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Altered responsiveness of the kidney to activation of the renal nerves in fat-fed rabbits

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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 wk, responses to reflex activation of renal
sympathetic nerve activity (RSNA) with hypoxia and electrical stim-
ulation 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 en-
hanced by fat feeding. For example, 1 Hz RNS reduced sodium
excretion by $79 \pm 4\%$ in fat-fed rabbits and $46 \pm 13\%$ in controls.
RNS (2 Hz) reduced medullary perfusion by $38 \pm 11\%$ in fat-fed
rabbits and $9 \pm 4\%$ 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 indistin-
guishable 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.

hypertension; hypoxia; kidney circulation; obesity; kidney medulla;
renal sympathetic nerves; renin-angiotensin system; sodium excretion

THE SYMPATHETIC NERVOUS SYSTEM is often activated in over-
weight and obese individuals (16). For example, muscle sym-
pathetic 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). More-
over, muscle sympathetic nerve activity is increased by even
modest weight gain in nonobese humans (21), while both
muscle sympathetic nerve activity and whole body norepineph-
rine 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 impor-
tant 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 hyperten-
sion 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 predisposition of obese subjects to development of hyper-
tension is driven at least partly by altered neural control of the
kidney. Increased renal sympathetic nerve activity (RSNA),
driven through activation of specific central nervous system
pathways, obviously makes a major contribution to altered
renal function in obesity. However, to understand how inte-
grated 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 (RNS). In the present
study we used this approach to test the hypothesis that modest
increases in adiposity can enhance responsiveness of the kid-
ney 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). The

experimental protocols were approved in advance by the Animal Ethics Committee of the School of Biomedical Sciences, Monash University. Rabbits entered the study at 18 wk of age. After a 2-wk 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 wk. 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 wk 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 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 pentobarbital sodium (90–150 mg plus 30–50 mg/h iv; Sigma, St. Louis, MO). Rabbits were artificially ventilated throughout the surgery, and experiment and extracellular fluid volume was maintained by intravenous infusion ($0.18 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) 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 model DP4s; Moor Instruments, Millwey, Devon, UK) (23) and on the cortical surface (model 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}C]-labeled inulin (bolus dose of 10 μCi plus 45 nCi·kg^{-1}·min^{-1}, PerkinElmer Life Sciences, Boston, MA) 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 P_{O_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 midpoint of each urine collection period, samples of arterial blood (1 ml each) were taken for measurement of *1*) [^{14}C]inulin and sodium concentrations and *2*) 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 resuspended erythrocytes from previous blood samples.

In preparation for *part B*, the renal nerves were sectioned cranially, a transit-time ultrasound flow probe (model 2SB; Transonic Systems,

Ithaca, NY) 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 minutes 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*.

Postmortem Measurements

At the completion of *part B*, rabbits were killed with an overdose of pentobarbital sodium. 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 2-s 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 microvolts.

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 nanograms per minute. Thus, 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 flow probe. 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 with online publication of this article).

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 −20°C for subsequent analysis. [^{14}C]inulin and sodium concentrations in plasma and urine were determined as previously described (17). Glomerular filtration rate (GFR) was determined as the clearance of [^{14}C]inulin.

Statistical Methods

Data are expressed as means ± SE. Hemodynamic variables and RSNA are presented as the average over the 15-min renal clearance periods, unless otherwise stated. Arterial blood P_{O_2}, NE kinetics, and PRA overflow data were derived from the blood samples collected at the midpoint of the 15-min clearance periods. Analysis of variance was employed for hypothesis testing. PRA values were $\log_{10}$ 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 vs. recovery from hypoxia or RNS, *part A* vs. *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 wk 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-wk 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

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

Fat Pad	Control Diet, g	High Fat Diet, g
Combined	101.7±9.6	175.4±15.1‡
Perirenal	58.7±4.9	90.7±7.2†
Mesenteric	32.4±5.9	64.6±7.1†
Gonadal	7.2±1.1	13.6±2.1*
Pericardial	3.5±0.4	6.4±0.9†

Data are means ± SE of measurements taken at postmortem after 9 wk of a high-fat diet ($n = 8$) or control diet ($n = 9$). *$P_{Diet} \leq 0.05$, †$P_{Diet} \leq 0.01$, ‡$P_{Diet} \leq 0.001$, (unpaired *t*-test). Combined fat-pad mass is the sum of the mass of the perirenal, mesenteric, gonadal, and pericardial fat pads.

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 in part B), urine flow (68 ± 25 μl/min greater in part B) and PRA overflow (46.5 ± 15.8 ng/min less in part B) (Table 3).

Responses to Hypoxia

Baseline arterial P_{O_2} was similar in the two groups of rabbits, averaging 103 ± 3 mmHg. Ventilation with 10% O_2 reduced arterial P_{O_2} similarly in both groups, to an average of 28 ± 1 mmHg. Arterial P_{O_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 ($P_{Diet} \geq 0.21$; 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 ($P_{Diet} \geq 0.38$; 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 ($P_{Diet} \geq 0.40$). In contrast, RNS-induced reductions in MLDF were greater in fat-fed than control rabbits ($P_{Diet} = 0.02$). For example, 2 Hz RNS reduced MLDF by 38 ± 11% in fat-fed rabbits but by only 9 ± 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_{Freq} always < 0.001). RNS-induced reductions in sodium excretion were significantly greater in fat-fed rabbits than in control rabbits ($P_{Diet} = 0.05$). For example, 1 Hz RNS reduced sodium excretion by 79 ± 4% in fat-fed rabbits but only by 46 ± 13% in controls. RNS-induced reductions in urine flow also tended to be greater in fat-fed rabbits (82 ± 3% at 1 Hz) than in controls (51 ± 14% at 1 Hz), although this apparent effect was not statistically significant ($P_{Diet} = 0.07$). There was a similar tendency for RNS-induced reductions in fractional sodium excretion to be greater in fat-fed rabbits (−59 ± 3% at 1 Hz) than in controls (−38 ± 10% at 1 Hz) ($P_{Diet} = 0.10$; Fig. 2).

Table 2. *Hemodynamic variables and plasma renin activity before and after the 9-wk dietary intervention in conscious rabbits*

Variable	Control Diet		High Fat Diet		P_{Diet}	P_{Time}	$P_{Diet*Time}$
	Before	After	Before	After			
Mean arterial pressure, mmHg	72.3±4.0	81.9±4.7	77.1±4.0	88.7±3.4	0.78	**0.006**	0.22
Heart rate, beats/min	207±7	207±5	202±8	196±8	0.43	0.57	0.47
Hematocrit, %	34.7±1.1	35.2±2.0	35.7±1.3	36.3±0.8	0.26	0.44	0.83
Plasma renin activity, ng/ml	2.45±0.39	2.94±0.48	2.51±0.62	4.47±1.61	0.90	**0.04**	0.55

Data are means ± SE of data from 9 control rabbits and 8 rabbits placed on a high-fat diet. *P* values are the outcomes of ANOVA, shown in bold when statistically significant.

RNS induced frequency-dependent increases in arterial PRA, renal venous PRA, arteriovenous PRA difference, and renal PRA overflow (P_{Freq} always ≤ 0.002). These responses were not significantly different in fat-fed compared with control rabbits (P_{Diet} ≥ 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-wk 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. First, the antinatriuretic response to RNS was enhanced by fat-feeding. Second, 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 prohypertensive 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 present 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 angiotensin $(AT)_1$- (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 inter-

Table 3. *Baseline levels of variables in control and fat-fed rabbits*

Variable	Control Diet		High-Fat Diet		Statistical Outcomes		
	Part A	*Part B*	*Part A*	*Part B*	P_{Diet}	P_{Part}	$P_{Diet*Part}$
Mean arterial pressure, mmHg	72±2	72±1	72±1	73±1	0.65	0.52	0.55
Heart rate, beats/min	240±9	247±15	241±9	264±14	0.45	0.22	0.53
Hematocrit, %	32.5±0.4	31.4±0.5	30.4±0.3	29.1±0.9	**0.001**	**0.05**	0.86
Renal blood flow, ml/min		27.5±2.3		26.1±2.3	0.86		
Cortical laser Doppler flux, units	303±28	262±25	317±26	253±28	0.90	0.06	0.67
Medullary laser Doppler flux, units	58±11	53±9	86±12	88±11	**0.02**	0.69	0.96
Renal sympathetic nerve activity, μV	23.7±5.5		26.2±3.5		0.71		
Urine flow, μl/min	177±32	312±38	238±39	305±50	0.51	**0.02**	0.41
Sodium excretion, μmol/min	33.0±7.1	46.9±5.4	37.0±5.3	42.7±5.8	0.98	0.11	0.50
Glomerular filtration rate, ml/min	3.01±0.74	3.68±0.85	4.21±0.61	3.70±0.81	0.44	0.91	0.45
Fractional sodium excretion, %	12.3±4.5	12.6±3.1	6.9±1.3	10.3±2.4	0.22	0.56	0.62
Arterial PRA, ng/ml	13.0±2.4	10.0±1.7	12.4±1.9	16.6±3.9	0.28	0.79	0.15
Renal venous PRA, ng/ml	13.3±2.9	11.5±2.2	15.3±2.2	17.7±4.2	0.16	0.91	0.46
Arteriovenous PRA difference, ng/ml	1.9±0.9	1.5±0.7	2.7±0.8	1.1±0.5	0.79	0.17	0.42
Renal PRA overflow, ng/min	55.5±25.1	38.9±16.2	89.1±24.4	22.1±9.4	0.68	**0.05**	0.23

Values are means ± SE of values averaged over the two control periods in each part of the experiment. *P* values are the outcomes of ANOVA, shown in bold when statistically significant. Responses to hypoxia were tested in *part A*, while responses to electrical stimulation of the renal nerves were tested in *part B*.
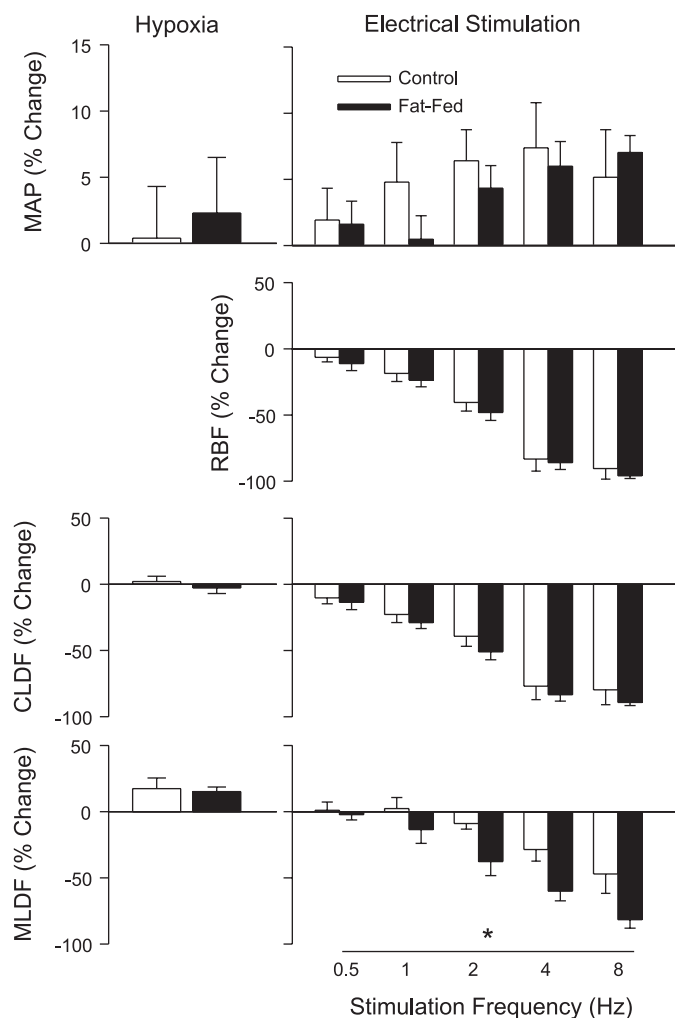
Fig. 1. Responses of arterial pressure and renal hemodynamics to hypoxia (*left*) and electrical stimulation of the renal nerves (*right*). Columns and error bars show responses of control ($n = 6$–9) and fat-fed ($n = 6$–8) rabbits as means ± SE of %change. MAP, mean arterial pressure; RBF, renal blood 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. *$P_{Diet} \leq 0.05$. 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 ($P_{Frequency} = 0.002$).

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 present study could contribute to the development of hypertension in fat-fed rabbits. Our present 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 presently have no evidence that the relationship between these phenomena is causal rather than just associative.

A limitation of our present study is that it provided little information regarding the precise mechanisms by which adiposity enhances the responses of sodium excretion and
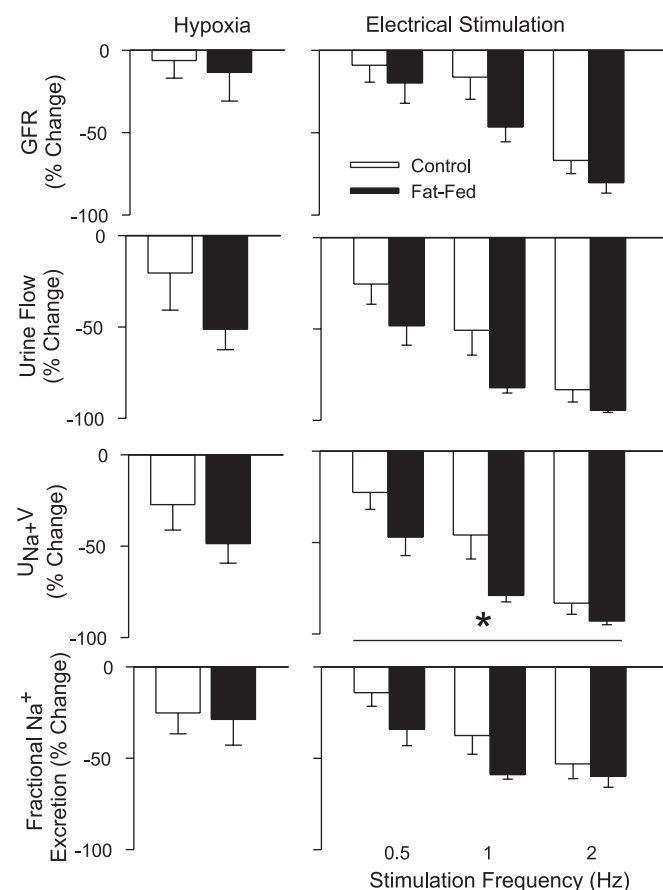

Fig. 2. Responses of renal excretory function to hypoxia (*left*) and electrical stimulation of the renal nerves (*right*). Columns and error bars are as for Fig. 1. GFR, glomerular filtration rate; $U_{Na+}V$, sodium excretion. *$P_{Diet} \leq 0.05$. Across both groups of rabbits, hypoxia significantly 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 ($P_{Frequency} < 0.001$).

actions 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,
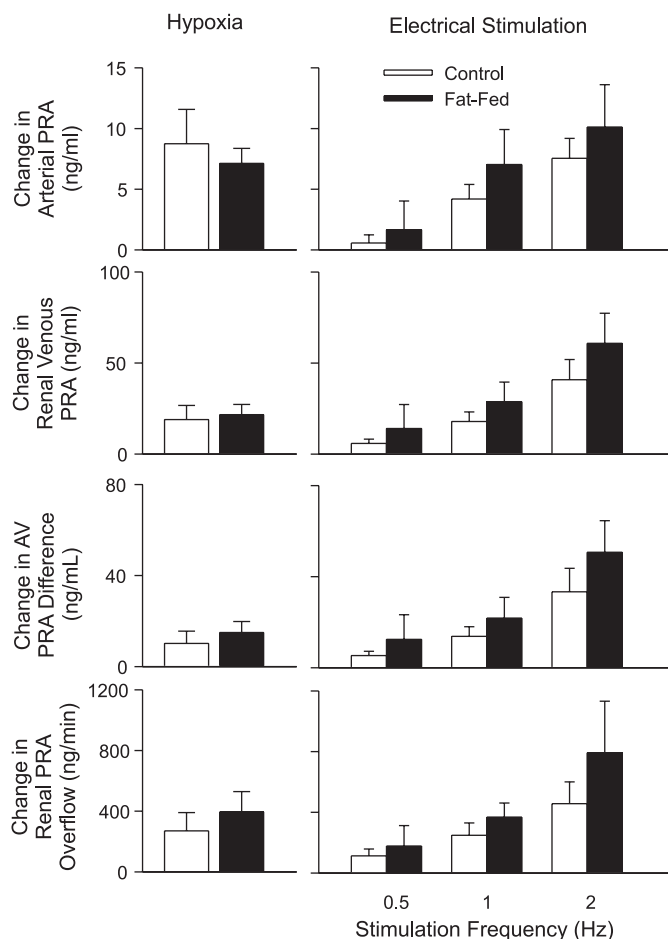
Fig. 3. Changes in plasma renin activity (PRA) and renal PRA overflow in response to hypoxia (*left*) and electrical stimulation of the renal nerves (*right*). Columns and error bars are as for Fig. 1. AV, arteriovenous. Across both groups of rabbits, both hypoxia and electrical stimulation of the renal nerves significantly increased arterial PRA ($P_{\text{Hypoxia}} < 0.001$, $P_{\text{Frequency}} = 0.001$), renal venous PRA ($P_{\text{Hypoxia}} < 0.001$, $P_{\text{Frequency}} < 0.001$), the arteriovenous PRA concentration difference ($P_{\text{Hypoxia}} < 0.001$, $P_{\text{Frequency}} < 0.001$), 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 with control rabbits (P_{Diet} always ≥ 0.36).

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 prejunctional 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 GFR 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 present 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 present 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.

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