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Original Article

ORIGINAL ARTICLE

# Kidney oxygenation, perfusion and blood flow in people with and without type 1 diabetes

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## ABSTRACT

**Background**. We used magnetic resonance imaging (MRI) to study kidney energetics in persons with and without type 1 diabetes (T1D).

**Methods**. In a cross-sectional study, 15 persons with T1D and albuminuria and 15 non-diabetic controls (CONs) underwent multiparametric MRI (3 Tesla Philips Scanner) to quantify renal cortical and medullary oxygenation ($R_2$*, higher values correspond to higher deoxyhaemoglobin concentration), renal perfusion (arterial spin labelling) and renal artery blood flow (phase contrast). Analyses were adjusted for age, sex, systolic blood pressure, plasma haemoglobin, body mass index and estimated glomerular filtration rate (eGFR).

**Results**. Participants with T1D had a higher median (Q1; Q3) urine albumin creatinine ratio (UACR) than CONs [46 (21; 58) versus 4 (3; 6) mg/g; $P < .0001$] and a lower mean ± SD eGFR (73 ± 32 mL/min/1.73 m$^2$ versus 88 ± 15 mL/min/1.73 m$^2$; $P = .12$), although not significantly. Mean medullary $R_2$* was lower in T1D (34 ± 6/s versus 38 ± 5/s; $P < .01$) corresponding to a higher oxygenation. $R_2$* was not different in the cortex. Cortical perfusion was lower in T1D (163 ± 40 versus 224 ± 49 mL/100 g/min; $P < .001$). Renal artery blood flow was lower in T1D than in CONs (360 ± 130 versus 430 ± 113 mL/min; $P = .05$). In T1D, lower cortical oxygenation and renal artery blood flow were both associated with higher UACR and lower eGFR ($P < .05$).

**Conclusions**. Participants with T1D and albuminuria exhibited higher medullary oxygenation than CONs, despite lower cortical perfusion and renal artery blood flow. This might reflect perturbed kidney energetics leading to a higher setpoint

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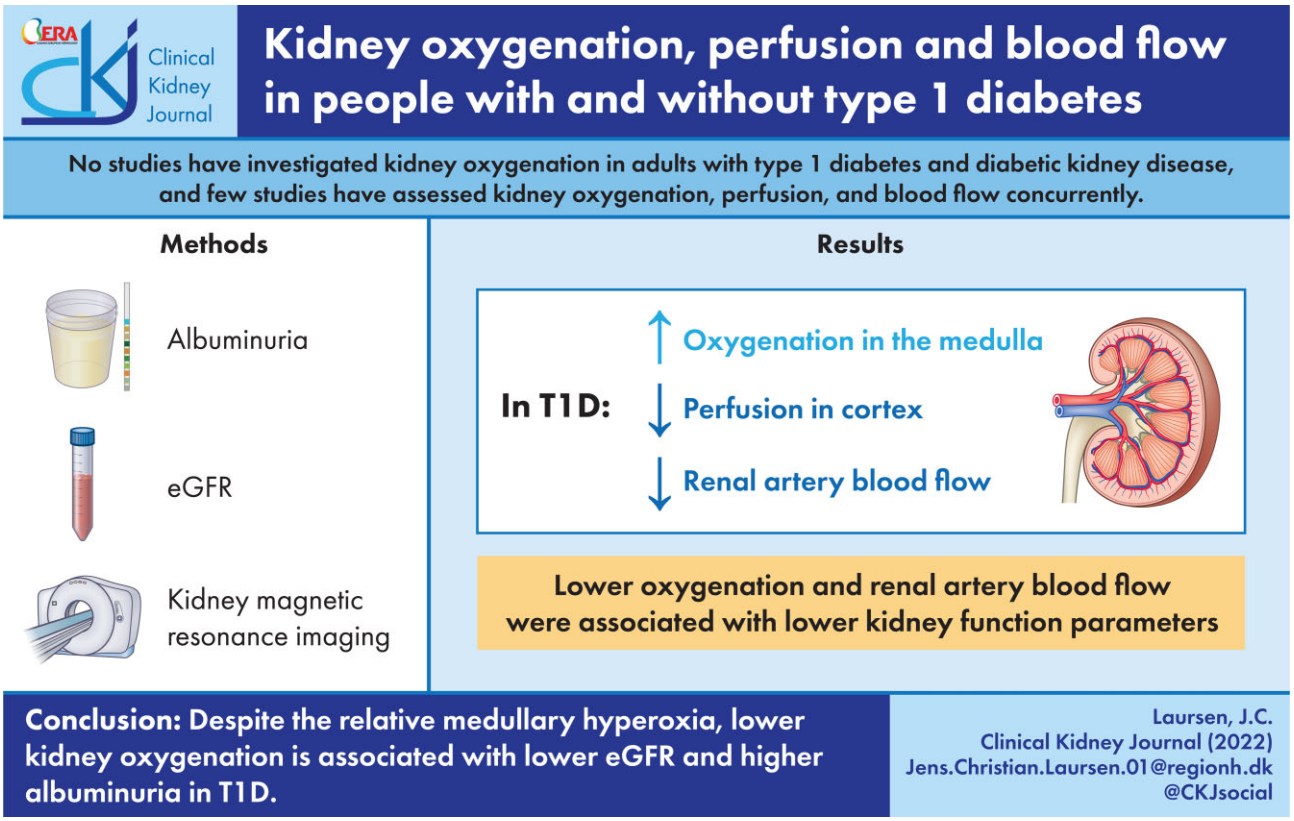
of medullary oxygenation in T1D. Lower cortical oxygenation and renal artery blood flow were associated with higher UACR and lower eGFR in T1D.

## GRAPHICAL ABSTRACT




*Keywords:* diabetic kidney disease, hypoxia inflammation, magnetic resonance imaging, renal haemodynamics, type 1 diabetes

## INTRODUCTION

Diabetes is the most common cause of chronic kidney disease (CKD) [1], a progressive disease seen in up to 10% of adults worldwide and responsible for an estimated 1.2 million deaths annually [2, 3]. Chronic kidney hypoxia has been suggested to be a unifying pathway in the pathogenesis of diabetic kidney disease, but human data remain scarce [4, 5].

Several studies have used blood-oxygen-level-dependent magnetic resonance imaging (BOLD-MRI) to estimate kidney oxygenation in individuals with varying degrees of diabetic kidney disease. Vinovskis *et al.* found relative renal hypoxia which was associated with albuminuria in adolescents with type 1 diabetes (T1D) compared with non-diabetic controls (CONs) [6]. Studies in adults with CKD with and without diabetes (both T1D and T2D) as compared with healthy controls have been inconsistent and documented either lower cortical oxygenation and no difference in medullary oxygenation [7, 8], no differences in cortical or medullary oxygenation [9–11], lower cortical oxygenation and higher medullary oxygenation [12], or no difference in cortical oxygenation and higher medullary oxygenation [13].

To our knowledge, no studies have investigated kidney oxygenation in adults with T1D and diabetic kidney disease, and few studies have assessed kidney oxygenation, perfusion and blood flow concurrently. Accordingly, the objective of this study was to compare kidney oxygenation, perfusion and blood flow between persons with T1D and albuminuria and CONs. Additionally, we sought to determine the associations among these magnetic resonance imaging (MRI) parameters, albuminuria and estimated glomerular filtration rate (eGFR). We hypothesized that cortical oxygenation would be lower and medullary oxygenation similar in persons with diabetes compared with CONs, and that lower cortical oxygenation would associate with higher albuminuria and lower eGFR in T1D. Moreover, we posited that renal perfusion and blood flow would be lower in T1D and associated with impaired kidney function.

To provide mechanistic insight into the pathophysiology leading to kidney complications in T1D and investigate the hypothesis that hypoxia is a feature of kidney disease, we explored the correlations among MRI measures and parameters important for kidney oxygenation: blood glucose [14] and blood oxygen saturation. We also evaluated the association with other suggested factors which have been implicated in the development of kidney disease. Autonomic nerve function is known

to be important for renal circulation [15] and thus, we also explored correlations with this parameter. Finally, as inflammation is thought to be a downstream consequence of kidney hypoxia [16], we explored associations between inflammation biomarkers and kidney oxygenation.

## MATERIALS AND METHODS

### Study design and participants

This cross-sectional study was conducted as part of a randomized controlled trial investigating the acute effect of dapagliflozin on kidney oxygenation in 15 persons with T1D and albuminuria [17]. Adults with T1D and albuminuria defined as a urine albumin creatinine ratio (UACR) ≥30 mg/g in two out of three consecutive samples prior to inclusion assessed from the hospital laboratory database were eligible to participate in the trial. This pre-specified sub-study included the addition of 15 age- and sex-matched CONs for comparison at baseline, i.e. before intervention with dapagliflozin or placebo. MRI measurements were performed at Rigshospitalet Glostrup, Copenhagen, Denmark, while the remaining assessments and procedures were performed at Steno Diabetes Center Copenhagen, Denmark. The study protocol was approved by the Regional Ethical Committee (H-1 952 662). All participants provided written consent. The study was registered in the EU Clinical Trials Register (EudraCT 2019–004557-92) and on ClinicalTrials.gov (NCT04193566). The study complied with the Declaration of Helsinki and Good Clinical Practice Guidelines.

### Outcomes

The primary outcome was the difference between T1D and CONs in kidney oxygenation ($R_2^*$) measured with quantitative MRI on a 3-T Philips Achieva scanner. $R_2^*$ measures were interleaved by measures of renal perfusion using arterial spin labelling (ASL) and measures of renal artery blood flow using phase-contrast sequences. $R_2^*$ is directly related to the tissue content of deoxyhaemoglobin and assuming that blood oxygenation is in equilibrium with the surrounding tissue oxygenation, it can be used as an indirect marker of intra-renal oxygenation [18]. Accordingly, numerous previous studies have validated $R_2^*$ or changes in dynamic $R_2^*$-weighted signal (BOLD) as an indirect measure of renal blood oxygenation in both patients and healthy participants [12, 19–21]. $R_2^*$ and perfusion maps were obtained for a 5 mm coronal slice covering both kidneys and measures of renal artery blood flow were obtained for the right kidney. Reported $R_2^*$ and perfusion values are the means of regions of interest covering the medulla and cortex of both kidneys. Details regarding the ASL, phase-contrast and $R_2^*$ MRI scanning protocols and data processing have been published previously [22, 23] and are in accordance with expert consensus on protocol optimization for quantitative measures of renal haemodynamic function [24–27]. ASL and phase-contrast MRI data were respiratory-gated whereas $R_2^*$ data were acquired during a single breath-hold. The mean scan duration was ∼1 h. Study participants fasted from food and liquid from midnight, the evening before the day of the scan and abstained from medication (except insulin), alcohol, tobacco use and strenuous exercise for 24 h before the visit. If participants experienced a low blood sugar (<3 mmol/L) during the fasting period they were instructed to take 10 g dextrose or drink 100 mL apple juice. All three measures were repeated three times on 1 day for CONs and three times on 2 days separated by 1 month for T1D participants (the two baseline scans in the randomized trial [17]). Means of the three measures were reported. Perfusion calculations were performed using the kinetic model presented by Buxton *et al.* [28] with a tissue-blood partition coefficient of 0.9, a labelling efficiency of 0.95 and a blood T1 value of 1.65 s. The intra-individual coefficients of variation for the measures have previously been reported [17] and were 4.3% for cortical $R_2^*$, 5.9% for medullary $R_2^*$, 7.3% for cortical perfusion, 15.4% for medullary perfusion and 3.8% for renal artery blood flow.

Detailed descriptions of methods for blood pressure, blood oxygen saturation, blood glucose and cardiovascular autonomic function have previously been reported [17, 29].

Inflammation is regarded as an important step in the pathogenesis of chronic kidney disease, i.e. chronic kidney hypoxia leading to inflammation and fibrosis [16]. Kidney tissue-specific inflammation biomarkers were not available and thus, a plasma inflammation biomarker panel was chosen as a proxy for kidney inflammation. Inflammation biomarkers were measured on peripheral blood samples with the Olink® Target 96 Inflammation panel (Olink, Uppsala, Sweden) including 92 inflammation-related human protein biomarkers [30]. Biomarker levels are shown as the Normalized Protein Expression (NPX) which is a relatively arbitrary unit on a log2 scale. Although NPX directly correlates with initial protein concentrations, no comparisons of absolute protein levels can be made.

### Statistical analysis

In the primary study, we estimated that a sample size of 15 participants per group was required to demonstrate a difference in kidney oxygenation between groups of 20%, which was considered clinically meaningful. The power calculation is described in the primary outcome paper of the randomized trial [17]. The goal of this sub-study was to demonstrate a similar difference when comparing participants with T1D with CONs.

Clinical characteristics of participants are presented as *n* (%), mean [standard deviation (SD)] or, if skewed distributions, as medians with interquartile range [interquartile range (IQR)]. For clinical characteristics, Chi-squared test and Fisher's exact test were used for categorical data and *t*-tests were used for quantitative data. Differences in MRI outcomes between the two groups and associations between MRI outcomes and kidney function in the T1D group were assessed using linear regression models. Models for differences between groups were adjusted for age, sex, systolic blood pressure, body mass index, haemoglobin and eGFR. Models for associations with kidney function were adjusted for the same variables, excluding eGFR in models with eGFR as an outcome and with eGFR in models with albuminuria as an outcome. Finally, linear regression models with kidney oxygenation (cortical or medullary) as the outcome and inflammation biomarkers as the exposure, adjusted for age, sex, systolic blood pressure, body mass index, haemoglobin and eGFR, were carried out and *P*-values were adjusted for multiple testing using the Benjamini–Hochberg method.

## RESULTS

### Clinical characteristics

Baseline characteristics are shown in Table 1. A total of 15 participants with T1D and 15 CONs were included in the study. The two groups had similar age and sex distribution. The T1D group had a mean ± SD diabetes duration of 39 ± 16 years. Systolic blood pressure was higher and baroreflex sensitivity was lower in the T1D group compared with the CONs group. The T1D group had a higher UACR and a lower eGFR, although not significantly for the latter.

**Table 1. Baseline characteristics by type 1 diabetes (T1D) or non-diabetic control (CONs) group**

| | T1D ($n = 15$) | CONs ($n = 15$) | P |
|---|---|---|---|
| Age (years) | 58 ± 14 | 56 ± 15 | .82 |
| Women, *n* (%) | 5 (33) | 5 (33) | 1 |
| Duration of diabetes (years) | 39 ± 16 | – | – |
| Body mass index (kg/m$^2$) | 26.8 ± 3.1 | 25.9 ± 7.1 | .64 |
| Smoking, *n* (%) | 2 (13) | 2 (13) | 1 |
| HR (beats/min) | 69 ± 11 | 63 ± 9 | .08 |
| Systolic BP (mmHg) | 138 ± 16 | 125 ± 12 | .02 |
| Diastolic BP (mmHg) | 80 ± 12 | 78 ± 7 | .56 |
| Blood oxygen saturation (%) | 95.6 ± 1.8 | 96.4 ± 1.4 | .14 |
| Baroreflex sensitivity (ms/mmHg) | 6 (4; 10) | 10 (6; 18) | <.001 |
| Hba1c (%) | 8.0 ± 0.6 | 5.3 ± 0.2 | – |
| Hba1c (mmol/mol) | 61 ± 7 | 34 ± 3 | – |
| Blood glucose (mmol/L) | 9.6 ± 1 | 5.8 ± 0.4 | – |
| UACR (mg/g) | 46 (21; 58) | 4 (3; 6) | <.0001 |
| eGFR (mL/min/1.73 m$^2$) | 73 ± 32 | 88 ± 15 | .12 |
| Plasma creatinine (μmol/L) | 108 ± 55 | 79 ± 11 | .06 |
| Plasma LDL (mmol/L) | 2.1 ± 1.1 | 2.5 ± 0.3 | .21 |
| Plasma haemoglobin (mmol/L) | 8.2 ± 1.0 | 8.9 ± 0.9 | .03 |
| RAAS blocking agent, *n* (%) | 13 (87) | – | – |
| Insulin, *n* (%) | 15 (100) | – | – |
| Lipid-lowering therapy, *n* (%) | 11 (73) | 1 (7) | <.001 |
| Acetylsalicylic acid, *n* (%) | 9 (60) | – | – |

Shown are mean ± SD, *n* (%) and median (quartile 1; quartile 3). T1D, type 1 diabetes; CONs, non-diabetic controls; BP, blood pressure; Hba1c, haemoglobin A1C; UACR, urine albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; RAAS, renin-angiotensin-aldosterone system.

### Kidney oxygenation, perfusion and blood flow

The mean renal cortical $R_2^*$ was for T1D 22 ± 5/s and for CONs 22 ± 3/s corresponding to a mean difference [95% confidence interval (CI)] of 0 (3 to −3)/s ($P = .94$). Renal medullary $R_2^*$ was lower in T1D (corresponding to a higher medullary oxygenation) with a renal medullary $R_2^*$ of 34 ± 6/s compared with 38 ± 5/s in CONs which corresponded to a difference of 6 (2–9)/s ($P < .01$). The difference was still significant after adjustment ($P < .001$). As a sensitivity analysis, we tried adjusting further for renal medullary perfusion and renal artery blood flow and changed neither the estimate nor the significance of the model. The renal cortical perfusion was lower in T1D compared with CONs [T1D 163 ± 40 mL/100 g/min; CONs 224 ± 49; difference 59 (30–88); $P < .001$] and the difference was still significant after adjustment ($P < .001$). The renal medullary perfusion was no different when comparing the groups, but the renal artery blood flow was lower in the T1D group [T1D 360 ± 130 mL/min; CONs 430 ± 113 mL/min; difference 70 (−7 to 147); $P = .07$], although not significantly. The difference did however become borderline significant after adjustment ($P = .05$) (Table 2).

### Associations between MRI parameters and kidney function parameters

A higher UACR was associated with a lower eGFR in the T1D group (Supplementary data, Fig. S1). In T1D, a higher UACR was associated with a higher renal $R_2^*$ (corresponding to a lower oxygenation) in the cortex and medulla as well as a lower renal artery blood flow. The associations between cortical oxygenation and renal artery blood flow remained significant after adjustment (Table 3; Fig. 1A). As a sensitivity analysis, we tried adjusting these models further for eGFR, which changed the estimates and *P*-values considerably ($R_2^*$ cortex: $\beta = 0.04$, $P = .90$; renal artery blood flow: $\beta = -0.5$, $P = .04$).

A lower eGFR was also associated with a higher $R_2^*$ in the cortex and medulla as well as a lower renal artery blood flow, and the associations with cortical oxygenation and renal artery blood flow remained significant after adjustment (Table 3; Fig. 1C).

### Associations among MRI measures and with parameters important for oxygenation

Cortical oxygenation was not associated with cortical perfusion ($\beta = -0.0$, $P = .26$) and medullary oxygenation was not associated with medullary perfusion ($\beta = -0.1$, $P = .53$). Likewise, kidney oxygenation parameters did not associate significantly with renal blood flow (cortex: $\beta = -0.0$, $P = .13$; medulla: $\beta = -0.0$, $P = .90$). Cortical and medullary oxygenation was not associated with blood glucose, blood oxygen saturation or with baroreflex sensitivity ($P > .1$).

### Associations with inflammation biomarkers

Higher levels in three biomarkers were associated with higher cortical oxygenation (SCF, TRANCE and FGF-21). Higher levels in nine biomarkers were associated with medullary hypoxia (CCL1, CXCL10, GDNF, IL-10, IL-13, IL-20, IL20RA, MCP-1 and MCP-4), meaning that a higher level of the inflammation biomarker correlated with lower medullary oxygenation (Supplementary data, Table S1).

## DISCUSSION

### Principal findings

In this study, we found that persons with T1D and albuminuria exhibited higher medullary oxygenation than CONs in the setting of lower renal cortical perfusion and renal artery blood flow. Despite the relative medullary hyperoxia, lower cortical oxygenation correlated with lower eGFR and higher albuminuria in T1D. Thus, we posit that the apparent medullary hyperoxia is a function of perturbed kidney energetics and represents a higher setpoint of medullary oxygenation in T1D. The aetiology of this relative medullary hyperoxia in the setting of impaired perfusion in T1D remains unclear.

### Previous work on renal oxygenation

No previous renal MRI studies dedicated to T1D with albuminuria or kidney disease were found. However, one recent study by Vinovskis *et al.* demonstrated relative kidney hypoxia in adolescents with T1D, which was also associated with albuminuria [6]. They did not report absolute levels of renal oxygenation as in the present study, but the association between renal hypoxia and albuminuria is in line with our findings.

In studies on CKD with and without diabetes (mixed T1D and T2D), results are conflicting. One study by Milani *et al.* demonstrated lower cortical oxygenation and higher medullary oxygenation in persons with CKD compared with healthy controls [12]. An important difference between their study and ours is that they assessed the oxygenation in 12 layers of renal tissue and reported the three most outer layers as cortical layers, and the three most inner layers as medullary layers, instead of dividing the kidney manually into cortical and medullary regions of interest like we did.

**Table 2. Magnetic resonance imaging outcomes by groups of type 1 diabetes or non-diabetic controls**

| | T1D ($n = 15$) | CONs ($n = 15$) | Difference (95% CI) | P | Adjusted P |
|---|---|---|---|---|---|
| $R_2^*$ cortex (/s) | 22 ± 5 | 22 ± 3 | 0 (3 to −3) | 0.94 | .49 |
| $R_2^*$ medulla (/s) | 34 ± 6 | 38 ± 5 | 6 (2 to 9) | <0.01 | <.001 |
| Perfusion cortex (mL/100 g/min) | 163 ± 40 | 224 ± 49 | 59 (30 to 88) | <0.001 | <.001 |
| Perfusion medulla (mL/100 g/min) | 43 ± 11 | 44 ± 15 | −0 (−8 to 7) | 0.89 | .99 |
| Renal blood flow (mL/min) | 360 ± 130 | 430 ± 113 | 70 (−7 to 147) | 0.07 | .05 |

Shown are mean ± SD and differences with 95% confidence intervals calculated by an unadjusted linear regression model using the magnetic resonance outcome as outcome and group (T1D versus CONs) as exposure. The model was further adjusted for age, sex, systolic blood pressure, body mass index haemoglobin and eGFR. T1D, type 1 diabetes; CONs, non-diabetic controls.

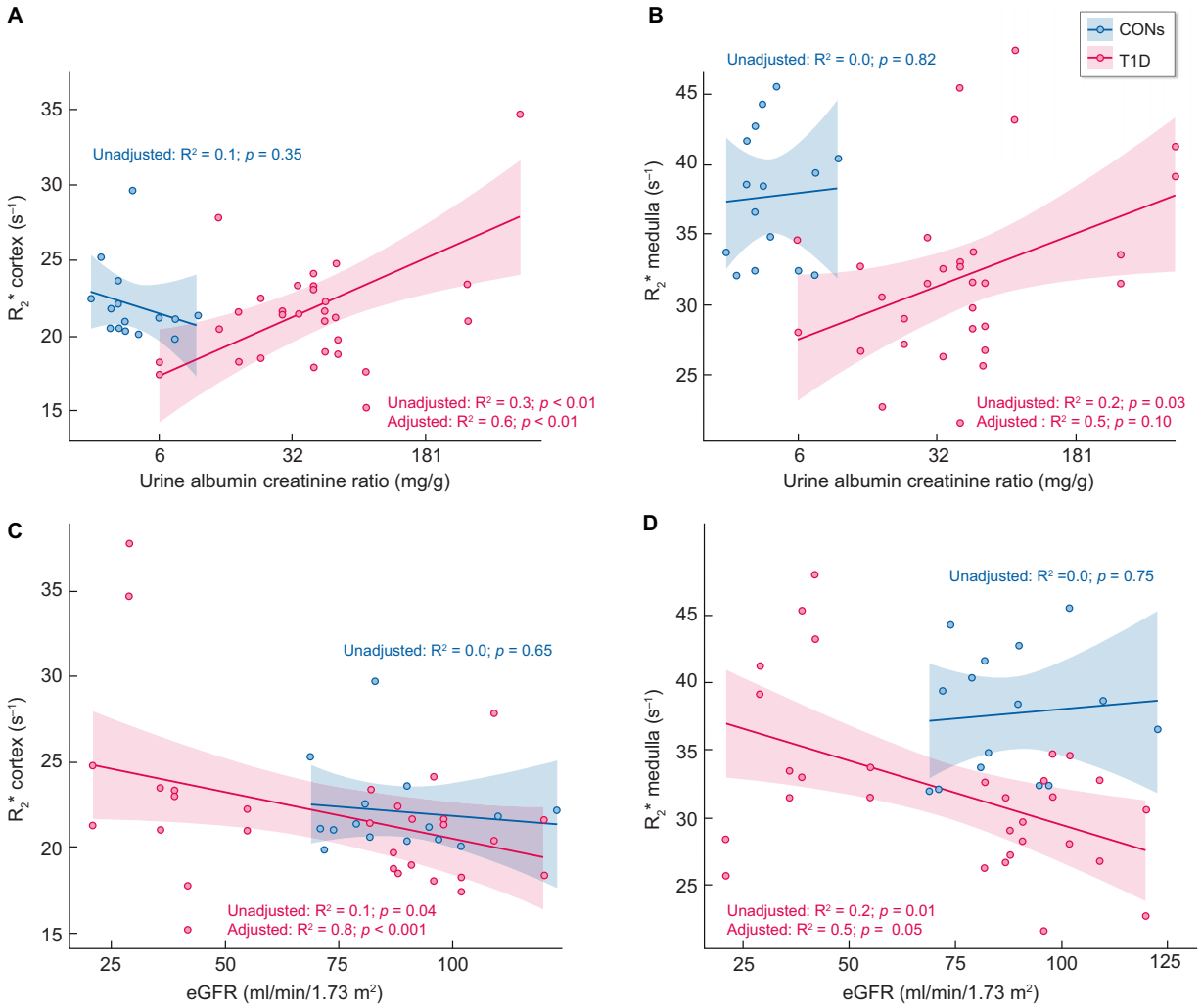



**FIGURE 1:** Associations between kidney function and kidney oxygenation in non-diabetic controls and type 1 diabetes. Shown are plots from unadjusted linear regressions between (**A**) urine albumin creatinine ratio (log2-transformed to obtain normality of residuals) as outcome and renal cortical oxygenation as exposure, (**B**) urine albumin creatinine ratio as outcome and renal medullary oxygenation as exposure, (**C**) estimated glomerular filtration rate (eGFR) as outcome and renal cortical oxygenation as exposure and (**D**) eGFR as outcome and renal medullary oxygenation as exposure. Significant associations were further adjusted for age, sex, systolic blood pressure, body mass index and haemoglobin. $R^2$, coefficient of determination; P, P-value, CONs, non-diabetic controls (blue); T1D, type 1 diabetes (red).

A study by Prasad *et al.* used the manual method like we did, and they observed lower cortical oxygenation (although not significant) and no difference in medullary oxygenation in persons with CKD compared with healthy controls [10]. Lower medullary oxygenation was associated with lower eGFR, but lower cortical oxygenation was not. Of note, they also observed a lower arterial spin labelling-assessed cortical blood flow (in the present study termed cortical perfusion) in CKD compared with healthy controls which is in line with our observations in T1D. The renal cortical perfusion levels and magnitude of difference between groups were similar to our results.

**Table 3. Associations between magnetic resonance imaging outcomes and kidney function in type 1 diabetes**

| | Unadjusted | | | Adjusted | | |
|---|---|---|---|---|---|---|
| | B | $R^2$ | P | $\beta$ | $R^2$ | P |
| Urine albumin creatinine ratio | | | | | | |
| $R_2^*$ cortex | 0.8 | 0.3 | <.01 | 0.8 | 0.6 | <.01 |
| $R_2^*$ medulla | 0.6 | 0.2 | .03 | 0.6 | 0.5 | .10 |
| Perfusion cortex | −0.3 | 0.0 | .39 | −0.2 | 0.4 | .58 |
| Perfusion medulla | 0.5 | 0.1 | .14 | 0.4 | 0.4 | .17 |
| Renal artery blood flow | −1.1 | 0.5 | <.001 | −1.1 | 0.7 | <.001 |
| Estimated glomerular filtration rate | | | | | | |
| $R_2^*$ cortex | −11 | 0.1 | .04 | −20 | 0.8 | <.001 |
| $R_2$ medulla | −15 | 0.2 | <.01 | −12 | 0.5 | .05 |
| Perfusion cortex | 2 | 0.0 | .81 | 3 | 0.4 | .63 |
| Perfusion medulla | −8 | 0.0 | .24 | −6 | 0.4 | .34 |
| Renal artery blood flow | 19 | 0.4 | <.001 | 18 | 0.6 | <.01 |

Shown are standardized beta-estimates ($\beta$) with coefficients of determination ($R^2$) and P-values from unadjusted linear regression analyses using the kidney function parameter as outcome and standardized magnetic resonance parameters as exposures. Models were further adjusted for age, sex, systolic blood pressure, body mass index and haemoglobin.

An important study by Pruijm *et al.* using the 12-layer technique observed lower cortical oxygenation and no difference in medullary oxygenation in patients with CKD compared with healthy controls [8]. In contrast to our study, they did not demonstrate an association with baseline eGFR, but interestingly, they observed a strong association between lower cortical oxygenation and a yearly decrease in eGFR, indicating an important role of renal cortical hypoxia in the progression of CKD.

We found two studies dedicated to T2D. The first study showed that medullary oxygenation was lower in T2D compared with CONs, but they found no difference between groups in cortical oxygenation. They also found that a higher medullary oxygenation was associated with higher albuminuria, which is in contrast to our study [31]. The other study showed lower renal cortical and medullary oxygenation in T2D compared with CONs and found that lower eGFR correlated with lower cortical oxygenation, but also with higher medullary oxygenation [32]. Finally, a study in patients with diabetic nephropathy (diabetes type not reported) showed no difference in cortical oxygenation, but higher medullary oxygenation in patients with diabetes and albuminuria compared with CONs [13], which is in line with our findings.

Thus, MRI studies assessing renal oxygenation in non-diabetic CKD and diabetic kidney disease show conflicting results, but there is a consistent trend, that renal cortical hypoxia seems to play an important role in the progression of CKD. In this study, we did not find proof of renal cortical hypoxia in persons with T1D and albuminuria, but we found a strong correlation between lower renal cortical oxygenation and lower kidney function in T1D, supporting the hypothesis of chronic hypoxia being a unifying pathway for diabetic kidney disease [4, 5]. Moreover, in the primary randomized trial, we presented evidence that sodium-glucose cotransporter 2 (SGLT2) inhibition, which is known to slow the progression of CKD [33], acutely increases cortical oxygenation in persons with T1D and albuminuria [17]. The importance of this previous finding is emphasized by our present finding that cortical oxygenation is strongly associated with lower kidney function.

A somewhat surprising, but also important finding was that persons with the T1D and albuminuria exhibited higher medullary oxygenation than non-diabetic controls which is in line with some of the above-mentioned studies in CKD with or without diabetes [12, 13, 32]. A higher renal medullary tissue oxygenation can be a consequence of a higher oxygen supply, a lower oxygen demand, or a mix of both. Although the medullary perfusion was not higher in T1D group, the renal artery blood flow and cortical perfusion were lower compared with CONs and the medullary perfusion was the same, which can be interpreted as a relative medullary hyper-perfusion, indicating a higher medullary oxygen supply. Moreover, increased proximal reabsorption of glucose due to proximal tubule SGLT2 upregulation in the T1D group [34] could have reduced the distal tubule workload and consequently, lowered the medullary workload and thereby, lowered the medullary oxygen demand.

Studies are needed to investigate changes in kidney energetics along the course of diabetic kidney disease, e.g. large studies comparing T1D patients with normo-, micro- and macroalbuminuria or even better, cohort studies following patients during the progression from normoalbuminuria to macroalbuminuria. Based on the present findings, we hypothesize that these studies might show renal cortical normoxia and medullary hyperoxia at early stages of diabetic kidney disease and renal cortical hypoxia and medullary hypoxia at late stages. Interestingly, a recent study in 24 patients with diabetes (21% T1D) and CKD showed that medullary hyperoxia (lower medullary $R_2^*$) was associated with a higher annual loss in eGFR, indicating that medullary hyperoxia might be important for the progression of diabetic kidney disease [35].

## Previous work on kidney perfusion and blood flow

Our results on renal perfusion agree well with previous MRI studies using arterial spin labelling. Two studies observed that renal cortical perfusion was lower in patients with CKD [10, 36] and one study found the same in persons with T2D [37]. Though we did observe a similar lower renal cortical perfusion in T1D, all three studies also found that lower cortical perfusion was associated with lower eGFR or with annual loss in eGFR, which we were not able to demonstrate in this study. Only one of these studies measured medullary perfusion and in contrast to our study, lower medullary perfusion was observed in individuals with diabetic kidney disease (diabetes type was not reported) compared with healthy controls [36]. In this study population, mean eGFR was however lower (50 ± 14 mL/min/1.73 $m^2$) than in the present study (73 ± 32 mL/min/1.73 $m^2$), which might in part explain our differing results.

Renal artery blood flow has previously been observed to be lower, both in individuals with CKD and in T1D with microalbuminuria when compared with healthy controls [9, 38]. To our knowledge, we are the first to show an association between lower renal artery blood flow and higher albuminuria in T1D.

## MRI measures and associations with parameters important for kidney oxygenation

Renal perfusion is regarded as a primary determinant of renal oxygenation, but the interplay between perfusion and renal tissue oxygenation is complex, with increased perfusion causing increased oxygen supply, but at the same time an increased glomerular filtration rate, which causes an increased oxygen consumption [5]. In this study, we found no association between renal perfusion and renal $R_2^*$ in either the medulla or the cortex and likewise, no association between renal artery blood flow and renal $R_2^*$. This does not mean that renal perfusion or renal

artery blood flow are not important for oxygenation, but measurements of the GFR would have been helpful for the interpretation. We previously demonstrated that blood oxygen saturation is lower and that cardiovascular autonomic function is impaired in T1D [39, 40] and we speculated that these parameters might correlate with renal oxygenation, but we found no evidence supporting this. We measured blood glucose immediately before each MRI scan as blood glucose has previously been shown to correlate with renal cortical oxygenation [11], but we found no association between renal oxygenation and blood glucose.

### Associations with inflammation biomarkers

Inflammation is an important feature of CKD, and anti-inflammatory agents have been evaluated in the treatment of CKD [41, 42]. Thus, we explored the associations between MRI measures and a selection of biomarkers related to inflammation. Higher levels of several of the biomarkers were associated with lower renal medullary oxygenation, but the association with Monocyte Chemoattractant Protein-1 (MCP-1) is particularly interesting as urine levels of MCP-1 have been found to predict albuminuria and kidney disease progression in T2D [43] and inhibition of MCP-1 has shown beneficial effects on albuminuria in T2D [44].

### Implications and further studies

Inhibitors of the SGLT2 have been found to slow the progression of diabetic kidney disease in persons with T2D [45] and to improve hard renal outcomes in persons with CKD with and without diabetes [33]. The exact mechanisms are unknown, but we recently published a paper demonstrating that a single 50 mg dose of the SGLT2 inhibitor dapagliflozin improves renal cortical oxygenation compared with a placebo after 6 h in persons with T1D with albuminuria [17]. This improved cortical oxygenation could play an important role in the renoprotective mechanisms of SGLT2 inhibitors, but our finding needs to be reproduced with lower doses and with chronic treatment. There are several ongoing trials using BOLD MRI to assess the effect of SGLT2 inhibitors or GLP1 receptor agonists on renal oxygenation, perfusion and blood flow. These include The Adolescent Type 1 diabetes Treatment with SGLT2i for hyperglycEMia and hyPerfilTration (ATTEMPT) trial (NCT04333823) studying the effects of 16 weeks of dapagliflozin 5 mg daily in adolescents with T1D and The Renal Oxygenation, Oxygen Consumption and Hemodynamic Kinetics in Type 2 DIabetes: an Ertugliflozin Study (ROCKIES) trial (NCT04027530) studying the effects of 4 weeks of ertugliflozin 15 mg daily in persons with T2D. Similarly, the The Research Study to Find Out How Semaglutide Works in the Kidneys Compared to Placebo, in People With Type 2 Diabetes and Chronic Kidney Disease (REMODEL) trial (NCT04865770) is evaluating the effect of semaglutide on kidney MRI parameters in persons with T2D and CKD. These and other future trials will utilize non-invasive MRI methods to investigate the mechanism of action of kidney protective medications.

### Strengths and weaknesses

A limitation is that the T1D participants were relatively healthy and homogenous in terms of kidney function. Studying a larger group, including more individuals with macroalbuminuria and low eGFR might have revealed important differences, which the observed strong association between renal oxygenation and low kidney function also indicates. Eighty-seven percent of T1D participants were on a renin-angiotensin-aldosterone system (RAAS) blocking agent and even though all study participants were abstinent from medication except insulin on the day of the scan, this means that the present study findings should be interpreted within the context of RAAS inhibition. The T1D population in the present study had a relatively long diabetes duration (39 $\pm$ 16 years) along with a preserved eGFR. This limits the generalizability of our findings. $R_2^*$ is not a direct measure of renal oxygen tension and it depends on the concentration of deoxyhaemoglobin which probably makes it more suitable as a method of assessing intra-individual changes than inter-individual differences. Furthermore, shifts in the oxygen dissociation curve would make the interpretation of oxygen tension more uncertain. A reduced cortical blood volume due to a reduced capillary network could mimic increased oxygenation with lower $R_2^*$ [46]. Thus, there is a risk that we underestimate potential cortical hypoxia in the current study. Using arterial spin labelling is considered less precise to assess medullary perfusion than cortical perfusion [27], which we also observed in the present study where medullary ASL had an intra-individual coefficient of variation of 15.4% compared with 7.3% for cortical ASL. Of note, the association between cortical oxygenation and albuminuria lost significance in the sensitivity analysis with further adjustment for eGFR, but as can be appreciated in Supplementary data, Fig. S1, albuminuria and eGFR were closely associated in this population and collinearity could be the explanation. The primary strength of our study is that we evaluated renal oxygenation, perfusion and blood flow simultaneously.

## CONCLUSION

Renal medullary oxygenation was higher, and renal cortical perfusion and renal artery blood flow were lower in persons with T1D and albuminuria compared with CONs. In persons with T1D, lower renal cortical oxygenation and renal artery blood flow were associated with lower kidney function and higher albuminuria. Medullary hyperoxia in T1D might be a consequence of perturbed kidney energetics and represent a higher setpoint of medullary oxygenation in T1D.

## SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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J.C.L. is the guarantor of this work and had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. We thank all study participants and the laboratory technicians Maja Lis Dybdahl Halkjaer, Tina Nielsen, Dorthe Riis, Tina Ragnholm Juhl and Jessie Armand Hermann from Steno Diabetes Centre Copenhagen. Also thank you for the valued contribution from research assistants Mathilde Overgaard Lauersen, Gidega Vijayakumar, Linnéa Haugen and Büsra Köylü from Rigshospitalet Glostrup.

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## AUTHORS' CONTRIBUTIONS

J.C.L. contributed with conceptualization, study design, data curation, formal analysis, investigation, methodology, project administration, data validation, visualization, writing—original draft and writing—review and editing. N.S.-H. and I.K.B.R. contributed with investigation and writing—review and editing. B.H. contributed with formal analysis, investigation, methodology, project administration, software, supervision, data validation and writing—review and editing. C.S.H., H.B.W.L., P.-H.G., P.B. and M.F.-M. contributed with conceptualization, resources, supervision, writing—review and editing. U.B.A. and P.R. contributed with conceptualization, methodology, resources, supervision, visualization and writing—review and editing.

## CONFLICT OF INTEREST STATEMENT

J.C.L. reports having given a lecture for Boehringer Ingelheim, the fee was given to Steno Diabetes Centre Copenhagen. P.R. reports having received research grants from AstraZeneca and Novo Nordisk and given lectures for AstraZeneca, Mundipharma and Boehringer Ingelheim, and has served as a consultant for AstraZeneca, Bayer, Eli Lilly, Boehringer Ingelheim, Astellas, Gilead, Sanofi Aventis Vifor and Novo Nordisk, all fees given to Steno Diabetes Centre. P.-H.G. has received lecture honoraria from Astellas, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, PeerVoice, Sanofi and Sciarc, and is an advisory board member of AbbVie, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Eli Lilly, Medscape, MSD, Mundipharma, Novo Nordisk and Sanofi. N.S-H. reports having stock equity in Novo Nordisk A/S and Akcea Therapeutics Inc. C.S.H. has received lecture honoraria to Steno Diabetes Centre Copenhagen from Novo Nordisk. P.B. has acted as a consultant for AstraZeneca, Bayer, Bristol-Myers Squibb, Boehringer Ingelheim, Eli-Lilly, LG Chem, Sanofi, Novo Nordisk and Horizon Pharma. P.B. serves on the advisory boards for AstraZeneca, Bayer, Boehringer Ingelheim, Novo Nordisk and XORTX. B.H., I.K.B.R., H.B.W.L., M.F.-M. and U.B.A. declare no competing interests.

## DATA AVAILABILITY STATEMENT

Individual, de-identified participant data are not freely available because of the risk of patient re-identification, but interested parties can request access to de-identified participant data or anonymized clinical study reports through submission of a request for access to the corresponding author, provided that the necessary data protection agency and ethical committee approvals are provided in compliance with relevant legislation.

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