Impaired vascular responses to relaxin in diet-induced overweight female rats

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thelium-dependent vasodilation and compliance and decreasing myogenic reactivity. Diet-induced overweight and obesity are associ-
ated with impaired endothelial dysfunction and vascular remodeling leading to a reduction in arterial diameter. In this study, we tested
the hypothesis that local vascular responses to relaxin are impaired in diet-induced overweight female rats on a high-fat cafeteria-style diet
for 9 wk. Rats were chronically infused with either relaxin or placebo for 5 days, and vascular responses were measured in isolated mesen-
teric arteries and the perfused kidney. Diet-induced overweight signif-
icantly increased sensitivity to phenylephrine (by 17%) and vessel wall thickness, and reduced renal perfusion flow (RPPF; by 16%), but
did not affect flow-mediated vasodilation, myogenic reactivity, and vascular compliance. In the normal weight rats, relaxin treatment
significantly enhanced flow-mediated vasodilation (2.67-fold), decreased myogenic reactivity, and reduced sensitivity to phenylephrine
(by 28%), but had no effect on compliance or RPPF. NO blockade by L-NAME diminished most relaxin-mediated effects. In diet-induced
overweight rats, the vasodilator effects of relaxin were markedly reduced for flow-mediated vasodilation, sensitivity to phenylephrine,
and myogenic response compared with the normal diet rats, mostly persistent under L-NAME. Our data demonstrate that some of the
vasodilator responses to in vivo relaxin administration are impaired in isolated mesenteric arteries and the perfused kidney in diet-induced
overweight female rats. This does not result from a decrease in Rspj1 (relaxin family peptide receptor) expression but is likely to result from
downstream disruption to endothelial-dependent mechanisms in diet-
induced overweight animals.

relaxin; mesenteric arteries; vasodilation; myogenic reactivity; arterial
compliance

RELAXIN, A MEMBER OF THE INSULIN-LIKE GROWTH FACTOR SUPERFAMILY, IS AN IMPORTANT VASODILATORY HORMONE OF PREGNANCY (6, 10, 22, 31). Mostly based on renal vascular studies, four mecha-
nisms are thought to be involved in relaxin-mediated vasodi-
lation: upregulated endothelium-dependent NO pathway (3, 12), blunted responsiveness to vasoconstrictive stimuli (27),
decreased myogenic reactivity (26), and increased compliance (19). Chronic administration of relaxin to nonpregnant female rats induces vascular adaptations comparable to those observed in pregnancy (9, 12) through mechanisms involving matrix
gelatinases, metalloproteases, endothelin B receptors, and vascular endo-
thelial growth factor (8), whereas relaxin neutralizing antibodies
completely abolish the vascular vasodilator effects in rat pregnancy (25). These observations suggest a pivotal role of
relaxin in gestational vascular adaptation.

A high-fat diet, inducing mild overweight in rats, predis-
poses one to endothelial dysfunction (1) and reflects the human dietary etiology of overweight (17), which clinically translates
into an increased risk for gestational hypertensive disease (28). It has been proposed that attenuated adaptation of the above-
mentioned mechanisms may precede gestational hypertensive
disorders (16, 35). However, it is unknown whether diet-
induced overweight affects normal pregnancy-like relaxin-
induced vascular changes. The mesenteric and renal vascular beds predominately contribute to peripheral resistance. In non-
pregnant healthy conditions, they receive ~25% and 30% of
total cardiac output (5, 34), whereas during pregnancy renal plasma flow (RPF) and mesenteric vascular perfusion increases by
40% and 65% (4, 7). We hypothesized that diet-induced
overweight induces relaxin-resistance, defined as the inability
to produce comparable vascular responses to relaxin exposure
as observed in healthy controls. To this end, we performed an
experimental ex vivo study on relaxin-induced vascular re-
sponses in isolated mesenteric arteries and kidney in normal-
weight and diet-induced overweight female rats.

MATERIALS AND METHODS

Animals

The experimental protocol was approved by the Animal Exper-
iments Committee of the Radboud University Nijmegen Medical
Centre and was performed in accordance with the European guide-
lines on animal experiments. Virgin female Wistar Hannover rats
(Harlan Netherlands, Horst, The Netherlands) received either a regu-
lar diet [ssniff R/M-H, n = 20, used in a previous cohort (37)] or a
high-fat cafeteria-style diet [ssniff EF R/M acc. D12451 (II), 45% KJ
as fat, n = 20] ad libitum from an age of 21 days onward. A comparable high-fat diet has previously been shown to result in
nonsignificant mild overweight, normal lipid profile, and insulin
resistance (2, 32). Rats were housed in filter-top cages on a 12-h
light/dark cycle. At an age of 73–85 days, rats were randomly
assigned (independent from estrous stage) to chronic infusion for 5
days with either placebo or recombinant human H2 relaxin (both
containing 5 mM sodium acetate, pH 5.0; Corthera, San Mateo, CA).
This readily available human relaxin induces vascular responses in nonpregnant rats comparable to those attributed to endogenous relaxin in preg-
nant rats (12, 25). On day 0, an osmotic minipump (Alzet, model 2001, DURECT, Cupertino, CA) was implanted subcutane-
ously under isoflurane anesthesia. The osmotic minipump infused
human relaxin at a dose of 4 μg/h for 5 days. This dose of relaxin
results in plasma relaxin concentrations equivalent to mid-pregnant
rats: 30 ng/ml (12, 30). On day 5, the rats were anesthetized with an intraperitoneal injection of 6 mg/100 g pentobarbital (Apharno, Arnhem, The Netherlands). Furosemide 1 mg/100 g (Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) was also injected intraperitoneally to achieve maximal urethral distention for optimal catheter placement. Blood was withdrawn from the vena cava to measure plasma human H2 relaxin concentration levels (Human Relaxin-2 Quantikine ELISA kit DRL200, R & D Systems, Minneapolis, MN). Continuation of chronic relaxin exposure was maintained during all experiments by exposing both mesenteric arteries and isolated kidney to perfuse and tissue bath solutions containing 30 ng/ml of the trial-medication supplied (relaxin or a comparable amount of placebo; incubation time of 30 min).

**Pressure-Perfusion Myograph**

The flow-mediated vasodilation, the myogenic reactivity to pressure and compliance were analyzed in a pressure-perfusion myograph (Pressure Myograph System-Model P100, J. P. Trading, Aarhus, Denmark). The responses were determined in basic PBS (in mM: 119 NaCl, 4.69 KCl, 25 NaHCO3, 1.17 MgSO4, 1.18 KH2PO4, 5.5 glucose, and 10 HEPES), with additional 2 mM EGTA and 0.01 mM Na-nitroprusside (for calcium-free-PBS) to measure compliance and 2.5 mM CaCl2, 0.027 mM Na2EDTA (for calcium-PBS) to assess flow-mediated vasodilation and myogenic reactivity. The buffers were oxygenated with 95% O2 and 5% CO2 at a temperature of 37°C. Second-order mesenteric arteries (150–400 μm) were mounted on two opposing glass cannulae (125 μm). Vascular inner diameter was measured through video recording (Vessel View, J. P. Trading). Arteries were equilibrated at an intraluminal pressure of 60 mmHg during 20 min. Subsequently, thromboxane A2 agonist U46619 (9,11-dideoxy-11,9-dihydroxy-11,9-epoxymethanoprostaglandin F2α, Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) was added (bath concentration of 10−7–10−6 M) to preconstrict vessels to 30–40% of their basal diameter.

**Flow-mediated vasodilation.** Flow-mediated vasodilation is defined as the vasodilator response to a certain flow increase. After reaching a stable contraction, the flow in the vessel was increased every 2 min in 16.7 μl/min steps from 0 to 100 μl/min (comparable to shear-stress range of 0–10 dyne/cm2) in the absence and presence of 100 μM of the NO antagonist L-nitro-arginine methyl ester (L-NAME; Sigma-Aldrich Chemie), while maintaining a mean intraluminal pressure of 60 mmHg. Vessels attaining <20% preconstriction and <10% vasodilation over the completed flow range or with unstable pressure due to fluid leakage were excluded from the study. Flow-mediated vasodilation was expressed as a percentage of the preconstriction status.

**Myogenic reactivity and vascular compliance.** Myogenic reactivity is defined as the vasoconstrictive response to a certain pressure increase in the presence of calcium. Compliance is defined as the vasodilator response to a certain pressure increase in the absence of calcium to avoid smooth muscle cell contraction. A new mesenteric vessel was isolated and prepared as described above. Intraluminal pressure was increased every 2 min in 10-mmHg steps from 20 mmHg to 110 mmHg to determine successive myogenic reactivity and compliance. For the latter, outer and inner vessel diameters and wall thickness were measured, and stress-strain relationship was obtained.

**Wire Myograph (Response to Phenylephrine)**

We studied the vasoconstrictor response to phenylephrine (Sigma-Aldrich Chemie) with a Mulvany Halpern myograph (dual wire myograph model 400A, J. P. Trading). The bath was filled with physiological salt solution [PSS (in mM): 119 NaCl, 4.69 KCl, 2.5 CaCl2, 25 NaHCO3, 1.17 MgSO4, 1.18 KH2PO4, 5.5 glucose, and 0.027 EDTA] and oxygenated with 95% O2 and 5% CO2 at a temperature of 37°C. Four second-order mesenteric arteries (150–400 μM) were mounted in two wire myographs, stabilized as previously described (23), and set at a tension equivalent generated at 90% of the inner circumference of 100 mmHg. The response to 124 mM KCl was measured (used for normalization of the phenylephrine response). The response to phenylephrine was determined in eight steps over a range from 10−7 M to 10−5 M in the absence or presence of 100 μM L-NAME. The data of the two arteries in each myograph were averaged when available.

**Isolated Perfused Rat Kidney Model**

Vascular adaptation of the isolated kidney to relaxin was assessed in the isolated perfused rat kidney (IPRK) model. Within the time frame of our protocol, this model shows stable renal perfusion flow (RPFF) and provides an indication of renal function (20) but may not reflect effective renal plasma flow responses in vivo. Briefly, the right renal artery was cannulated via the left renal artery and aorta. The right ureter was cannulated for urine collection. Perfusion was started in situ, and the kidney was removed and placed in a perfused bath at a temperature of 37°C. The kidney was perfused at a constant pressure of 90 mmHg with oxygenated cell-free Krebs-Ringer-Henselstein [containing (in mM) 113 NaCl, 4.8 KCl, 25 NaHCO3, 1.4 KH2PO4, 2.2 CaCl2, 1.4 MgCl2, 5 glucose] and 1.7 mM pluronic F-108 (oxygen carrier and oncotic agent, BASF, Arnhem, The Netherlands). RPFF was recorded in real time with the use of a computer system [Midac testorganizer, W95 (V3.0), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands].

Stabilization of the RPFF was accomplished during a 40-min period. From 40 to 60 min, the basal renal plasma flow (RPFFbaseline) was determined. At 60 min, L-NAME was added at a final concentration of 100 μM to the perfusate to investigate the contribution of NO to the relaxin (or placebo)-evoked vasodilator response up to 160 min (RPFF160). RPFF was normalized for body weight (ml/min·1·100 g body wt−1).

**Rxfp1 Gene Expression**

Rxfp1 gene expression was analyzed by quantitative polymerase chain reaction (qPCR), as described previously (38). Total RNA was extracted from frozen mesenteric arteries with Trizol (GIBCO), according to the manufacturer’s instructions. Forward/reverse primers and 6-carboxy fluorescein (FAM)-labeled TaqMan probes were specific for the full-length rat Rxfp1 from Biosearch Technologies (Novato, CA). Rxfp1 gene expression was compared with expression of the ribosomal 18S reference gene and presented as normalized ΔCT, transformed by 2−n, where n = −ΔCT (38).

**Statistical Analysis**

The data are expressed as means ± SE. Flow-mediated vasodilation, stress-strain relationship, response to phenylephrine, and RPFF were analyzed by nonlinear regression curve fitting (GraphPad Prism 4.0, Institute for Scientific Information, San Diego, CA). Subsequent curve fit estimates were Diamax (maximal vascular diameter after flow change), Flow50% (flow rate inducing 50% dilation, flow-sensitivity), K1 and K2 (exponential constants for stress-strain relationship), Rmax (maximum response to phenylephrine), and C50% (phenylephrine concentration inducing a 50% response), RPFFbaseline (basal RPFF), and RPFF160 (RPFFbaseline minus RPFF160). Overall myogenic reactivity (MRoverall, corrected for percentage preconstriction) and overall vascular compliance (VCoverall) were analyzed by using ANOVA for repeated measures (Greenhouse-Geisser correction; SPSS 16.0.2, SPSS, Chicago, IL). Selective straight-line curve fitting was performed for both myogenic reactivity (MRoverall) and compliance (VCoverall), estimating the slope over the range of 60–110 mmHg. Baseline characteristics were analyzed with Student’s t-test. In all analyses, we compared relaxin vs. placebo in normal-weight rats, relaxin vs. placebo in diet-induced overweight rats, and normal-weight vs. diet-induced overweight placebo-treated rats. A P value of <0.05 was considered to be statistically significant.
Table 1. Baseline vascular characteristics of mesenteric arteries in normal weight and diet-induced overweight female rats, pretreated with relaxin (4 μg/h for 5 days) or placebo

<table>
<thead>
<tr>
<th>Flow-mediated vasodilation</th>
<th>Normal Weight</th>
<th>Diet-Induced Overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n)</td>
<td>Relaxin (n)</td>
</tr>
<tr>
<td></td>
<td>Placebo (n)</td>
<td>Relaxin (n)</td>
</tr>
<tr>
<td>Basal diameter, μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-NAME absence</td>
<td>296 ± 16 (6)</td>
<td>314 ± 15 (7)</td>
</tr>
<tr>
<td>Precontraction, %</td>
<td>42 ± 4 (6)</td>
<td>37 ± 2 (7)</td>
</tr>
<tr>
<td>L-NAME presence</td>
<td>311 ± 16 (5)</td>
<td>330 ± 18 (4)</td>
</tr>
<tr>
<td>Precontraction, %</td>
<td>38 ± 2 (5)</td>
<td>36 ± 5 (4)</td>
</tr>
<tr>
<td>Myogenic reactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal diameter, μm</td>
<td>304 ± 11 (8)</td>
<td>319 ± 11 (10)</td>
</tr>
<tr>
<td>Precontraction, %</td>
<td>41 ± 3 (8)</td>
<td>45 ± 3 (10)</td>
</tr>
<tr>
<td>Vascular compliance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to phenylephrine</td>
<td>309 ± 8 (8)</td>
<td>318 ± 12 (9)</td>
</tr>
<tr>
<td>L-NAME absence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal diameter, μm</td>
<td>251 ± 9 (8)</td>
<td>234 ± 10 (10)</td>
</tr>
<tr>
<td>KCl contraction, mN</td>
<td>12.8 ± 0.9 (8)</td>
<td>12.7 ± 0.4 (10)</td>
</tr>
<tr>
<td>L-NAME presence</td>
<td>240 ± 9 (9)</td>
<td>243 ± 10 (10)</td>
</tr>
<tr>
<td>KCl contraction, mN</td>
<td>13.1 ± 0.5 (9)</td>
<td>12.5 ± 0.5 (10)</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE; n = number of animals. *Significant difference between placebo- and relaxin-treated rats (P < 0.05).

RESULTS

The study population contained 20 normal-weight female rats on the normal diet and 20 diet-induced female rats on the high-fat diet with average ages of 82 ± 1 and 84 ± 1 days, respectively. One rat on the high-fat diet failed to thrive for unknown reasons and was excluded from the study. Overall, the high-fat diet induced mild overweight, as rats on the high-fat diet were 13% heavier than those on the normal diet (234 ± 4 vs. 208 ± 2 g; P < 0.001). Plasma concentrations of human H2 relaxin in the relaxin-treated female rats were comparable in both weight groups (74 ± 16 ng/ml in normal weight rats vs. 61 ± 5 ng/ml in diet-induced overweight rats; P = 0.44) and were significantly elevated compared with placebo-treated female rats (<15.6 pg/ml). Plasma relaxin concentrations were comparable after correction for weight (36 ± 8 ng·ml⁻¹·100 g⁻¹ in normal weight rats vs. 27 ± 3 ng·ml⁻¹·100 g⁻¹ in diet-induced overweight rats; P = 0.30) and did not differ significantly between the two groups.

Basal diameter of the mesenteric vessels and percentage precontraction to U46619 did not differ significantly between normal-weight and diet-induced overweight female rats or between those chronically infused with relaxin and placebo (Table 1). For the most part, this was also seen in the presence and absence of L-NAME in the different experimental settings. However, under L-NAME, the response to 124 mM KCl was significantly increased in the relaxin-treated diet-induced overweight group compared with placebo-treated diet-induced overweight female rats (15.1 ± 0.7 vs. 12.1 ± 0.6 mN, respectively).

Flow-Mediated Vasodilation

Flow-mediated vasodilation in the mesenteric arteries of placebo-treated female rats did not differ significantly between normal weight and diet-induced overweight animals either in the absence (P = 0.32) or presence (P = 0.08) of L-NAME (Table 2). Similarly, although the maximum response to flow (Diam max) was slightly higher in the diet-induced overweight female rats, it was not significant compared with normal-weight female rats in the absence or presence of L-NAME. In normal-weight female rats, chronic infusion with relaxin significantly (P < 0.01) increased physiological flow (Flow phys) compared with placebo treatment. In diet-induced overweight female rats, chronic infusion with relaxin significantly (P < 0.01) increased physiological flow (Flow phys) compared with placebo treatment.

Table 2. Flow-mediated vasodilation curve-fit estimates in mesenteric arteries of normal weight and diet-induced overweight female rats pretreated with relaxin (4 μg/h for 5 days) or placebo

<table>
<thead>
<tr>
<th>Flow-Mediated Vasodilation</th>
<th>Placebo (n)</th>
<th>Relaxin (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight</td>
<td>48 ± 9 (6)</td>
<td>18 ± 4* (7)</td>
</tr>
<tr>
<td>Diet-induced overweight</td>
<td>35 ± 7 (6)</td>
<td>54 ± 16 (7)</td>
</tr>
<tr>
<td>Normal weight (L-NAME)</td>
<td>28 ± 6 (5)</td>
<td>30 ± 10 (4)</td>
</tr>
<tr>
<td>Diet-induced overweight (L-NAME)</td>
<td>42 ± 5 (6)</td>
<td>38 ± 6 (8)</td>
</tr>
<tr>
<td>Diam max, %</td>
<td>Normal weight</td>
<td>31 ± 5 (6)</td>
</tr>
<tr>
<td>Diet-induced overweight</td>
<td>46 ± 6 (6)</td>
<td>40 ± 12 (7)</td>
</tr>
<tr>
<td>Normal weight (L-NAME)</td>
<td>27 ± 3 (5)</td>
<td>31 ± 5 (4)</td>
</tr>
<tr>
<td>Diet-induced overweight (L-NAME)</td>
<td>35 ± 3 (6)</td>
<td>40 ± 4 (8)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. Experiments were performed in the pressure-perfusion myograph in absence and presence of 100 μM L-NAME. Curve-fit estimates: amount of flow inducing a 50% response (Flow50%) and maximum response to flow (Diammax) as a percentage of precontraction to U46619. *Significant difference between placebo- and relaxin-pretreated rats (P < 0.05).
flow sensitivity 2.67-fold (Flow_{50\%} from 48 ± 9 to 18 ± 4 μl/min) but had the opposite effect in diet-induced overweight female rats (35 ± 7 to 54 ± 16 μl/min), although this was not significant (P = 0.22; Fig. 1 and Table 2). There was no significant effect of relaxin infusion on mesenteric artery Diam_{max} in either weight group (Table 1). NO blockade blunted the different flow-mediated responses to relaxin in both weight groups but had no effect on Diam_{max} (Table 2).

**Myogenic Reactivity and Vascular Compliance**

Myogenic reactivity was recorded in the presence of calcium (Fig. 2). We detected a significant (P = 0.02) increase in MR_{overall} in the mesenteric arteries of placebo-treated diet-induced overweight female rats compared with those in the normal-weight group. This was probably due to smaller basal diameter, since MR_{<60} was not significantly affected (from −1 ± 2 to 0 ± 2 μm/10 mmHg; P = 0.67). Chronic infusion of relaxin in normal-weight female rats reduced both MR_{overall} (P = 0.01) and MR_{>60} (from −1 ± 2 to 7 ± 3 μm/10 mmHg; P = 0.03), whereas it did not significantly affect MR_{overall} (P = 0.1) and MR_{>60} (from 2 ± 2 to 3 ± 2 μm/10 mmHg; P = 0.43) in diet-induced overweight female rats.

Mesenteric arterial compliance (measured in the absence of calcium) did not differ significantly between the diet-induced overweight and normal-weight placebo-treated female rats (Fig. 3). However, vessel wall thickness in diet-induced overweight female rats was significantly increased (P = 0.05) compared with normal weight female rats (Fig. 3C). Chronic infusion of relaxin did not significantly alter stress-strain curves, vessel wall thickness, VC_{overall}, or VC_{>60} in either weight group.

**Response to Phenylephrine**

Mesenteric arteries of placebo-treated diet-induced overweight female rats had significantly (P = 0.04) increased sensitivity to phenylephrine (C_{50\%}) by 17% (from 1.39 ± 0.08 to 1.19 ± 0.02 μM) compared with normal-weight female rats (Table 3). Pretreatment with L-NAME also significantly (P < 0.01) diminished R_{max} to phenylephrine in placebo-treated diet-induced overweight female rats. Relaxin treatment significantly (P < 0.01) decreased the sensitivity to phenylephrine (C_{50\%}) by 28% (from 1.39 ± 0.08 to 1.78 ± 0.10 μM) in normal-weight female rats (Table 3). Under NO blockade, this effect was reduced but still significant (from 0.89 ± 0.04 to 1.06 ± 0.07 μM; P = 0.04). In diet-induced overweight female rats, relaxin had no effect on C_{50\%}. However, in the presence of L-NAME, there was a significant (P < 0.01) desensitizing effect of relaxin (from 0.84 ± 0.02 to 1.10 ± 0.05 μM). Relaxin did not affect the maximum response (R_{max}) in either weight group, although pretreatment with L-NAME increased R_{max} in diet-induced overweight female rats (P = 0.05).

**IPRK Model**

The IPRK experiments resulted in RPFF response curves and corresponding curve fit estimates of normal-weight and diet-induced overweight female rats (Fig. 4). Comparison of the two placebo-treated weight groups demonstrated a significant (P < 0.001) reduced RPFF_{baseline} by 16% (from 6.9 ± 0.2 to 5.8 ± 0.1 ml·min\(^{-1}\)·100 g\(^{-1}\)) in the diet-induced overweight

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**Fig. 1.** Flow-mediated vasodilator response (left) and corresponding Flow_{50\%} (right) in normal weight (n_{RLX} = 7 and n_{PLAC} = 6) and diet-induced overweight (n_{RLX} = 7 and n_{PLAC} = 6) female rat mesenteric arteries, pretreated with relaxin (RLX; 4 μg/h for 5 days) or placebo (PLAC). §Significant difference between placebo- and relaxin-treated rats (P < 0.05).

**Fig. 2.** Myogenic reactivity (in presence of calcium) in normal weight (n_{RLX} = 10 and n_{PLAC} = 8) and diet-induced overweight (n_{RLX} = 9 and n_{PLAC} = 9) female rat mesenteric arteries, pretreated with RLX (4 μg/h for 5 days) or PLAC. §Significant difference between placebo- and relaxin-treated rats (P < 0.05). 1 and 2 Differences in the within-subjects factor and the between-subjects factor, respectively.
female rats but no effect on NO-mediated vasodilation (RPFF$_{\text{delta}}$ from 3.9 ± 0.4 to 3.6 ± 0.1 ml·min$^{-1}$·100 g$^{-1}$). Chronic infusion with relaxin had no effect on RPFF$_{\text{baseline}}$ (from 6.9 ± 0.2 to 6.7 ± 0.1 ml·min$^{-1}$·100 g$^{-1}$; $P = 0.38$) and RPFF$_{\text{delta}}$ (from 3.9 ± 0.4 to 4.1 ± 0.3 ml·min$^{-1}$·100 g$^{-1}$; $P = 0.78$) in normal-weight female rats. In the diet-induced overweight female rats, relaxin lowered RPFF$_{\text{baseline}}$ by 3% (from 5.8 ± 0.1 to 5.6 ± 0.1 ml·min$^{-1}$·100 g$^{-1}$; $P = 0.01$) but also lowered RPFF$_{\text{delta}}$ 14% (from 3.6 ± 0.1 to 3.1 ± 0.1 ml·min$^{-1}$·100 g$^{-1}$; $P < 0.001$).

**Rxfp1 Gene Expression**

To confirm that *Rxfp1* was expressed in the mesenteric arteries and test the hypothesis that diet or relaxin treatment could affect expression, we used quantified *Rxfp1* expression. *Rxfp1* was expressed in the mesenteric arteries, with a high degree of variation (Ct value range of 7.79 –19.53). We did not find differences in 18S expression among the weight and treatment groups. There was no significant difference in expression between the two weight groups and no effect of relaxin treatment (Fig. 5).

**DISCUSSION**

Chronic relaxin exposure stimulates mesenteric vasodilation by affecting various independent responses involved in local vascular control. These effects are largely NO dependent. The ex vivo kidney is insensitive to relaxin. High-fat diet-induced

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**Table 3. Phenylephrine response curve-fit estimates in mesenteric arteries of normal-weight and diet-induced overweight female rats pretreated with relaxin (4 μg/h for 5 days) or placebo**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Relaxin</th>
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<tbody>
<tr>
<td></td>
<td>($n$)</td>
<td>($n$)</td>
</tr>
<tr>
<td>$C_{50%}$, μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>1.39 ± 0.08</td>
<td>1.78 ± 0.10*</td>
</tr>
<tr>
<td>Diet-induced overweight</td>
<td>1.19 ± 0.02†</td>
<td>1.21 ± 0.04</td>
</tr>
<tr>
<td>Normal weight (l-NAME)</td>
<td>0.89 ± 0.04</td>
<td>1.06 ± 0.07*</td>
</tr>
<tr>
<td>Diet-induced overweight (l-NAME)</td>
<td>0.84 ± 0.02</td>
<td>1.10 ± 0.04*</td>
</tr>
<tr>
<td>$R_{\text{max}}$, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>115 ± 4</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Diet-induced overweight</td>
<td>112 ± 3</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>Normal weight (l-NAME)</td>
<td>127 ± 3</td>
<td>122 ± 4</td>
</tr>
<tr>
<td>Diet-induced overweight (l-NAME)</td>
<td>114 ± 3†</td>
<td>124 ± 3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$ = number of animals. Experiments were performed in the wire myograph in absence and presence of 100 μM l-NAME. Curve-fit estimates: concentration of phenylephrine inducing a 50% response ($C_{50\%}$) and maximum response ($R_{\text{max}}$) as a percentage of the maximum response to 124 mM KCl. *Significant difference between placebo- and relaxin- pretreated rats ($P < 0.05$). † Significant difference between normal-weight and diet-induced overweight rats ($P < 0.05$).
overweight impairs normal mesenteric in vitro vasodilation and lowers RPFF in response to high levels of relaxin. Chronic relaxin-treatment improves the vasodilator state of normal weight female rat mesenteric arteries by affecting several independent pathways. We observed that it raises flow-mediated vasodilation and lowers sensitivity to phenylephrine, both (partially) by enhanced NO-mediated vasodilation. This is in line with observations made by others on the relaxin effects on NO-dependent vasodilation and angiotensin-II sensitivity (12). In vivo experiments in healthy normal-weight female rats have shown that relaxin raises renal plasma flow (RPF) up to 40% by increasing NO availability (12). In our ex vivo experiments, relaxin had no effect on the RPFF in normal-weight female rats. Whole organ perfusion is affected by perfusate viscosity and solutions’ oxygen delivery capacity. As we added oxygen-carrier pluronic to the perfusate and since we were able to detect small changes in renal function between the weight groups, we think that the absence of a response to relaxin is unlikely due to the IPRK model. Our data therefore suggest that, in healthy female rats, in the absence of humoral and/or autonomic control, relaxin does not intrinsically adjust kidney function. Being mildly overweight in young subjects induces renal hyperfiltration through hyperinsulinemia (36). In the isolated setting, we observed that diet-induced overweight reduced RPFF without affecting NO synthase activity (29). The reduced basal RPFF may be explained by enhanced renal myogenic tone, as observed in overweight-related dyslipidemia (18). Above the diet-induced overweight-related renal impairment, relaxin pretreatment decreased basal RPFF and lowered NO-mediated renal vasodilation.

Our study has several potential limitations. First, our high-fat diet induced only mild overweight. On the analogy of human diet-related overweight, this high-fat diet model has been used in literature to investigate overweight-related vas-
cular changes in rats (17). As reported by others (2, 14), the moderate increase in body weight was sufficient to cause significant differences in vascular responses. Second, the human relaxin plasma levels we detected were a little higher than 30 ng/ml, as reported by others (12). This may be explained by volume distribution, relaxin metabolism, or clearance differences among different rat strains. As the acute effects of relaxin predominate (19) and since we exposed both the ex vivo kidney and mesenteric artery experiments to the same amount of trial medication as used by others, it is unlikely that this may have confounded our results. Third, rats were enrolled into the experiments independently of their estrous cycle. We think that our randomization procedure has distributed the estrous stages equally among groups, and therefore the cycle stage does not explain the observed effects of relaxin.

Relaxin is thought to act through the G-protein-coupled Rxfp1 receptor, activating a vasoconstrictive mitogen-activated protein kinase (MAPK) pathway (39) and the normally predominant NO-mediated vasodilator phosphatidylinositol 3-kinase (PI3K) pathway (13), possibly by interference of endothelin (ET) and its receptors (ETA and ETB) (10). High-fat diet, similar to that used in the present study, induces overweight-related insulin resistance (33), which leads to inhibition of the PI3K pathway and overexpression of the MAPK pathway (24). Although insulin and relaxin induce vasodilation through activation of different receptors (tyrosine kinase and the Rxfp1 receptor, respectively), they both use similar downstream signaling pathways. Our study showed comparable Rxfp1 receptor expression between normal diet and high-fat diet female rats. In analogy to insulin resistance, a downregulated PI3K pathway and an upregulated MAPK pathway could explain our findings on relaxin resistance in high-fat diet-induced overweight female rats, which results in a relaxin-induced vasoconstrictive state by a relatively impaired NO pathway.

In summary, diet-induced overweight impairs normal mesenteric endothelium-dependent and -independent in vitro vasodilation and reduces RPFF in response to relaxin exposure. This suggests the presence of relaxin resistance in high-fat diet-induced overweight female rats.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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