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Diet-induced hypertension in rats is associated with increased renal vasoconstrictor response to angiotensin II after imitated endothelial dysfunction

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ABSTRACT

The mechanisms behind development of diet-induced hypertension remain unclear. The kidneys play a paramount role in blood volume and blood pressure regulation. Increases in renal vascular resistance lead to increased mean arterial blood pressure (MAP) due to reduced glomerular filtration rate and Na+ excretion. Renal vascular resistance may be increased by several factors, e.g. sympathetic output, increased activity in the renin-angiotensin system or endothelial dysfunction. We examined if a 14-week diet rich in fat, fructose or both led to increased renal vascular resistance and blood pressure. Sixty male Sprague-Dawley rats received normal chow (Control), high-fat chow (High Fat), high-fructose in drinking water (High Fructose), or a combination of high-fat and high-fructose diet (High Fat + Fruc) for 14 weeks from age 4-weeks. Measurements included body weight (BW), telemetry blood pressures, renal blood flow in anesthetized rats, plasma concentrations of atrial natriuretic peptide and glucose, as well as vessel myography in renal segmental arteries. Body weight increased in both groups receiving high fat, whereas MAP increased only in the High Fat + Fruc group. Renal blood flow did not differ between groups showing that renal vascular resistance was not increased by the diets. After inhibiting nitric oxide and prostacyclin production, renal blood flow reductions to Angiotensin II infusions were exaggerated in the groups receiving high fructose. MAP correlated positively with heart rate in all rats tested. Our data suggest that diet-induced hypertension is not caused by an increase in renal vascular resistance. The pathophysiological mechanisms may include altered signaling in the renin-angiotensin system and increases in central sympathetic output in combination with reduced baroreceptor sensitivity leading to increased renal vasoconstrictor responses.

1. Introduction

The incidence of obesity (body mass index (BMI) >30 kg/m²) is epidemic. Obesity is linked to hypertension, diabetes, and development of chronic renal failure. The mechanisms behind obesity-induced elevations of blood pressure are not fully clarified. In humans, obesity increases fractional proximal Na⁺ reabsorption (Strazzullo et al., 2006) indicating that hypertension in obesity may be due to increased renal Na⁺ retention. Increased renal Na⁺ reabsorption will have at least two consequences: 1) increased extracellular volume activating the atrial natriuretic peptide (ANP) system and reducing sympathetic output via atrial volume receptors; 2) reduced distal tubular Na⁺ reabsorption (NaCl) increasing renin secretion and angiotensin II (Ang II) levels (Hall et al., 2019) and deactivating the tubuloglomerular feedback (TGF) thus increasing glomerular filtration rate (GFR) and renal blood flow (RBF). However, obese subjects have significantly reduced

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plasma ANP levels (Wang et al., 2004) which may further contribute to the increased renal Na\(^+\) reabsorption and possibly also increased renal vascular resistance. Furthermore, ANP is known to suppress renin release and central sympathetic activity (Schlueter et al., 2014). A reduced ANP activity can therefore lead to increased renin secretion and sympathetic activity in obese subjects.

The increased activity in the sympathetic nervous system observed in obese subjects may lead to further activation of the renin-angiotensin system (RAS) (Krieger and Landsberg, 1988). Consequently, renal Na\(^+\) reabsorption and renal vascular resistance will increase in obese subjects. In dogs fed a high-fat diet for 9 weeks, an increase in plasma renin activity was found (Henegar et al., 2001). Blood pressure, renal blood flow (RBF) and glomerular filtration rate (GFR) was increased suggesting that increased activity in RAS caused the hypertension (Henegar et al., 2001). In Sprague Dawley rats fed fructose for 8 weeks, Ang II-induced renal vasoconstriction was reduced possibly due to desensitization (Abdulla et al., 2012). GFR was unchanged whereas cortical single nephron GFR and blood flow was reduced (Sanchez-Lozada et al., 2007) suggesting increased vascular resistance in cortical afferent arterioles.

On the other hand, in genetically induced obese Zucker rats with hypertension, a decreased level of renin activity and increased RBF was found (Stepp et al., 2007) whereas vascular responses to Ang II were significantly increased. Others have shown increased afferent arteriolar diameter in obese Zucker rats (Roos et al., 2008), consistent with vascular outward remodeling observed in obese Zucker rats at 24–37 weeks of age (Lohr et al., 2015). Increased renal perfusion pressure (RPP) in Obese Zucker rats led to reduced afferent arteriolar constriction indicating reduced autoregulatory ability (Hayashi et al., 2002). Thus, the effect on afferent arteriolar resistance and the renal vascular response to Ang II seems to differ in diet-induced hypertension compared to genetically induced obesity.

Aortic endothelial dysfunction was observed in rats fed a high fat/high fructose diet for 16 weeks (Panchal et al., 2011). Afferent arterioles from rats fed a high-fat diet for 6 weeks had an impaired vasodilatory response to acetylcholine (ACh) (Elmarakby and Imig, 2010) suggesting renal endothelial dysfunction. This was also shown in afferent arterioles from obese Zucker rats (Hayashi et al., 2002).

In this study, we examined the development of diet-induced hypertension and the relevance of ANP, renal vascular responsiveness and endothelial function. Our initial hypothesis was that an increase in renal vascular resistance drives the development of hypertension irrespective of changes in plasma ANP levels, sympathetic activity and vascular responses to Ang II.

2. Methods

2.1. Animals and diets

All experiments were approved by the Animal Experiments Inspectorate of the Danish Ministry of Justice and performed in compliance with the guidelines from Directive 2010/63/EU on the protection of animals used for scientific purposes. Sixty male Sprague-Dawley (SD) rats (Taconic, Lille Skensved, Denmark) were used in the study. Young (4-week) rats were randomly divided into 4 groups. Three groups were exposed to experimental diets. The remaining group served as age-matched controls and received water and normal rat chow (13 kcal/100 kcal saturated fat, Altromin 1319, Brogaarden, Lyng, Denmark) ad libitum (Control group). One group (High Fat) received a high-fat chow (60 kcal/100 kcal saturated fat, D12492, Research Diets, New Brunswick, USA) and tap water. Another group (High Fructose) was fed normal rat chow and drinking water, to which 10% w/v fructose was added (F0127, Sigma-Aldrich, Brondby, Denmark). The last group (High Fat + Fruc) received the high-fat diet and 10% fructose in the drinking water. The diets were maintained for a total of 14 weeks, beginning at age 4-week until age 18-week. The diet and control groups were kept in cages adjacent to each other and received simultaneous feeding and cleaning procedures to minimize handling bias.

2.1.1. Substudies

Three different sets of experiments were performed in separate groups of animals (A-C):

A) Measurement of systolic blood pressure (SBP) and heart rate (HR) using tail cuffs. Fasting glucose and ANP plasma concentrations were measured at diet weeks 0, 7 and 14. In vivo measurement of renal blood flow responses were performed in anesthetized rats at diet week 14. This experimental set included 9 Control rats, 5 High Fat rats, 5 High Fructose rats and 5 High Fat + Fruc rats.

B) Measurement of mean arterial blood pressure (MAP), SBP and HR using telemetry. This experimental set included 5 Control rats, 5 High Fat rats, 6 High Fructose rats and 6 High Fat + Fruc rats.

C) Measurement of renal vascular responses using wire myography. This experimental set included 7 Control rats and 7 High Fat + Fruc rats.

2.2. Body weight

Body weight (BW) was measured weekly in all rats used for blood pressure measurements (experiment A and B). In experiment C body weight was measured only at diet week 14.

2.3. Systolic blood pressure and heart rate

SBP was measured every week in experiment A using the tail-cuff method. For SBP comparisons, the High Fat group (N = 5) was matched with Control group 1 (N = 3), the High Fructose group (N = 5) was matched with Control group 2 (N = 3) and the High Fat + Fruc group (N = 5) was matched with Control group 3 (N = 3). All 8 rats in matched groups had SBP measured the same day by the same technician. This was done to avoid un-intentional changes in blood pressure as the rats were sensitive to noise, smell, temperature etc. For all other determinations (glucose and ANP plasma concentrations and RBF measurements), Control rats (N = 9) were pooled. For SBP measurements, rats were placed in a size-matched restrainer and maintained at constant ambient temperature (32 °C) (Model 303SC, IITC Inc., Woodland Hills, CA). A tail-cuff consisting of a metal tube lined with a thin, inflatable rubber bladder, a light source, and a photo cell sensor, was placed at the base of the tail. The tail-cuff was connected to a cuff pump (Model 31, IITC Inc.) and a semiautomatic blood pressure analyzer. The cuff was inflated to a pressure exceeding expected SBP (approx. 210 mmHg), and was subsequently deflated over 30 s. Recordings of the blood pressure pulsations during this 30-s period was stored on a PC for later offline analysis. The SBP was determined as the blood pressure at which pulsations returned during cuff deflation. Rats were accustomed to the SBP measurements from the first diet week in order to minimize the influence of stress on the SBP recordings.

2.4. Mean arterial blood pressure and heart rate

To allow for continuous monitoring of arterial blood pressure in freely moving animals, a telemetry transmitter (model PA-C40; Data Science International, St. Paul, MN, USA) was aseptically implanted in the abdominal aorta, under isoflurane anesthesia. After a ten day recovery period, blood pressure data was acquired continuously at 500 Hz over a 48-h period using PONEMAH 5.20 software (Data Science International). MAP, SBP and HR were derived from the blood pressure curve by the software and used for analysis. MAP was calculated on a beat-to-beat basis as the average pressure in one cardiac cycle. HR was calculated as the inverse of the time interval (PI) for the cardiac cycle multiplied by 60, as estimated from the pressure data. Thus, HR at time n was found as HR(n) = 1/PI(n) x 60. Data acquisition was started on
Friday afternoon and data used for analysis collected from Saturday 06:00 to Monday 06:00, to ensure minimal disturbance of the animals.

2.5. Plasma concentrations of atrial natriuretic peptide and glucose

At diet-weeks 0, 7 and 14 blood samples were drawn from the tail to measure plasma concentrations of atrial natriuretic peptide (ANP) and glucose. The samples were drawn after overnight fasting. ANP was measured by a commercial enzyme immunoassay (EIA) kit against rat plasma. Glucose was measured with an Accu-Chek glucose meter (Roche, Germany).

2.6. Renal blood flow measurements: Surgical procedure for in vivo experiments

Rats were anesthetized with isoflurane delivered in 65% nitrogen and 35% oxygen. These rats had previously had their SBP and HR measured using the tail cuff method (experiment A above). A polyethylene (PE-50) catheter was placed in the left carotid artery for measurements of arterial pressure using a Statham P23-dB pressure transducer (Gould, Oxnard, CA, USA). Two PE-10 catheters in the right jugular vein allowed for continuous i.v. infusions. Isotonic saline was given continuously at a rate of 20 μl/min. The muscle relaxant Cisatracurium (Nimbex, 0.85 mg/ml; GlaxoSmithKline, Brondby, Denmark) was administered as a 0.5 ml bolus, followed by continuous infusion (17 μg/min in physiological saline (20 μl/min)). A tracheotomy was performed and the rat was ventilated (tidal volume 0.8 ml/100 g body weight) at a frequency of ~70 breaths/min. A body temperature of 37°C was maintained by placing the rat on a servo-controlled heating table. An isotonic saline concentration of ~2% was needed to maintain sufficient anesthesia and stable blood pressure levels. After midline and subcostal incisions, the abdominal aorta and left kidney were exposed. A tapered and curved PE-10 catheter was introduced through the left iliac artery and advanced through the abdominal aorta and ~1 mm into the left renal artery. Test agents were administered through this catheter directly into the renal artery to minimize systemic effects. The catheter did not interfere with RBF measurements. A perivascular flow probe (1PRB; Transonic T 420) was placed around the left renal artery to measure RBF. The left ureter was catheterized (PE-10 connected to PE-50) to ensure free urine flow. After the surgical procedure was completed, the rat was allowed to recover for ~30 min before the experimental protocol was initiated.

2.7. Experimental protocol in vivo

Before administration of vasoactive substances, the infusion rate in the renal artery catheter was increased from 10 to 144 μl/min, to ensure that the substances reached the renal vasculature almost instantly (Steendahl et al., 2004). An Upchurch six-port injection valve (Upchurch Scientific, Oak Harbor, WA USA) was used for administration of Ang II (bolus 2 ng/10 μl) and ACh (calculated renal plasma concentration 0.5 μM given for 90 s). After the initial Ang II and ACh responses were recorded indomethacin (5 mg/kg i.v. Sigma-Aldrich, Copenhagen, Denmark) was given as a bolus followed by N (G)-nitro-L-arginine methyl ester (L-NAME, Sigma-Aldrich; 10 mg/kg i.v. for 30 min). After 30 min equilibration, the responses to Ang II and ACh were measured again.

2.8. Isometric myograph force measurement

Rats (Control N = 7; High Fat + Fruc N = 7) were euthanized using spinal cord dislocation. Both kidneys were excised and bathed in cold dissection buffer (in mmol/l: NaCl 135, KCl 5, MgSO4 7H2O 1, HEPES 10, glucose 5, CaCl2 1 and albumin 5 g/l, pH 7.4). Under microscope, segmental arteries (length ~ 2 mm) were dissected free and cleaned from surrounding renal tissue. The arteries were threaded onto two stainless steel wires (0.40 mm) and transferred to a preheated (37°C) myograph chamber (Danish Myo Technology A/S, Aarhus, Denmark) containing a physiological saline solution (PSS in mmol/l: NaCl 130, NaHCO3 14.9, KCl 4.7, MgSO4 7H2O 1.17, KH2PO4 1.18, glucose 5.5, CaCl2 1.6, EDTA 0.026) aerated with 95% O2 and 5% CO2, resulting in a pH of 7.4. The period between isolation and the start of experiments was less than 2 h. The vessels were normalized to a tension equivalent to 0.9 times the tension found at a transmural pressure equivalent to 100 mmHg (Mulvany and Halpern, 1977).

2.8.1. Myograph protocol

All protocols were initiated with two successive exposures to PSS containing 60 mmol/l K+ and 10 μmol/l norepinephrine (NE) serving as viability test. Subsequently, the vessels were pre-constricted with NE (1 μmol/l) and cumulative dose-response curves to ACh (1 nmol/l to 10 μmol/l) and GLP-1 (1 pmol/l – 1 μmol/l) were performed. ACh or GLP-1 were added to the chamber every 90 s. The vessels were allowed to equilibrate for 20 min between experiments. Thereafter, at time control curve was generated by adding 1 μmol/l NE without any vasodilators for an equivalent duration as in the concentration-response experiments.

2.9. Solution and drugs

Myograph experiments: All compounds were prepared as stock solutions in water and diluted in PSS. Norepinephrine, ACh and GLP-1 were both added directly to the myograph chamber, according to the concentrations stated above. Viability tests were made using KPSS (in mmol/l): NaCl 74.7, NaHCO3 14.9, KCl 60, MgSO4 7H2O 1.17, KH2PO4 1.18, glucose 5.5, CaCl2 1.6, EDTA 0.026. Myograph bath buffers were heated to 37°C before use.

2.10. Data handling and statistical analyses

Increases in body weight were followed over time in experiments A and B and pooled with results from experiment C at 14 weeks. Changes in blood pressure and heart rate were measured during the last 7 diet weeks. Changes in RBF after intrarenal infusion of Ang II and ACh were calculated as an average of 30 s during the maximum responses. Changes in tension after addition of ACh or GLP-1 were calculated as the maximum response to each concentration normalized to the maximum NE contraction. Data are presented as Δ values where the baseline values were calculated as the last 30 s before infusion of vasoactive substances. Changes in isometric tension were calculated as % of the NE-induced contraction.

Statistical analyses were performed using SigmaPlot 14 (Systat Software, Germany) or GraphPad Prism 5 (GraphPad Software, Inc.). Differences in BW between groups for experiments A and B at diet week 14 were compared using a one-way ANOVA with a Student-Newman-Keuls posthoc test. Body weight (diet week 14, all rats) was analyzed using a two-way ANOVA with Fat vs. Fructose as factors, and an interaction term. Differences over time and between groups were analyzed using a two-way ANOVA with repeated measures using a Bonferroni posthoc test. The Control group at diet week 8 was used as control when comparing telemetry blood pressure and HR over time. Tail BP data were tested using a two-way ANOVA with rat group vs. diet week as factors, and an interaction term. Baseline MAP, RBF and renal vascular resistance (RVR) data were compared using a one-way ANOVA. MAP vs. HR data correlation was performed as fit to data using a linear model and reporting a slope with 95% confidence intervals, a goodness of fit (R2), and a P-value for testing slope deviation from zero. P < 0.05 was considered significant. Values are reported as mean ± SEM unless otherwise stated.
3. Results

3.1. Body weight

BW over time in rats fed a control, high fat, high fructose or high fat + fructose diet are shown in Fig. 1A. All groups had comparable BW prior to initiation of the different diets (Control: 74 ± 3 g; High Fat: 73 ± 3 g; High Fructose: 73 ± 4 g; High Fat + Fruc: 66 ± 3 g). After 14 weeks of diet, all groups had a significantly increased BW (Control: 452 ± 14 g; High Fat: 520 ± 20 g; High Fructose: 449 ± 7 g; High Fat + Fruc: 522 ± 18 g; P < 0.01). The increase in BW was, however, significantly larger in the High Fat and High Fat + Fruc groups compared to Control and High Fructose (P < 0.05).

The two-way ANOVA factor analysis showed no effect of fructose on BW at 14 weeks of diet, but a highly significant effect of high fat (P < 0.001) (Fig. 1B).

3.2. Systolic blood pressure, mean arterial blood pressure and heart rate

Before onset of feeding, rats in the individual groups had comparable systolic blood pressure (Control: 118 ± 5 mmHg; High Fat: 120 ± 5 mmHg; High Fructose: 126 ± 7 mmHg; High Fat + Fruc: 117 ± 6 mmHg). Matching the individual diet groups with their corresponding Control groups (1–3) in the tail plethysmography determinations of SBP revealed a highly significant increase in SBP in the High Fat + Fruc group, only (Fig. 2). Due to the growth rate of the rats (from 4 to 18 weeks of age), telemetry measurements were initiated in diet week 8 (i.e. at 12 weeks of age). Telemetry-measured MAP in freely moving rats increased significantly over time (using diet week 8 as baseline) in all diet groups but not in the Control group (Fig. 3A). SBP exhibited a similar pattern (Fig. 3B). HR decreased significantly over time in the High Fructose group, only (Fig. 3C). Comparing between groups, MAP and SBP increased significantly only in the High Fat + Fruc group compared to Control (Fig. 3A and B). The increase was already significant at diet week 8. HR was significantly increased in both diet groups receiving fructose compared to Control (Fig. 3C). However, in the High Fructose group, HR decreased again at diet week 13. MAP correlated positively with heart rate from all rats (Slope = 0.34 ± 0.08; r² = 0.44; P < 0.001).

3.3. Plasma concentrations of atrial natriuretic peptide and glucose

All diet groups and the Control group had similar levels of plasma ANP and glucose in diet week 0 (Fig. 4A and B). Over time plasma ANP did not change significantly in any group. Over time plasma glucose increased in the High Fat group from diet week 0 to 7 and then decreased again. Compared to Control, plasma glucose was significantly increased in the High Fat group after 7 weeks and in the High Fructose group after 14 weeks of diet. However, as plasma levels of glucose after fasting did not increase above 5 mmol/L none of the diet groups appeared hyperglycemic over time.

3.4. Renal blood flow measurements

MAP, RBF and RVR measured before and after infusion of L-NAME and indomethacin were comparable between groups, when evaluated at

![Fig. 1. Body weight from control rats and rats kept on a diet for 14 weeks. A) Body weight measured over time in rats from experiments A and B; Control (N = 14), High Fat (N = 10), High Fructose (N = 11) and High Fat + Fruc (N = 11) rats. Rats fed High Fat or High Fat + Fruc increased significantly in body weight at diet week 14 compared to Control and to High Fructose (*, P < 0.05; one-way ANOVA). B) Body weight at diet week 14 in all rats (Control (N = 21), High Fat (N = 10), High Fructose (N = 11) and High Fat + Fruc (N = 18). Body weights were compared using a two-way ANOVA with factor analysis. Data presented as mean ± SEM.](image)
diet week 14 (Table 1). The treatment with L-NAME and indomethacin induced a significant increase in MAP and RVR and a significant decrease in RBF in all groups. RVR seemed to be higher in the control group than in the diet groups after L-NAME and Indomethacin but this difference is caused by one rat. Removing this rat resulted in an RVR of 22.2 \( \pm \) 2.2 mmHg/ml/min in the Control group, which was not significantly different from the diet groups.

An intrarenal bolus injection of Ang II reduced RBF significantly in all groups (Fig. 5A). There were no significant differences in the RBF response between groups. After treatment with L-NAME and indomethacin the reduction in RBF induced by Ang II was significantly larger in the groups receiving fructose (High Fructose: 73 \( \pm \) 10\% vs. 93 \( \pm \) 3\%, \( P < 0.05 \); High Fat + Fruc: 71 \( \pm \) 8\% vs. 99 \( \pm \) 3\%, \( P < 0.05 \)) as demonstrated by the factor analysis (Fig. 5B) showing a significant main effect (\( P < 0.05 \)) of high fructose on Ang II-induced RBF responses after treatment with L-NAME and indomethacin.

Intrarenal infusion of ACh increased RBF significantly in all groups (Fig. 5C) and there were no significant differences in the vasodilatory RBF response between groups. After treatment with L-NAME and indomethacin the ACh-induced increase in RBF was significantly reduced in all groups, with no significant differences between the groups (Fig. 5D).

Fig. 2. Systolic blood pressure (SBP) measured over time in the High Fat group (\( N = 5 \)) including corresponding Control (\( N = 3 \)), High Fructose (\( N = 5 \)) and Control (\( N = 3 \)) rats. A significant increase in SBP was found in the High Fat + Fruc only during weeks 8–14 (two-way ANOVA). Data are presented as mean \( \pm \) SEM.
**3.5. Isometric myograph force measurement**

Vessel diameter did not differ between the Control (N = 7) and High Fat + Fruc group (N = 7) (468 ± 19 μm vs. 434 ± 16 μm). Maximal isometric contraction in response to KPSS and 10 μmol/l NE were similar between segmental arteries isolated from Control and High Fat + Fruc rats (22.5 ± 1.2 mN/2 mm vs. 25.0 ± 0.9 mN/2 mm, respectively). The initial pre-contraction induced by 1 μmol/l NE was also comparable (14.8 ± 1.1 mN/2 mm vs. 15.4 ± 0.7 mN/2 mm). Addition of ACh or GLP-1 significantly reduced tension in segmental arteries from both groups (Fig. 6A and B, respectively). The pEC\textsubscript{50} for ACh was 6.1 ± 0.8 and 6.2 ± 0.8 in segmental arteries from Control rats and High Fat + Fruc rats, respectively. For GLP-1, pEC\textsubscript{50} was 6.7 ± 1.4 and 5.3 ± 3.2. There were no significant differences in the vasorelaxing responses between the two diet groups.

**4. Discussion**

The mechanisms behind diet-induced hypertension are not fully elucidated. In this study, rats fed a High Fat + Fruc diet for 14 weeks had significantly elevated BW, MAP, SBP and HR compared to Control. This co-existed with an enhanced renal vasoconstrictor response to Ang II infusions during endothelial dysfunction induced by blocking nitric oxide and prostacyclin production. In rats fed a High Fat or High Fructose diet, MAP also increased over time, but less so than in the High Fat + Fruc group. Moreover, rats fed only a High Fructose diet had no significant increase in BW compared to controls. However, High Fructose rats did have elevated HR, which also co-existed with an enhanced renal vasoconstrictor response to Ang II infusions during induced endothelial dysfunction. BW increased more in rats receiving high fat in their diets compared to rats not receiving fat. Blood pressure was not directly correlated with BW since rats receiving high fat developed a modest increase in blood pressure compared to the High Fat + Fruc group despite identical increases in BW. Prior to this study, our hypothesis was that an increase in RVR drives the development of diet-induced hypertension, for example by increased vascular responsiveness to Ang II or noradrenaline (sympathetic activity), reduced activity in the ANP system, or endothelial dysfunction. However, our data showed no obvious change in RVR despite a significantly increased MAP and SBP after 14 weeks on a High Fat + Fruc diet. As RVR was measured in anesthetized rats, these data may not reveal the RVR present in conscious rats.

Instead, three alternative hypotheses may be suggested, namely that diet-induced hypertension is caused by 1) local factors either released or lacking from the renal endothelium causing exaggerated renal vasoconstrictions 2) enhanced activity and/or expression of components of RAS causing exaggerated renal Ang II induced vasoconstrictions, or 3) enhanced central sympathetic output causing increases in heart rate and renal sympathetic nerve activity. Hypothesis 1) is supported by our data showing that NO and PGI\textsubscript{2} seem to protect against Ang II induced vasoconstriction in rats receiving high fructose. Although hypotheses 2 and 3 could be caused by reduced activity in the ANP system (Schlueter et al., 2014), no significant changes in plasma ANP levels were found in any of the diet groups arguing against a role for ANP in diet-induced hypertension. However, we cannot exclude effects via downstream signaling events in the ANP pathway.

In vivo and ex vivo renal vascular relaxations to the endothelium-dependent vasodilator ACh were not impaired in the High Fat + Fruc group. This finding is consistent with a study using rats receiving the same diet for 28 weeks, in which only the EDH-type relaxations after blocking nitric oxide and prostacyclin production were impaired at study end (Gradel et al., 2018). This finding argues against endothelial dysfunction as the primary cause of increased blood pressure in the 14-week High Fat + Fruc diet rats in the present study. Possibly, the observed increase in blood pressure in our diet groups is not severe enough to elucidate significant endothelial dysfunction. Furthermore, the renal vascular response to GLP-1 was not reduced in the High Fat + Fruc diet rats developing hypertension. In other hypertensive models, the renal expression of GLP-1 receptors has been shown to be significantly decreased, which may impair renal vasodilatation elicited by GLP-1 and thereby increase RVR (Jensen et al., 2020; Liu et al., 2015; Ronn et al., 2017).

Renal endothelial dysfunction may still be a secondary factor in the increased blood pressure. Exaggerated RBF reductions to Ang II were revealed in the diet groups receiving high fructose but only in the presence of induced endothelial dysfunction. An increased production of NO and/or PGI\textsubscript{2} may protect against increased renal vascular Ang II sensitivity caused by the high fructose. However, this is not supported by the observed increase in baseline RVR in anesthetized rats after treatment with L-NAME and indomethacin. The effect of induced endothelial dysfunction was similar in all diet groups indicating that the production of NO and/or PGI\textsubscript{2} was not increased by any of the diets. At 11 months of age mRNA expression of the AT\textsubscript{1}-R in renal cortex was increased in obese
Zucker rats compared to age-matched control rats (Xu et al., 2005), which is in accordance to a study showing that obese Zucker rats had an augmented renal vascular response to Ang II (Stepp et al., 2007). Furthermore, wild-type mice fed a high fructose diet (66% by calorie) for 8 weeks had increased aortic AT$_{1a}$-receptor expression (Shinozaki et al., 2004). In rats receiving 10% fructose in drinking water for 8 weeks beginning at weaning, MAP, RVR, and intra-renal levels of Ang I and II were increased (Yokota et al., 2018). A recent study of rats on a high fructose (20%) and high fat (35% lard) diet for 120 days showed increased SBP and RVR, together with increased expression in the kidney cortex of RAS components (renin, angiotensinogen, Ang I, Ang II) (Pessoa et al., 2020). Taken together, these data are consistent with AT$_{1}$-receptors and other RAS signaling components being upregulated by high fructose and high fat feeding, which then causes increased renal vasoconstrictor responses, reduced RBF, and increased blood pressure. In our RBF measurements, the increased RAS component expressions caused by a diet containing high fructose might be masked by shear stress-dependent release of NO or prostacyclin. However, when the production of these two paracrine substances are blocked, the renal Ang II infusions causes exaggerated vasoconstrictor responses in the High Fructose group (two-way ANOVA RM). *, P < 0.05 vs. week 0, †, P < 0.05 vs. Control at corresponding week. Data are presented as mean ± SEM.

Table 1
Physiological status of anesthetized rats before and after infusion of L-NAME and indomethacin.

|                | Baseline MAP (mmHg) | Baseline RBF (ml/min) | Baseline RVR (mmHg/ml/min) | L-N/indo MAP (mmHg) | L-N/indo RBF (ml/min) | L-N/indo RVR (mmHg/ml/min) |
|----------------|---------------------|-----------------------|------------------------------|--------------------|----------------------|---------------------------|
| Control (N = 9)| 107 ± 4             | 9.0 ± 0.8             | 14.1 ± 2.9                   | 136 ± 7            | 5.5 ± 0.7            | 35.4 ± 13.3               |
| High Fat (N = 5)| 107 ± 4             | 9.5 ± 0.7             | 11.4 ± 0.9                   | 137 ± 5            | 6.9 ± 0.6            | 20.8 ± 2.3                |
| High Fructose (N = 5)| 94 ± 2         | 9.3 ± 0.8             | 10.3 ± 0.7                   | 118 ± 3            | 5.7 ± 0.7            | 22.0 ± 2.9                |
| High Fat + Fruc (N = 5)| 96 ± 4          | 8.6 ± 0.6             | 11.4 ± 0.9                   | 118 ± 4            | 5.8 ± 0.7            | 22.1 ± 3.6                |

MAP: mean arterial pressure; RBF: renal blood flow; RVR: renal vascular resistance; L-N/indo: treatment with L-NAME and indomethacin. Data are presented as mean ± SEM. †, P < 0.01 vs. baseline.

Fig. 4. Plasma concentration of atrial natriuretic peptide (ANP; A) and glucose (B) measured over time in Control (N = 9), High Fat (N = 5), High Fructose (N = 5) and High Fat + Fruc (N = 5) rats. ANP did not change significantly in any diet group. Plasma glucose increased significantly over time in the High Fat group at diet week 7. Plasma glucose increased significantly compared to Control in the High Fat group at diet week 7 and in the High Fructose group at diet week 14 (two-way ANOVA RM). *, P < 0.05 vs. week 0. †, P < 0.05 vs. Control at corresponding week. Data are presented as mean ± SEM.
Fructose and High Fat + Fruc groups.

Some studies have shown a direct stimulating effect of fructose on the proximal Na\textsuperscript{+}/H\textsuperscript{+} exchanger NHE3 (Cabral et al., 2014; Queiroz-Leite et al., 2012). Furthermore, fructose was shown to potentiate the sodium retaining effect of Ang II on NHE3 after 8 days on a 20% fructose diet (Gonzalez-Vicente et al., 2018) and after 12 weeks on a high fructose diet the activity of the intra-renal RAS was significantly increased (Xu et al., 2017). These effects could account for the increase in blood pressure over time seen in the High Fructose group. Unfortunately, we do not have data on urinary sodium excretion from the rats in our study. Furthermore, high fructose diets seem to increase renal sympathetic nerve activity, but only when accompanied by increased salt intake (Komnenov et al., 2019).

Diets containing high fat have also been shown to increase renal sympathetic nerve activity (Khan et al., 2015; Matthews et al., 2017) and the activity in RAS (Fiorino et al., 2016). An increased mass of adipose tissue may contribute to increased sympathetic output and blood pressure via leptin signaling and central melanocortin 3/4 receptors (da Silva et al., 2008; Dunbar and Lu, 1999). From our in vivo data, it does not seem likely that the renal sympathetic nerve activity was increased by the diets containing high fructose, as RBF was comparable between groups. Furthermore, high fructose diets seem to increase renal sympathetic nerve activity, but only when accompanied by increased salt intake (Komnenov et al., 2019).

In conclusion, we found that a medium-long term (14-week) high fat
A fructose diet led to elevated blood pressure in healthy rats. A diet of high fructose or high fat alone did also lead to blood pressure increases over time although not as severe as when the two diet factors were combined. Increased body weight, as found in both groups fed high-fat diets, exaggerated the development of increased blood pressure. Increased renal vasoconstrictor responses, being buffered by endothelial factors, may be involved as indicated by increased renal vascular responses to Ang II during blockade of endothelial vasodilator production. Future experiments aimed at clarifying the mechanisms for development of diet-induced hypertension should include measurement of sodium transporter activity, sympathetic activity and RAS activity.

CRediT authorship contribution statement

Lars J Jensen Conceptualization, Methodology, Validation, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization.

Morten AV Lund Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Review & Editing.

Max Salomonsson Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Jens P Goetze Validation, Resources, Writing - Review & Editing.

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Declaration of competing interest

No disclosures.

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