ALTERED RESPONSIVENESS OF THE KIDNEY TO
ACTIVATION OF THE RENAL NERVES IN FAT-FED RABBITS

Sylvia Michaels¹, Gabriela A. Eppel¹, Sandra L. Burke², Geoffrey A. Head², James
Armitage²,³, Joan F. Carroll⁴, Simon C. Malpas⁵ & Roger G. Evans¹

Departments of ¹Physiology and ²Anatomy and Developmental Biology, Monash
University, Melbourne, Australia.
³Baker IDI Heart and Diabetes Institute, Melbourne, Australia.
⁴University of North Texas Health Science Center, Fort Worth, TX, USA.
⁵Department of Physiology, University of Auckland, New Zealand.

Running title: Fat intake and renal response to nerve activation

Author for correspondence:
Dr Roger Evans
Department of Physiology
PO Box 13F, Monash University, Victoria 3800, Australia
Tel: 61 3 9905 1466
Fax: 61 3 9905 2566
Email: Roger.Evans@med.monash.edu.au
Abstract

We tested whether mild adiposity alters responsiveness of the kidney to activation of the renal sympathetic nerves. After rabbits were fed a high-fat or control diet for 9 weeks, responses to reflex activation of renal sympathetic nerve activity (RSNA) with hypoxia and electrical stimulation of the renal nerves (RNS) were examined under pentobarbital anesthesia. Fat pad mass and body weight were respectively 74% and 6% greater in fat-fed rabbits than controls. RNS produced frequency-dependent reductions in renal blood flow, cortical and medullary perfusion, glomerular filtration rate, urine flow and sodium excretion and increased renal plasma renin activity (PRA) overflow. Responses of sodium excretion and medullary perfusion were significantly enhanced by fat-feeding. For example, 1 Hz RNS reduced sodium excretion by 79 ± 4% in fat-fed rabbits and 46 ± 13% in controls. Two Hz RNS reduced medullary perfusion by 38 ± 11% in fat-fed rabbits and 9 ± 4% in controls. Hypoxia doubled RSNA, increased renal PRA overflow and medullary perfusion, and reduced urine flow and sodium excretion, without significantly altering mean arterial pressure (MAP) or cortical perfusion. These effects were indistinguishable in fat-fed and control rabbits. Neither MAP nor PRA were significantly greater in conscious fat-fed than control rabbits. These observations suggest that mild excess adiposity can augment the antinatriuretic response to renal nerve activation by RNS, possibly through altered neural control of medullary perfusion. Thus, sodium retention in obesity might be driven not only by increased RSNA, but also by increased responsiveness of the kidney to RSNA.

Abstract word-count: 249

Keywords: Hypertension, hypoxia, kidney circulation, obesity, kidney medulla, renal sympathetic nerves, renin-angiotensin system, sodium excretion.
Introduction

The sympathetic nervous system is often activated in overweight and obese individuals (16). For example, muscle sympathetic nerve activity (46) and renal norepinephrine spillover (50) are directly correlated with body mass index and are especially elevated in human visceral obesity (2, 50). Moreover, muscle sympathetic nerve activity is increased by even modest weight gain in non-obese humans (21), while both muscle sympathetic nerve activity and whole-body norepinephrine spillover are reduced by modest weight loss in subjects with the metabolic syndrome (48). The precise mechanisms that link excess adiposity and sympathetic activation remain unknown, although hyperleptinemia clearly makes an important contribution (14, 41).

Arterial pressure is also positively associated with adiposity (24). Indeed, it has been argued that excess adiposity is the single most common cause of essential hypertension (26, 55). The kidney appears to play a critical role in the development of obesity-induced hypertension. This proposition is based on the observations that the pressure-natriuresis relationship is shifted to higher levels of arterial pressure in numerous experimental models of obesity (20, 22, 49) and obesity-induced hypertension is associated with renal sodium retention (25). The renal sympathetic nerves appear to provide a major mechanistic link in obesity-induced sodium retention and hypertension, based chiefly on the observation that renal denervation blunts the development of sodium retention and hypertension in dogs fed a high-fat diet (27).

The evidence discussed above has led to the proposition that the pre-disposition of obese subjects to development of hypertension is driven at least partly by altered neural control of the kidney. Increased RSNA, driven through activation of specific central nervous system pathways, obviously makes a major contribution to altered renal function in obesity. But to understand how integrated neural control of the kidney is altered in states of excess adiposity, we also must know whether the responsiveness of the kidney to any given level of RSNA is
altered. Such changes are plausible, given the observations of altered adrenoceptor density (7) and renal responsiveness to angiotensin II (47) in rat models of obesity and altered renal structure in obese mice (10) dogs (1) and rabbits (13). We have recently developed a method that allows assessment of the responsiveness of various renal parameters (blood flow, sodium excretion and renal renin release) to renal sympathetic activation (4, 17). Our approach allows us to examine the responses of these parameters to reflex activation of the renal nerves induced by hypoxemia and electrical stimulation of the renal nerves. In the current study we used this approach to test the hypothesis that modest increases in adiposity can enhance responsiveness of the kidney to a given level of RSNA, which could act synergistically with increased RSNA to promote salt and water retention, and so the development of hypertension.

Methods

Animals and diets

Male New Zealand White rabbits (n = 17), from a specific pathogen free colony bred at Monash University Animal Services, were studied according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition). Rabbits entered the study at 18 weeks of age. After a 2-week acclimatization period, during which they consumed the control diet, rabbits were randomly assigned to receive either the high fat diet (n = 8) or control diet (n = 9) for the next 9 weeks. The control and high fat diets contained, respectively, 4.2 and 13.7% total fat, 18.2 and 17.2% protein, 14.4 and 12.7% crude fiber, and 11 and 14 MJ/kg digestible energy. Sodium content of both diets was 0.2%. The high fat diet was available ad libitum, but the control diet was restricted to a maximum of 90 g per day. Average daily food intake was similar for the control (86 ± 3 g) and high fat diet (90 ± 4 g).
Measurements in conscious rabbits

Prior to, and 9 weeks after, commencement of the dietary intervention, mean arterial pressure (MAP) and heart rate (HR) were measured via an ear artery catheter in conscious rabbits for a 60 min period. At the end of this period a 1 ml sample of arterial blood was collected for measurement of plasma renin activity (PRA).

Terminal experiment under anesthesia

The experiment comprised two parts. In part A, responses to reflexive increases in RSNA, induced by hypoxia, were tested. The renal nerves were then sectioned, and in part B we examined responses to electrical stimulation of the renal nerves (RNS).

Preparative Surgery: The central arteries and marginal veins of both ears were catheterized under local analgesia (1% lidocaine; Xylocaine; AstraZeneca; North Ryde, NSW, Australia) (8). Anesthesia was induced and maintained with sodium pentobarbital (90-150 mg plus 30-50 mg/h i.v.; Sigma Chemical Company, St Louis, MO). Rabbits were artificially ventilated throughout the surgery and experiment and extracellular fluid volume was maintained by intravenous infusion (0.18 ml kg\(^{-1}\) min\(^{-1}\)) of a 4:1 mixture of compound sodium lactate and polygeline/electrolyte solution. A heated table and infrared heating lamp were used to maintain body temperature between 36.5 and 37.5 °C.

The left kidney was exposed via a flank incision and placed in a cup secured to the operating table. Catheters were placed in the left renal vein and left ureter (9). The renal nerves were then isolated and placed across a recording electrode. Laser Doppler flow probes were placed in the renal inner medulla (9 mm below the cortical surface; 26 gauge, DP4s, Moor Instruments Ltd., Millwey, Devon, UK) (23) and on the cortical surface (DP2b, Moor Instruments) to provide measurements of medullary and cortical laser Doppler flux (MLDF and CLDF respectively) as indices of regional kidney perfusion. An intravenous infusion of \[^{14}\text{C}\]-inulin (bolus dose of 10 µCi plus 45 nCi kg\(^{-1}\) min\(^{-1}\), Perkin Elmer Life Sciences,
Boston, MA, USA) then commenced and continued for the entire experiment. During the 60 min equilibration period, before the experiment proper commenced, ventilation parameters were adjusted so that arterial blood $PO_2$ was 90 to 110 mmHg.

**Part A - responses to hypoxia:** This protocol comprised three 20 min periods, during which the rabbits were ventilated with room air, 10% oxygen, and room air, respectively. Thus, the period of hypoxia was bracketed by two control periods. Urine produced by the left kidney was collected during the final 15 min of each period. At the mid-point of each urine collection period, samples of arterial blood (1 ml each) were taken for measurement of (a) $[^{14}\text{C}]$-inulin and sodium concentrations and (b) PRA. A 0.3 ml sample of arterial blood was also taken for determination of arterial blood gas status (ABL510; Radiometer, Copenhagen, Denmark). Simultaneously, a 1 ml blood sample was taken from the renal vein for measurement of PRA. Blood was replaced with that from a donor rabbit, mixed with re-suspended erythrocytes from previous blood samples.

In preparation for part B, the renal nerves were sectioned cranially, a transit-time ultrasound flow probe (2SB, Transonic Systems Inc., Ithaca) was placed around the renal artery for measurement of renal blood flow (RBF), and the RSNA recording electrode was replaced with a stimulating electrode. The renal nerves were stimulated at 4 Hz for brief (30 s) periods at a range of voltages (1-10 V). The lowest voltage giving a maximal reduction in RBF was used in the subsequent experiment.

**Part B – responses to RNS:** This protocol initially comprised four 20 min periods; a control period followed by periods of RNS at progressively greater frequencies of 0.5, 1, and 2 Hz respectively. This was followed by two 3 min periods of RNS at 4 and 8 Hz respectively. Thirty min was allowed for recovery before a final 20 min control period commenced. Thus, as for part A, the periods of activation of the renal nerves were bracketed by control periods.
Urine and blood samples were taken during the final 15 min of each of the five 20 min experimental periods, as for part A.

Post-mortem measurements

At the completion of protocol B rabbits were killed with an overdose of sodium pentobarbital. The heart, left kidney, and major fat pads (mesenteric, perirenal, pericardiac and gonadal) were removed and weighed.

Hemodynamic variables

Signals were processed and digitized (15) to provide 2s averages of MAP (mmHg), HR (determined from the arterial pressure pulse; beats/min), RBF (ml/min), CLDF (perfusion units), and MLDF (perfusion units). Levels of MLDF and CLDF measured after the rabbit was killed were subtracted from the values obtained during the experiment, before data analysis was performed. Postganglionic RSNA was processed as previously described (17) and is presented in μV.

Renal PRA overflow was calculated as the product of renal plasma flow (ml/min) and the arteriovenous difference in PRA (ng/ml) and is given in units of ng/min. Thus, in order to calculate renal PRA overflow, we required an estimate of RBF. RBF was not measured directly during part A of the experiment, to avoid the possibility of damaging the renal nerves by placement of a perivascular flowprobe. Instead, it was estimated by calibrating CLDF to RBF measured during part B of the experiment, as we have described previously (17). The validity of this approach is evidenced by the strong correlation between RBF and CLDF ($r^2 = 0.93; P < 0.001$, see data supplement).

Analysis of blood and urine samples

Hematocrit was determined by the capillary tube method. Blood for PRA determination was collected (17) and assayed (40) as previously described. Urine volume was measured
gravimetrically, and aliquots were stored at -20º C for subsequent analysis. [14C]-inulin and sodium concentrations in plasma and urine was determined as previously described (17). Glomerular filtration rate (GFR) was determined as the clearance of [14C]-inulin.

Statistical Methods

Data are expressed as mean ± SEM. Hemodynamic variables and RSNA are presented as the average over the 15 min renal clearance periods, unless otherwise stated. Arterial blood PO₂, NE kinetics and PRA overflow data were derived from the blood samples collected at the mid-point of the 15 min clearance periods. Analysis of variance was employed for hypothesis testing. PRA values were log₁₀ transformed to reduce heteroskedacity. The between-subjects factors were rabbit and diet (control or high fat). The within-subjects factors were the various phases of the experiment (control versus recovery from hypoxia or RNS, part A versus part B of the experiment) and the experimental manipulations (hypoxia and frequency of RNS). We tested whether baseline levels of the measured variables differed between the two groups of rabbits (P_Diet) and whether they were altered by the various experimental manipulations (P_Time, P_Part, P_Hypoxia, P_Frequency). We also tested whether responses to these within-subjects factors differed according to diet (P_Diet, P_Diet*Frequency). When repeated measurements were made in the same rabbits, P values were conservatively adjusted to account for compound asymmetry using the Greenhouse-Geisser correction (31). Two sided $P \leq 0.05$ was considered statistically significant.

Results

Body weight, organ weight and fat pad weight

Over the 9 weeks of the dietary intervention, body weight increased significantly more in rabbits on the high fat diet (from 2.41 ± 0.06 to 2.83 ± 0.05 kg; 18.3 ± 1.6%) than in the control group (from 2.35 ± 0.07 to 2.66 ± 0.06 kg; 13.5 ± 1.5%) (P_Diet*Time = 0.05). Despite
the relatively small differences in weight gain between the groups, there were major differences in the weight of the major fat pads. Total fat mass was 74% greater in rabbits subjected to the high fat diet than control rabbits, due to marked differences in the weight of perirenal (56%), mesenteric (93%), gonadal (86%) and pericardial (79%) fat pads (Table 1). Heart weight and left kidney weight were indistinguishable in the two groups of rabbits ($P_{Diet} \geq 0.4$).

**MAP, HR, hematocrit and PRA in conscious rabbits**

Over the course of the 9 week dietary intervention, MAP averaged over all rabbits increased by 11 ± 3 mmHg and PRA increased slightly. However, the profiles of change in MAP and HR were indistinguishable in the two groups of rabbits. HR and hematocrit changed little over time and were similar in the two groups of rabbits (Table 2).

**Baseline variables in anesthetized rabbits**

In part A of the experiment, most variables returned to their initial control level after hypoxia, except CLDF (5 ± 2% less across both groups) and sodium excretion (41 ± 16% greater). Hematocrit also fell from 32.3 ± 0.4 to 30.5 ± 0.4%. In part B of the experiment, all variables returned to their initial control level after RNS except for GFR (33 ± 16% greater). These baseline changes were indistinguishable between the two groups of rabbits.

Most baseline variables were indistinguishable between control and fat-fed rabbits except for hematocrit which was slightly less in fat-fed rabbits (29.9 ± 0.4% averaged across parts A and B) than in control rabbits (32.0 ± 0.4%) and MLDF which was 42% greater in fat-fed rabbits than in control rabbits. Most variables remained relatively stable between parts A and B of the experiment, except for hematocrit (fell from 30.7 ± 0.4% to 29.3 ± 0.6%), CLDF (21 ± 3% less), urine flow (68 ± 25 μL/min greater) and PRA overflow (46.5 ± 15.8 ng/min less) (Table 3).
Responses to Hypoxia

Baseline arterial PO$_2$ was similar in the two groups of rabbits, averaging 103 ± 3 mmHg. Ventilation with 10% O$_2$ reduced arterial PO$_2$ similarly in both groups, to an average of 28 ± 1 mmHg. Arterial PO$_2$ returned to 96 ± 4 mmHg upon restoration of ventilation with room air. MAP was not significantly altered by hypoxia (Fig. 1), but HR increased similarly in the two groups of rabbits, by an average of 30 ± 4 beats/min. RSNA increased similarly in control (97 ± 29% above baseline) and fat fed (104 ± 17%) rabbits. CLDF was little affected by hypoxia (-1 ± 3% change) while MLDF increased (+16 ± 4% change). Responses of CLDF and MLDF to hypoxia were indistinguishable in control and fat-fed rabbits (P$_{Diet} \geq 0.41$).

Hypoxia was associated with little change in GFR (-10 ± 10% change) but marked decreases in urine flow (-36 ± 12% change), sodium excretion (-39 ± 9% change) and the fractional excretion of sodium (-27 ± 9% change). These effects were indistinguishable in control and fat-fed rabbits (P$_{Diet}$ ≥ 0.21; Fig. 2). Hypoxia markedly increased arterial PRA (+7.9 ± 1.4 ng/ml), renal venous PRA (+20.4 ± 4.5 ng/ml), the arteriovenous PRA difference (+12.7 ± 3.5 ng/ml) and renal PRA overflow (+333 ± 88 ng/min). These effects were also indistinguishable in control and fat-fed rabbits (P$_{Diet}$ ≥ 0.38; Fig. 3).

Responses to RNS

RNS was accompanied by small but statistically significant increases in MAP (P$_{Freq}$ = 0.03), which were indistinguishable in control and fat-fed rabbits. For example, at 2 Hz RNS, MAP increased by 5 ± 1 mmHg relative to its control level. RNS was also accompanied by frequency-dependent reductions in RBF, CLDF and MLDF (P$_{Freq}$ always < 0.001). The magnitude of reductions in RBF and CLDF were similar, and were indistinguishable in control and fat-fed rabbits (P$_{Diet}$ ≥ 0.40). In contrast, RNS-induced reductions in MLDF were
greater in fat-fed than control rabbits ($P_{\text{Diet}} = 0.02$). For example, 2 Hz RNS reduced MLDF by $38 \pm 11\%$ in fat-fed rabbits but by only $9 \pm 4\%$ in control rabbits (Fig. 1).

RNS was accompanied by marked and frequency-dependent reductions in GFR, urine flow, sodium excretion and fractional sodium excretion ($P_{\text{Freq}}$ always $< 0.001$). RNS-induced reductions in sodium excretion were significantly greater in fat-fed rabbits than in control rabbits ($P_{\text{Diet}} = 0.05$). For example, 1 Hz RNS reduced sodium excretion by $79 \pm 4\%$ in fat-fed rabbits but only by $46 \pm 13\%$ in controls. RNS-induced reductions in urine flow also tended to be greater in fat-fed rabbits ($82 \pm 3\%$ at 1 Hz) than in controls ($51 \pm 14\%$ at 1 Hz), although this apparent effect was not statistically significant ($P_{\text{Diet}} = 0.07$). There was a similar tendency for RNS-induced reductions in fractional sodium excretion to be greater in fat-fed rabbits ($-59 \pm 3\%$ at 1 Hz) than in controls ($-38 \pm 10\%$ at 1 Hz) ($P_{\text{Diet}} = 0.10$; Fig. 2).

RNS induced frequency-dependent increases in arterial PRA, renal venous PRA, arteriovenous PRA difference and renal PRA overflow ($P_{\text{Freq}}$ always $\leq 0.002$). These responses were not significantly different in fat-fed compared to control rabbits ($P_{\text{Diet}} \geq 0.36$). Responses of these variables to 1 Hz RNS were of a similar magnitude to those to hypoxia (Fig. 3).

**Discussion**

After a 9 week dietary intervention, fat-fed rabbits weighed only ~6\% more than controls. But there were marked differences in the weight of major fat pads, so that total fat-pad mass was 74\% greater in fat-fed than control rabbits. Relative to control rabbits, fat-fed rabbits were not hypertensive and did not have significantly elevated arterial PRA when conscious or under pentobarbital anesthesia. Nevertheless, renal responses to activation of the renal sympathetic nerves differed between fat-fed and control rabbits, in two important respects. Firstly, the antinatriuretic response to RNS was enhanced by fat-feeding. Secondly, while
RNS-induced reductions in RBF and CLDF were similar in fat-fed and control rabbits, the response of MLDF was enhanced by fat-feeding. It is tempting to speculate that these two phenomena are mechanistically linked, given the established role of the medullary circulation in regulation of renal sodium excretion (19, 34). Regardless, our findings suggest that the pro-hypertensive effects of adiposity, and in particular the impact of increased RSNA on renal excretory function, could be augmented by enhanced sensitivity of renal excretory function to a given level of RSNA.

Our current finding, that the response of medullary perfusion to RNS is augmented in fat-fed rabbits, is strikingly similar to our previous finding of augmented MLDF responses to RNS in angiotensin II-induced hypertension and in both the clipped and contralateral kidney in two kidney, one clip (2K1C) hypertension (4). Endogenous angiotensin II appears to enhance the responsiveness of the medullary circulation to RNS, through activation of both AT$_1$- (44) and AT$_2$-receptors (42). Endogenous nitric oxide, in contrast, acts to blunt responses of the medullary circulation to RNS (15, 43, 44, 51). In both angiotensin II-induced hypertension and in renovascular hypertension the intrarenal renin-angiotensin system is activated (38) and nitric oxide bioavailability is reduced, secondary to oxidative stress (45, 52-54). Thus, it seems reasonable to speculate that these mechanisms might contribute to the enhanced responsiveness of the medullary circulation to RNS in models of hypertension associated with activation of the renin-angiotensin system. Could similar mechanisms also explain this effect in fat-fed rabbits? There is certainly accumulating evidence that adipokine dysfunction, and in particular reduced levels of adiponectin, promotes the development of oxidative stress and reduced nitric oxide bioavailability in obesity (28, 37) and contributes to development of hypertension (39), at least in part through interactions with the renin-angiotensin system (29). Our measurements of arterial PRA in conscious rabbits and the responses of renal PRA overflow to hypoxia and RNS in anesthetized rabbits do not support the notion that neurally-mediated renin release is enhanced in these fat-fed rabbits, although the variability in these
data would impede detection of subtle effects. We also cannot discount the possibility that the intrarenal renin-angiotensin system is activated in this model. This latter possibility merits investigation, since it could provide a unified theory for development of enhanced responsiveness of the medullary circulation in moderate adiposity, angiotensin II-induced hypertension, and 2K1C hypertension. Finally, renal structural changes, including accumulation of intrarenal fat (36) and increased deposition of extracellular matrix, particularly in the renal medulla (13, 55), may alter the vascular and tubular responses to activation of the sympathetic nerves.

There is now strong evidence that the medullary circulation is a crucial player in long-term regulation of blood pressure, through the impact of medullary blood flow on tubular sodium reabsorption (18). The fat-fed rabbits we studied were not hypertensive, but there is unequivocal evidence that fat-feeding does eventually lead to development of hypertension in this species (3, 5, 6). Thus, the enhanced antinatriuretic response to activation of the renal nerves observed in the current study could contribute to the development of hypertension in fat-fed rabbits. Our current observations raise the intriguing possibility that the enhanced neurally mediated antinatriuresis in fat-fed rabbits is mediated at least partly by the greater responsiveness of the medullary circulation, although we currently have no evidence that the relationship between these phenomena is causal rather than just associative.

A limitation of our current study is that it provided little information regarding the precise mechanisms by which adiposity enhances the responses of sodium excretion and medullary perfusion to activation of the renal nerves. One possibility is that sympathetic neurotransmitter bioavailability at the neuromuscular junction is enhanced through either augmented release or diminished uptake and metabolism. However, given that responses of most renal parameters to RNS were relatively normal in fat-fed rabbits, this possibility seems unlikely. Nevertheless, without measurement of renal norepinephrine kinetics, a pre-
junctional site of action cannot be discounted. The variability of our measurements of renal PRA overflow must also be considered a limitation, since they would impede our ability to detect subtle but biologically important effects. The use of general anesthesia allowed us to make carefully controlled measurements of all facets of neural control of the kidney. However, we cannot necessarily generalize our findings to the conscious state. Clearly, therefore, further studies are required to show whether the antinatriuretic response to increased RSNA is augmented by adiposity in conscious rabbits.

As we found previously (17), hypoxia markedly increased RSNA and renal PRA overflow and reduced urine flow and sodium excretion. These effects appear to be entirely dependent on the presence of intact renal nerves (30, 33). The effects of hypoxia on renal excretory function and PRA overflow resembled those of RNS at 1 Hz. Yet hypoxia had relatively little effect on cortical perfusion and GFR and produced sustained increases in medullary perfusion, whereas 1 Hz RNS reduced cortical perfusion and glomerular filtration rate but had little or no impact on medullary perfusion (at least in control rabbits). Differences between responses to activation of the renal nerves induced by electrical stimulation and hypoxia are likely attributable both to the direct effects of hypoxemia and to differences in the nature of nerve recruitment in response to these stimuli. For example, medullary hyperemia during hypoxia is most likely a response to medullary hypoxia. The greater response of GFR to RNS than hypoxia may reflect the fact that hypoxia recruits renal nerves in a functional fashion, whereas during RNS all renal nerves are activated (11, 12, 32).

In contrast to the situation with RNS, we were unable to detect significantly enhanced antinatriuretic responses to hypoxia in fat-fed rabbits. We have no adequate explanation for this, although our inability to detect an effect could simply reflect the variability of the renal excretory response to hypoxia. It certainly cannot be explained by differential effects of hypoxia on RSNA in the two groups of rabbits, since the hypoxia-induced increases in RSNA
in control and fat-fed rabbits were very similar. Another possibility is that the enhanced antinatriuretic response to RNS in fat-fed rabbits is mechanistically linked to the associated enhanced reduction in medullary perfusion. Because hypoxia causes medullary hyperemia, this mechanistic link might be lost during this maneuver.

Levels of most variables we measured remained remarkably stable across the course of the entire experiment. Responses to hypoxia and RNS were fully reversible, so that, with only a few exceptions, all measured variables returned to their control levels after each maneuver. There was a small degree of hemodilution across the course of the study, but this phenomenon occurred to a similar extent in fat-fed and control rabbits. Basal PRA overflow was reduced, and urine flow was increased, in Part B of the experiment compared with part A, no doubt reflecting the effects of renal denervation, as we have observed previously (17).

**Perspectives and Significance**

Our current findings potentially provide a new perspective on the pathogenesis of obesity-induced hypertension. There is good evidence that the renal nerves contribute to the development of obesity-induced hypertension (27), but conventional wisdom has been that this role is attributable mainly to increases in RSNA mediated through signaling cascades initiated by leptin and other adipokines (24, 35, 41). Our current observations provide the first evidence that changes in the responsiveness of the kidney to RSNA might also contribute to the development of obesity-induced hypertension.

**Acknowledgements**

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References


**Table 1**: Mass of the perirenal, mesenteric, gonadal and pericardial fat pads.

<table>
<thead>
<tr>
<th>Fat Pad</th>
<th>Control Diet (g)</th>
<th>High Fat Diet (g)</th>
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<tbody>
<tr>
<td>Combined</td>
<td>101.7 ± 9.6</td>
<td>175.4 ± 15.1***</td>
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<tr>
<td>Perirenal</td>
<td>58.7 ± 4.9</td>
<td>90.7 ± 7.2**</td>
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<tr>
<td>Mesenteric</td>
<td>32.4 ± 5.9</td>
<td>64.6 ± 7.1**</td>
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<tr>
<td>Gonadal</td>
<td>7.2 ± 1.1</td>
<td>13.6 ± 2.1*</td>
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<tr>
<td>Pericardial</td>
<td>3.5 ± 0.4</td>
<td>6.4 ± 0.9**</td>
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Data are the mean ± SEM of measurements taken at post-mortem after 9 weeks of a high fat diet (n = 8) or control diet (n = 9). *** $P_{Diet} \leq 0.001$, ** $P_{Diet} \leq 0.01$, * $P_{Diet} \leq 0.05$ (unpaired t-test). Combined fat pad mass is the sum of the mass of the perirenal, mesenteric, gonadal and pericardial fat pads.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Diet</th>
<th>High Fat Diet</th>
<th>P&lt;sub&gt;Diet&lt;/sub&gt;</th>
<th>P&lt;sub&gt;Time&lt;/sub&gt;</th>
<th>P&lt;sub&gt;Diet*Time&lt;/sub&gt;</th>
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<td>Before</td>
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<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>72.3 ± 4.0</td>
<td>81.9 ± 4.7</td>
<td>77.1 ± 4.0</td>
<td>88.7 ± 3.4</td>
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<td>Heart Rate (beats/min)</td>
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<tr>
<td>Hematocrit (%)</td>
<td>34.7 ± 1.1</td>
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<td>36.3 ± 0.8</td>
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<td>Plasma Renin Activity (ng/ml)</td>
<td>2.45 ± 0.39</td>
<td>2.94 ± 0.48</td>
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<td>4.47 ± 1.61</td>
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Data are mean ± SEM of data from 9 control rabbits and 8 rabbits placed on a high fat diet. P values are the outcomes of analysis of variance.
Table 3: Baseline levels of variables in control and fat-fed rabbits.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Diet</th>
<th>High Fat Diet</th>
<th>Statistical Outcomes</th>
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<tbody>
<tr>
<td></td>
<td>Part A</td>
<td>Part B</td>
<td>Part A</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>72 ± 2</td>
<td>72 ± 1</td>
<td>72 ± 1</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>240 ± 9</td>
<td>247 ± 15</td>
<td>241 ± 9</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>32.5 ± 0.4</td>
<td>31.4 ± 0.5</td>
<td>30.4 ± 0.3</td>
</tr>
<tr>
<td>Renal Blood Flow (ml/min)</td>
<td>27.5 ± 2.3</td>
<td>26.1 ± 2.3</td>
<td>26.1 ± 2.3</td>
</tr>
<tr>
<td>Cortical Laser Doppler Flux (units)</td>
<td>303 ± 28</td>
<td>262 ± 25</td>
<td>317 ± 26</td>
</tr>
<tr>
<td>Medullary Laser Doppler Flux</td>
<td>58 ± 11</td>
<td>53 ± 9</td>
<td>86 ± 12</td>
</tr>
<tr>
<td>Renal Sympathetic Nerve activity (μV)</td>
<td>23.7 ± 5.5</td>
<td>26.2 ± 3.5</td>
<td>0.71</td>
</tr>
<tr>
<td>Urine Flow (μL/min)</td>
<td>177 ± 32</td>
<td>312 ± 38</td>
<td>238 ± 39</td>
</tr>
<tr>
<td>Sodium Excretion (μmol/min)</td>
<td>33.0 ± 7.1</td>
<td>46.9 ± 5.4</td>
<td>37.0 ± 5.3</td>
</tr>
<tr>
<td>Glomerular Filtration Rate (ml/min)</td>
<td>3.01 ± 0.74</td>
<td>3.68 ± 0.85</td>
<td>4.21 ± 0.61</td>
</tr>
<tr>
<td>Fractional Sodium Excretion (%)</td>
<td>12.3 ± 4.5</td>
<td>12.6 ± 3.1</td>
<td>6.9 ± 1.3</td>
</tr>
<tr>
<td>Arterial PRA (ng/ml)</td>
<td>13.0 ± 2.4</td>
<td>10.0 ± 1.7</td>
<td>12.4 ± 1.9</td>
</tr>
<tr>
<td>Renal Venous PRA (ng/ml)</td>
<td>13.3 ± 2.9</td>
<td>11.5 ± 2.2</td>
<td>15.3 ± 2.2</td>
</tr>
<tr>
<td>Arteriovenous PRA difference (ng/ml)</td>
<td>1.9 ± 0.9</td>
<td>1.5 ± 0.7</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Renal PRA overflow (ng/min)</td>
<td>55.5 ± 25.1</td>
<td>38.9 ± 16.2</td>
<td>89.1 ± 24.4</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of values averaged over the two control periods in each part of the experiment. P values are the outcomes of analysis of variance. Responses to hypoxia were tested in part A while responses to electrical stimulation of the renal nerves were tested in part B.
Figure Legends

**Fig. 1** Responses of arterial pressure and renal hemodynamics to hypoxia (left) and electrical stimulation of the renal nerves (right). Columns and error bars show responses of control (n = 6-8) and fat fed (n = 6-7) rabbits as mean ± SEM of percentage change. MAP = mean arterial pressure; RBF = renal blood flow; CLDF = cortical laser Doppler flux; MLDF = medullary laser Doppler flux. Note that responses of RBF to hypoxia were not measured directly, so are not shown. * P_Diet ≤ 0.05. Across both groups of rabbits, hypoxia significantly increased MLDF (P_{Hypoxia} = 0.002) but did not significantly alter MAP (P_{Hypoxia} = 0.75) or CLDF (P_{Hypoxia} = 0.41). Electrical stimulation of the renal nerves increased MAP (P_{Frequency} = 0.03) and reduced RBF (P_{Frequency} < 0.001), CLDF (P_{Frequency} < 0.001) and MLDF (P_{Frequency} = 0.002).

**Fig. 2** Responses of renal excretory function to hypoxia (left) and electrical stimulation of the renal nerves (right). Columns and error bars are as for Fig. 1. GFR = glomerular filtration rate; U_{Na+}V = sodium excretion. * P_Diet ≤ 0.05. Across both groups of rabbits, hypoxia significantly reduced urine flow (P_{Hypoxia} = 0.01), U_{Na+}V (P_{Hypoxia} = 0.001) and fractional Na+ excretion (P_{Hypoxia} = 0.008) but did not significantly alter GFR (P_{Hypoxia} = 0.34). Electrical stimulation of the renal nerves significantly reduced GFR (P_{Frequency} < 0.001), urine flow (P_{Frequency} < 0.001), U_{Na+}V (P_{Frequency} < 0.001) and fractional Na+ excretion (P_{Frequency} < 0.001).

**Fig. 3** Changes in plasma renin activity (PRA) and renal PRA overflow in response to hypoxia (left) and electrical stimulation of the renal nerves (right). Columns and error bars are as for Fig. 1. AV = arteriovenous. Across both groups of rabbits, both hypoxia and electrical stimulation of the renal nerves significantly increased arterial PRA (P_{Hypoxia} < 0.001, P_{Frequency} = 0.001), renal venous PRA (P_{Hypoxia} < 0.001, P_{Frequency} < 0.001), the arteriovenous PRA concentration difference (P_{Hypoxia} < 0.001, P_{Frequency} < 0.001) and renal
PRA overflow ($P_{\text{Hypoxia}} < 0.001$, $P_{\text{Frequency}} = 0.002$). Responses to hypoxia and electrical stimulation of the renal nerves were not significantly different in fat-fed compared to control rabbits ($P_{\text{Diet}}$ always $\geq 0.36$).
Figure 1

Hypoxia

Electrical Stimulation

MAP (% Change)

Control

Fat-Fed

RBF (% Change)

MLDF (% Change)

CLDF (% Change)

Stimulation Frequency (Hz)

*