidney was measured \textit{in situ}, while
its wet weight was determined after the experimental protocol had been
completed.

To visualize the microvessels, a small region of the transparent renal
cortex was transilluminated and immobilized with a light rod. Images of distal
ILAs, AAs and EAs were generated by means of a CCD camera (7020/20; Philips,
Eindhoven, The Netherlands) and recorded for off-line analysis using a VHS video
recorder (RTV 825; Blaupunkt, Hildesheim, Germany). The, images were digitized
using a computer equipped with an acquisition board (model IVG-128; Datacube,
Peabody, MA). Vessel diameters were determined with an automated custom
designed program (17). In vessel segments of \( \sim 5 \mu m \) length the distance between
vessel walls was scanned at \( \sim 1 \text{ Hz intervals for a period of } 30 \text{s. Measurements}
were obtained at each pixel and averaged. Each final value was derived from \( \sim 30 \)
of these measurements. ILAs were measured just before the bifurcation of AAs,
AAs just after branching from ILAs and EAs within 50 \( \mu m \) of the point where they
emerged from the glomeruli. Finally, before the experimental protocol was
started (see below) we looked for the presence of fat patches.

\textbf{Experimental protocol}

Kidneys were at first allowed to equilibrate for at least 60 minutes. Then
diameters were measured of as many ILAs, AAs and EAs visible in our field of
view. Subsequently, diameters of some of the preglomerular microvessels were
also determined at higher perfusion pressures. To this end two pressure-runs
were performed with step-wise increases (20 mm Hg) from 80 to 180 mm Hg. In
each run one of the best visible examples of both an ILA and AA were recorded.
Thereafter, renal perfusion pressure was returned to 80 mm Hg and kept there.

After a second equilibration period of 30 minutes, increasing
concentrations of AngII (Sigma; 0.01 nmol/L–1 nmol/L) were administered. It was
added directly to the perfusion medium in a cumulative way. Vessel diameter
measurements were started ten minutes after adding each concentration. For
the AngII concentration-response curves the same best visible pairs of ILA and AA
were used as previously mentioned.

\textbf{Statistical analysis}

All data are expressed as the mean±standard error of the mean (SEM).
The \( n \)-value refers to the number of kidneys examined. Since multiple vessels
were studied in a kidney, the mean values obtained for each vessel type per animal were used. A Students $t$ test with Bonferroni correction was performed on the raw data to analyze differences. Curve fitting was performed on the AngII concentration-response curves by utilizing the non-linear regression, sigmoidal dose-response option using hand-made restraints. All analyses were performed using GraphPad Prism version 4.02 for Windows, (GraphPad Software, San Diego California, USA). $P<0.05$ was considered statistically significant, when Bonferroni correction was applied $p$ values$<0.05/\sqrt{n}$ was considered significant.
Results

Figure 5.1 shows that the obese rats gained weight more rapidly than their lean counterparts, which was paralleled by an increasing difference in the consumption of rat chow. Interestingly, water consumption also increased progressively in the obese animals while it did not or hardly so in the leans.

Table 5.1. General characteristics of obese and lean Zucker rats.

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Lean</th>
<th>p*</th>
</tr>
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<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fasting glucose (mmol/L)</td>
<td>8.4 ± 0.8 (9)+</td>
<td>8.4 ± 0.4 (7)</td>
<td>0.93</td>
</tr>
<tr>
<td>Plasma insulin (mU/ml)</td>
<td>409.0 ± 65.5 (8)</td>
<td>77.5 ± 14.0 (8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein concentration (g/L)</td>
<td>4.3 ± 0.4 (5)</td>
<td>1.5 ± 0.1 (4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>128.5 ± 9.7 (7)</td>
<td>111.1 ± 8.8 (7)</td>
<td>0.21</td>
</tr>
<tr>
<td>Hydronephrotic kidney:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>3.6 ± 0.2 (5)</td>
<td>3.6 ± 0.2 (6)</td>
<td>0.75</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>1.0 ± 0.2 (5)</td>
<td>0.5 ± 0.1 (7)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*, p-value obese vs. lean Zucker rats. +, within parentheses number of animals.

Table 5.1 shows that the obese animals were not hyperglycemic but that their blood insulin levels had increased more than five-fold, indicating a hyperinsulinemic but still prediabetic state. They tended to have a higher mean arterial pressure, and clearly showed an increased urinary protein concentration.
(± 3-fold; table 5.1). The latter indicates, in combination with the increased water consumption (figure 5.1), the presence of proteinuria. This table also shows that the length of the hydronephrotic kidney in situ did not differ, while its weight was greater in the obese animals. Both parameters were not related to the duration of the hydronephrosis (data not shown). Inside the hydronephrotic kidney of the obese animals fat patches were observed, around glomeruli and microvessels.

**Microvessel diameters and response to AngII**

Figure 5.2 shows that the diameters of all three types of investigated microvessels differed in obese from lean animals in their experimental basal state. In obese animals the preglomerular arterioles had larger diameters compared to their lean counterparts (ILA: 25.7±0.3 vs. 23.0±0.4 µm and AA: 18.8±0.3 vs. 16.7±0.5 µm, respectively; p<0.01), while interestingly, the postglomerular efferent arteriole had smaller diameters in the obese (14.2±1.1 vs. 18.2±1.2 µm; p<0.05). Table 5.2 indicates that the difference in preglomerular diameters between obese and lean rats was also present at higher but still physiological pressures (100–140 mm Hg).
Table 5.2. Diameters (µm) of preglomerular microvessels at various perfusion pressures in obese (n=3) and lean (n=5) Zucker rats.

<table>
<thead>
<tr>
<th>Perfusion Pressure (mm Hg)</th>
<th>Distal interlobular artery</th>
<th>Afferent arteriole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obese</td>
<td>Lean</td>
</tr>
<tr>
<td>100</td>
<td>28.7 ± 1.0</td>
<td>22.6 ± 1.1</td>
</tr>
<tr>
<td>120</td>
<td>27.3 ± 2.1</td>
<td>22.7 ± 1.3</td>
</tr>
<tr>
<td>140</td>
<td>27.1 ± 1.6</td>
<td>22.8 ± 1.3</td>
</tr>
<tr>
<td>160</td>
<td>22.7 ± 0.5</td>
<td>22.6 ± 1.6</td>
</tr>
<tr>
<td>180</td>
<td>23.1 ± 1.2</td>
<td>22.3 ± 1.5</td>
</tr>
</tbody>
</table>

Figure 5.3 shows that in all three vessel types in both obese and lean animals AngII caused a vasoconstriction. In preglomerular microvessels, i.e. ILAs
and AAs, higher concentrations of AngII abolished the diameter difference between obese and lean animals. In all 3 microvessel types of obese rats, a trend for augmented vasoconstriction to AngII could be observed as indicated by figure 5.4.
Discussion

The present study indicates that in obese rats without hyperglycemia, but with hyperinsulinemia and which showed prominent proteinuria, a vasodilated preglomerular state was paralleled by a postglomerular vasoconstricted state. In addition, the ILA, AA and EA showed a trend for an augmented vasoconstriction to AngII in obese animals. Furthermore, obesity was attended with fat patches throughout the kidney.

The obese rats demonstrated a preglomerular vasodilated state accompanied by a postglomerular vasoconstricted state. Several studies have established the importance of preglomerular microvessels, i.e. ILA and AA, for the regulation of glomerular capillary pressure (35, 40). A decrease in their resistance will facilitate the relay of systemic blood pressure to the glomerulus. Moreover, constriction of the EA will also increase glomerular pressure (11). In our obese, pre-diabetic rats both factors were present, and, hence, might have caused an increase in glomerular capillary pressure. It has been suggested that the latter is the main determinant of the increased glomerular filtration rate (GFR) seen in obese, non-diabetic patient’s (7).

Furthermore, a trend for an augmented response to AngII was observed in obese rats. Higher concentrations of AngII abolished the diameter difference between obese and lean rats in preglomerular microvessels, but in postglomerular efferent arterioles AngII exaggerated the vasoconstricted state in obese animals. Hence, also in the presence of AngII the glomerular pressure might be increased in obese subjects. Several mechanisms could be involved in the increased response to AngII. Firstly, actions of AngII are mediated via the AT1 receptor, which is present on renal arteries and other segments of the nephron (12, 34). In proximal tubules of obese Zucker rats a redistribution of the AT1-receptor was observed with higher numbers in the brush border membranes. This coincided with a greater inhibition by AngII of cAMP accumulation (5). In the renal microcirculation, attenuation of an increase in cAMP would facilitate vasoconstriction (25). Secondly, in obese, non-hypertensive mice it was found that vascular contractility of the thoracic aorta due to AngII was increased and that this effect depended upon endothelin (3). The latter not only is a strong vasoconstrictor but may also contribute to vascular remodeling. Thirdly, in obese Zucker rats, albeit diabetic, AngII-induced vasoconstriction was enhanced in aortic rings, which was abolished when their aorta had been preincubated with the rho kinase inhibitor Y-27632, indicating an augmented vascular Ca\(^{2+}\) sensitization (22).
A prominent finding of the present study is the three-fold increase in both urinary protein concentration and water intake in the obese rats, indicating an even higher urinary protein excretion. This is in line with the findings of albuminuria in obese patients (7) as well as fat Zucker rats (15), and a possible increase in their glomerular capillary pressure (see above). Since obese patients and obese Zucker rats have both high blood levels of leptin (10, 26), this peptide hormone that is synthesized in adipocytes may play a role. Its receptor exists in a long and short isoform, which are both expressed in the kidney (29). The obese Zucker rat has a missense mutation in the hypothalamic leptin receptor, which is the long form (24, 30), and it is thus possible that in the kidney leptin can still act via the short form, among others, as a diuretic (29). Furthermore, chronic leptin infusion for 3 weeks into rats significantly enhances proteinuria (39). Moreover, in our obese animals we observed fat patches throughout the kidney, and it is thus possible that because of an increased number of intrarenal adipocytes local leptin levels were much higher than normal.

The animal model of obesity used in the present study was found to be prediabetic, no change in blood glucose levels was found, but levels of insulin were significantly increased. This finding is in accordance with previous studies in obese Zucker rats (5, 31, 37). There is some evidence that insulin resistance precedes the onset of established hypertension in high-risk patients (23). In our obese, hyperinsulinemic rats blood pressure tended to be higher. Other studies performing continuous blood pressure measurements showed an increase in blood pressure of 14-22 mm Hg in these animals (1, 18). The pressure difference observed in our study was of similar magnitude.

The differences in experimental basal diameter between obese and lean rats might reflect a remodeled renal microvascular bed. Obese Zucker rats are known to be hyperleptinemic (10, 26) and leptin is known to promote remodeling of the wall of carotid arteries (27). Whether leptin can also cause remodeling in the renal microcirculation is at present unknown. In humans, acute leptin infusion induces vasodilatation, which seems independent of nitric oxide (19, 21). Increased levels of leptin could have played a role in the observed vasodilated basal state of preglomerular microvessels in obesity. In addition, AngII is known to facilitate remodeling of microvessels and to be able to promote release of endothelin that also might contribute to vascular remodeling (see above). These latter two hormones will promote a vasoconstricted state, which in our experiments was observed in the EA.

In our obese animals we observed fat patches throughout the kidney. Adipocytes, which represent the main component of fat tissue, contain a fully functional local renin-angiotensin system (RAS) and have been reported to
synthesize angiotensinogen (4, 13, 38). Renin uses angiotensinogen to synthesize angiotensin I that is converted into Angll by angiotensin converting enzyme (ACE) (34). Moreover, in obese mice there was an increased ACE activity present in the kidney (3). Secondly, synthesis of angiotensinogen in the liver is stimulated by insulin (6), in our obese rats insulin levels had increased more than five-fold. In combination with an increase in local ACE-activity and RAS, this might in the kidney of obese animals have lead to increased levels of Angll. Besides its well-known acute contractile effect, Angll can cause a long-term increase in tone (20, 28). We hypothesize that this might have played a role in the constricted basal state of the postglomerular EA observed in obese animals.

The question remains why these, and possibly other, processes related to obesity (see above) have led in preglomerular arterioles to a vasodilated basal state and in postglomerular efferent arterioles to a vasoconstricted basal state. In this respect it may be mentioned that EAs differ in various ways from AAs and distal ILAs. A first example is that in preglomerular arterioles myogenic tone plays an important role in the regulation of glomerular perfusion, while postglomerular efferent arterioles are not sensitive to changes in pressure (2). A second example is the presence of both L-type, which are high voltage-gated, and T-type, which are low voltage-gated, calcium-channels in preglomerular arterioles while in the postglomerular efferent arterioles only T-type calcium-channels are present (9). Which molecular difference between EAs and preglomerular arterioles are actually involved in their dissimilar response to obesity requires further investigation.

In conclusion, our data indicate in obese rats without hyperglycemia, but with hyperinsulinemia, a vasodilated state in small preglomerular microvessels and vasoconstriction in the postglomerular efferent arteriole. Although Angll cancelled the former, the latter remained. Therefore, these data shed light on the periglomerular vascular changes that in obesity may play a role in the possibly increased glomerular capillary pressure, and which likely contribute to the increased glomerular filtration rate and proteinuria.

Acknowledgements

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