Kidney oxygenation under pressure

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CHAPTER 5

Sympathetic activation by lower body negative pressure decreases kidney perfusion without parallel reduction in oxygenation in healthy humans

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ABSTRACT

There is ample evidence that systemic sympathetic neural activity contributes to the progression of chronic kidney disease, possibly via limiting renal blood flow and thereby inducing renal hypoxia. Up to now there are no direct observations of this mechanism in humans. We studied the effects of systemic sympathetic activation by Lower Body Negative Pressure (LBNP) on renal blood flow (RBF) and renal oxygenation in healthy humans.

Eight healthy volunteers (age 19-31 years) were subjected to progressive LBNP. Brachial artery blood pressure was monitored intermittently. RBF was measured by phase contrast MRI in the proximal renal artery. Renal vascular resistance was MAP divided by RBF. Renal oxygenation (R2*) was measured for the cortex and medulla by Blood Oxygen Level Dependent (BOLD) MRI, using a mono-exponential fit.

During -30 mmHg LBNP, pulse pressure decreased from 50±10 to 43±7 mmHg; MAP did not change. RBF decreased from 1152 ± 80 to 1038 ± 83 mL/min to 950 ± 67 mL/min at -30 mmHg LBNP (p = 0.013). Heart rate and renal vascular resistance increased by 38±15% and 23±8% (p=0.04), respectively. There was no change in cortical or medullar R2* (20.3 ± 1.2 s⁻¹ vs 19.8 ± 0.43 s⁻¹; 28.6 ± 1.1 s⁻¹ vs 28.0 ± 1.3 s⁻¹).

Our results indicate that sympathetic activation decreases kidney perfusion without a parallel reduction of oxygenation in healthy humans.
INTRODUCTION

Systemic hyperactivity of the sympathetic nervous system is a hallmark of chronic kidney disease (CKD) \(^1\)\(^-\)\(^3\). Moreover, sympathetic nerve activity (SNA) is an independent predictor of kidney disease progression \(^4\). Also, therapies that limit sympathetic nervous system activity have shown reno-protective effects \(^5\). This has led to the pathophysiological paradigm that sympathetic activity is a causal factor in progression of CKD \(^1,4\)\(^-\)\(^7\). The mechanism by which this occurs is either via direct stimulation of pro-fibrotic factors \(^8\) or by inducing hypoxia \(^5,9\), which is the topic of the current study.

The renal parenchyma is characterized by a steep \(pO_2\) gradient and is thereby susceptible to hypoxia \(^10\)\(^-\)\(^12\). In animal models, renal sympathetic activation decreases renal blood flow \(^13\). Simultaneously, sympathetic nerves directly innervate the renal tubules inducing sodium reabsorption and thereby increasing metabolic demand \(^10,14\). The net effect of decreased renal blood flow and increased tubular demand is therefore a decrease in oxygenation \(^10,14,15\). However, in humans direct observations of the effect of sympathetic activation on renal oxygenation are lacking.

Lower Body Negative Pressure (LBNP) can be used to experimentally increase systemic SNA in humans \(^16\)\(^-\)\(^18\). Low-grade LBNP induces sustained sympathetic activation without systemic blood pressure effects \(^17\). Moderate-grade LBNP induces further sympathetic activation with moderate hemodynamic effects, while maintaining organ perfusion pressure \(^16,19,20\). In the kidneys, LBNP reduces blood flow and glomerular filtration rate while glomerular filtration fraction (FF) remains unaffected \(^19,21,22\). LBNP is therefore ideally suited to investigate the sympatho-renal effects on renal oxygenation.

Kidney oxygenation can reliably be assessed by blood oxygen level dependent (BOLD) MRI \(^23,24\). As BOLD MRI is sensitive to the blood deoxyhemoglobin level, the acquired signal is the composite result of oxygen extraction from the blood (i.e. metabolic demand) and the rate of oxygen delivery (i.e. perfusion) \(^25\). The technique was originally validated in a porcine model \(^26\). Also, in subsequent human studies the technique was shown to provide excellent intra-individual tracking of minor changes in kidney oxygenation. For example, we showed a 5% decrease of cortical oxygenation during Angiotensin II (Ang-II) infusion in healthy humans. These changes are caused by an Ang-II driven increase in FF, i.e increasing tubular workload relative to renal perfusion. \(^27\).

There seems to be a conflict between two (patho)physiologic observations regarding the role of SNA in the kidney hypoxia. On the one hand, sympathetic activation decreases renal blood flow and increases tubular metabolic load, affecting renal oxygenation negatively \(^15\).
On the other hand, sympathetic activity does not alter FF^{19,21,22}, thereby maintaining the balance between metabolic demand and oxygen supply. Against this background, we set out to explore the physiological effect of sympathetic activation by LBNP on cortical and medullar oxygenation by BOLD MRI in healthy human subjects. We hypothesized that with low to moderate-grades of LBNP, renal blood flow and medullar oxygenation decreases, while cortical oxygenation is only affected at moderate-grade LBNP. In addition we compared the renal oxygenation effects of LBNP to those induced by Ang-II using historic data.

**MATERIALS AND METHODS**

**Study population**
Eight healthy subjects were studied (age 19-31 years, 5/3 male/female, height 1.61 – 1.85 m, weight 62 – 76 kg). Their medical histories revealed no significant disease and none used medication. Written informed consent was obtained from all participants and the study protocol (protocol number NL53367.018.15) was approved by the Medical Ethics Review Committee of the Academic Medical Center (METC AMC, Amsterdam, The Netherlands). The study was conducted in accordance with the Declaration of Helsinki 2013.

**Study design**
Kidney MRI was performed at baseline and during consecutive LBNP levels at -15, -30 mmHg and recovery to 0 mmHg for 15 minutes each. Timeline of the protocol is given in Figure 1. Based on data form previous studies^{19,21,22}, the LBNP protocol was designed to reduce renal perfusion by LBNP to a similar extent as achieved by continuous Ang-II infusion (0.3, 0.9 ng/kg/min), in our previous study in a different group of eight similarly healthy subjects (age 19 – 22 years, 5/3 male/female, height 1.61 – 1.90 m, weight 63 – 82 kg) and with identical MRI acquisitions^{27}. This enabled comparison between the oxygenation responses of sympathetic activity (by LBNP) to Ang-II.

After being instrumented with electrocardiogram (ECG) electrodes and brachial artery cuff, the subjects were placed in a custom built LBNP box (department of Medical Technology, LUMC, The Netherlands) mounted on the MRI table. The box had a fixed saddle and was sealed around the subjects’ waists using a neoprene seal just above the iliac crest. Pillows, towels and sandbags were used to provide a fully comfortable body position. An anterior MRI coil was secured over chest and abdomen. During the LBNP-challenge, blood pressure and heart rate were monitored intermittently at, at least 2-minute intervals (Accutor Plus, Datascpe Corp., USA). For subject safety there was direct two way audio communication with the LBNP vacuum pump and MRI operators. Also, a physician was present inside the MRI room to directly observe the subjects for signs of discomfort and/or pre-syncope.
Magnetic Resonance Imaging

Magnetic Resonance Imaging was performed on a 3.0 Tesla MRI system (Ingenia, Philips Healthcare, Best, Netherlands) as described previously. Survey scans, including 3D T1-weighted multi echo gradient echo (T1W GE) with Dixon reconstruction, were used to locate the position of the kidneys and renal arteries. To account for LBNP associated motion of the subject, the Dixon reconstructed survey scan was repeated at every LBNP dose. BOLD and Phase Contrast (PC) MRI scans were subsequently acquired at baseline and during each LBNP dose.

Three-directional blood flow velocity was measured by PC MRI in a slice placed perpendicular to the right proximal renal artery, as described previously. In short, the PC MRI sequence parameters were as follows: the number of ECG-triggered cardiac phases was 30, field of view (FOV) 200x200 mm, resolution = 0.65x0.65 mm², slice thickness = 3 mm, repetition time (TR)/echo time (TE) = 8.5/5.7 ms, flip angle = 10°, SENSE factor = 2 (right-left direction), Venc = 100 cm/s in all directions. Acquisition time was 3:45 minutes and the sequence was acquired during free breathing. Off line image processing for PC MRI was performed using
dedicated software (GTFlow version 2.2.9, GyroTools LLC, Switzerland). After correction for background phase-offset errors, and aliasing, mean RBF (mL/s) was calculated using manual vessel segmentation in each cardiac phase. For further RBF analysis, equal perfusion of both right and left kidney was assumed.

Changes in the BOLD MRI signal were quantitatively assessed by measuring the transverse relaxation rate (R2*) within different regions of interest (ROIs). We measured R2* using a multi-echo single-slice gradient-echo MRI sequence with the following parameters: FOV = 400 × 400 mm², resolution = 1.2 × 1.2 mm², slice thickness = 4 mm, TR = 140 ms, flip angle = 70°, TE1 = 2 ms, ΔTE = 5 ms; number of echoes = 16. Image acquisition was performed in 18 seconds during a single inspiratory breath hold in a coronal slice where the kidney cross section was largest and cortical/medullary definition best visible on the survey scans. For BOLD MRI analysis, circular ROIs with a diameter of eight voxels were defined at regular intervals throughout each kidney’s cortex and medulla in the baseline scan. For each subject, the resulting masks were then transferred to the three subsequent BOLD images. Renal R2* values were calculated for cortex and medulla separately, using mono-exponential fits, using routines written in Matlab (The MathWorks, Natrick, USA).

**Study power and statistical analysis**

The study was powered based on previous data using the same MRI protocols in healthy humans. In that study we found that a 20% perfusion reduction resulted in an 2.4 s⁻¹ increase in cortical R2*. Anticipating a potential lesser effect or potentially more variance in the current experiment, this study was powered at 0.81 to detect an R2* increase of 1.4 s⁻¹ in n=8.

All data are presented as mean with standard error. Renal vascular resistance (RVR) was calculated as the mean arterial pressure (MAP) divided by the renal blood flow (RBF). Shapiro-Wilk’s test was used to verify normal distribution of the data. One-way ANOVA for repeated measures was used to assess the dose effect of LBNP on systemic (blood pressure, heart rate) and renal (RVR, RBF) hemodynamic parameters, cortical and medullar R2*. A Z-test was used to compare the LBNP response to the historic positive control (Ang-II response). The z-score is reported with a two-tailed p-value. All statistical analyses were performed using SPSS Statistics 22 (IBM, Chicago, USA). A significance level of α=0.05 was used.
RESULTS

Subjects
All subjects tolerated the LBNP doses well and reported no periods of discomfort or light-headedness. Baseline measurements are given in Table 1.

Table 1 Baseline characteristics per subject

<table>
<thead>
<tr>
<th>Subject (#,♂/♀)</th>
<th>Hemodynamics</th>
<th>MRI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>HR (bpm)</td>
</tr>
<tr>
<td>1 ♂</td>
<td>79</td>
<td>66</td>
</tr>
<tr>
<td>2 ♂</td>
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<tr>
<td>8 ♂</td>
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<td>61</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>85 (2.1)</td>
<td>71 (3.3)</td>
</tr>
</tbody>
</table>

bpm, beats per minute; CR2*, cortical R2*; HR, heart rate; MAP, mean arterial pressure; MR2*, medullary R2*; MRI, magnetic resonance imaging; RBF, renal blood flow; RVR, renal vascular resistance. Baseline characteristics after 15 minutes of supine rest.

Systemic hemodynamic changes induced by LBNP
The absolute values of the hemodynamic parameters during each stage of the experiment are listed in Table 2. Figures 1A-C depict the responses in percentage to baseline. As expected the arterial blood pressure did not change significantly at the chosen levels of LBNP. Only the pulse pressure decreased significantly from 50 ± 3.5 at baseline to 43 ± 2.3 mmHg at -30 mmHg, F(2,14) = 6.4, p = 0.043, (Fig. 2A). The heart rate increased by 38% ± 15%, at maximum LBNP (F(2,14) = 20.1, p = 0.004, Fig. 2B). These hemodynamic effects were present in all individual subjects. The LBNP intervention did not induce (pre-)syncope in any of the subjects.

Renal perfusion and oxygenation
The absolute values MRI derived perfusion and oxygenation parameters during each stage of the experiment are listed in Table 2. Figures 1D-F depict the responses in percentage to baseline. Renal vascular resistance increased LBNP-dose dependently by 23 ± 8% at -30 mmHg LBNP (F(2,14) = 27.8, p = 0.002, Fig. 2C). RBF decreased from 17.2% ± 2.3% at -30 mmHg LBNP, F(2,14) = 11.8, p = 0.013, Fig. 2D, black line). These renal hemodynamic effects were present in all individual subjects. Nor the Cortical R2* (CR2*), nor the medullar R2* (MR2*) changed during LBNP, (Fig. 2E and F, black lines).
Comparison to an Ang-II response

As a historical positive control experiment for the present study, we compared the perfusion and R2* changes induced by 0.3 and 0.9 ng/kg/min Ang-II infusion. We used data from a previous experiment on a different group of young healthy subjects who had been recruited from the same population as the subjects in the current study. These data are presented in gray in Figure 2. In those experiments, RBF decreased dose dependently from 1215±83 to 1025±89 mL/min (Fig. 2D, gray line) and cortical R2* increased from 17.4 ± 1.1 to 19.3 ± 0.8 sec⁻¹ (Fig. 2E, gray line). The average cortical R2* response was $\beta = 0.025 ± 0.17$ in the LBNP group and $\beta = 5.9 ± 2.4$ in the Ang-II group, these responses were significantly different with a z-score of $z = 2.5$ with a two-tailed p-value of $p = 0.014$.

DISCUSSION

Our findings can be summarized as follows. LBNP consistently increased heart rate and lowered pulse pressure while organ perfusion pressure (MAP) was unchanged in all subjects. This is consistent with selective sympathetic activation. LBNP induced a marked increase in renal vascular resistance and reduced renal perfusion substantially in all subjects. However, during selective sympathetic activation there was no discernable effect on kidney oxygenation, in the cortex or medulla. This is in contrast to the decreased cortical oxygenation by Ang-II infusion with similar changes in renal vascular resistance and flow. These explorative data do not support sympathetic activity as a causal factor of renal hypoxia under physiological circumstances.
Kidney oxygenation during sympathetic activation

Figure 2 Systemic and renal hemodynamic effects of low to moderate-grade LBNP. All graphs depict percent change compared to baseline (BL) at -15mmHg LBNP, -30 mmHg LBNP and during recovery (RC). Graphs depict results of the current study in black and results from our previous study using Ang-II infusion in gray, for comparison. That study assessed the same parameters as function of continuous infusion of Ang-II. Significant responses are assessed by repeated measures ANOVA are indicated by * and † for LBNP and Ang II infusion, respectively.

In light of the two conflicting concepts surrounding the possible influences of SNA on renal oxygenation (e.g. decreased RBF with increase metabolic load vs. a maintained FF and oxygenation balance) we observe the following. During our LBNP experiments, we found a renal vascular resistance increase and perfusion reduction virtually identical to those found by other authors, in previous studies involving healthy humans 19,21,22,31. In these studies, both ERPF and GFR (assessed by radioisotope measurements) decreased proportionally by approximately 10 and 20% at -15 and -30 mmHg respectively, while maintaining FF. Applying those observations to our study, indicates that the oxygenation balance was maintained, which explains the absence of an effect on renal oxygenation by LBNP induced sympathetic activation. This is further supported by a study of Würzner et al., who also reported on the fractional distal reabsorption of sodium, which was unaffected during LBNP 21. Distal sodium reabsorption in the medullary thin ascending limb of Henle’s loop, is metabolically most demanding 10 and it seems that this process is affected by LBNP in proportion to the renal perfusion reduction, as well 21. Thus, the two processes with the most influence on renal oxygenation status, i.e. filtration fraction and distal sodium reabsorption, are affected in the same direction and in equal proportion to the RBF reduction during LBNP. This would suggest that sympathetic activation per se, does not influence renal oxygenation in healthy humans.
These observations stand in contrast to the effects of Ang-II on renal oxygenation. Contrary to sympathetic neural activation, Ang-II directly changes the balance between oxygen supply and demand by vasoconstriction of the glomerulus’ efferent arterioles. Thereby Ang-II decreases renal perfusion while metabolic demand is maintained.

This is the first study that directly measured renal oxygenation during sympathetic activation in healthy humans. We successfully used an LBNP intervention as a sympathetic stimulus, in combination with renal MRI measurements. Although the imaging area was in close proximity of the LBNP box, we did not observe any distortion in the MR images. The premise of this study was a universal physiological phenomenon. The lack of effect on cortical and medullar R2* change raises the specific limitation of a potential Type II statistical error. However, a post-hoc power calculation on the point estimate from the current study, showed that to arrange for a statistical power of 80% to consolidate this effect, the needed number of subjects is 209. Apart from the questionable relevance of such a minor effect, it is not feasible to reach such a number of subjects. We speculate that in CKD patients the effects may be discernable in smaller sample sizes.

Further limitations of our study concern the absence of direct verification of an increased sympathetic tonus (e.g. by microneurography) and the degree of sodium retention during the experiments. However, previously these effects have extensively been documented and combining these measurements with MRI is not (yet) feasible. Another limitation is that we cannot rule out that other factors were superimposed on the sympathetic neural activation. Specifically, we have not measured hormones that regulate renal hemodynamics, while it is known that activation of the renin angiotensin system (RAS) occurs at medium grade LBNP. It has been documented that 10 minutes of -18 mmHg and lower LBNP induces detectable increases in plasma renin activity and Ang-II levels. Possibly, RAS activation is reflected in the delayed return to baseline in renal perfusion and renal vascular resistance after LBNP cessation that we found. However, the RAS activation induced by LBNP was insufficient to induce renal hypoxia.

Regarding the generalizability of our results to CKD patients, we are limited by the fact that this constitutes an acute experiment in a small group of young healthy subjects versus a chronic pathophysiological process observed in patients. However, a chronically sustained sympathetic activating intervention is not feasible in humans. Whether the renal oxygenation response to LBNP is different in patients, should be subject of further studies.

In conclusion, our exploratory data question the universal physiological concept that sympathetic hyperactivity per se decreases kidney oxygenation. We showed that selective induction of sympathetic activity by LBNP induces a substantial and consistent renal blood
flow reduction, without parallel cortical or medullar hypoxia. These data are in agreement with sympathetic activation suppressing renal oxygen demand and supply equally, thus maintaining adequate tissue oxygenation.

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Chapter 5

REFERENCES


