RESEARCH ARTICLE

Thyroid hormone increases oxygen metabolism causing intrarenal tissue hypoxia; a pathway to kidney disease

Ebba Sivertsson[1], Malou Friederich-Persson[1], Patrik Persson[1], Masaomi Nangaku[2], Peter Hansell[1], Fredrik Palm[1] *

1 Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden, 2 Department of Medicine, University of Tokyo, Tokyo, Japan

* Fredrik.Palm@mcb.uu.se

Abstract

The proposed mechanisms for the development of nephropathy are many, complex and often overlapping. Although recent literature strongly supports a role of kidney hypoxia as an independent pathway to nephropathy, the evidence remains inconclusive since the role of hypoxia is difficult to differentiate from confounding factors such as hyperglycemia, hypertension and oxidative stress. By increasing kidney oxygen consumption using triiodothyronine (T_3) and, thus, avoiding these confounding factors, the aim of the present study was to investigate renal hypoxia *per se* as a causal pathway for the development of nephropathy. Healthy Sprague-Dawley rats were treated with T_3 (10 μg/kg/day) and the angiotensin II AT_1-receptor antagonist candesartan (1 mg/kg in drinking water) to eliminate effects of T_3-induced renin release; and compared to a candesartan treated control group. After 7 weeks of treatment *in vivo* kidney function, oxygen metabolism and mitochondrial function were evaluated. T_3 did not affect glomerular filtration rate or renal blood flow, but increased total kidney oxygen consumption resulting in cortical hypoxia. Nephropathy, demonstrated as albuminuria and tubulointerstitial fibrosis, developed in T_3-treated animals. Mitochondria uncoupling mediated by uncoupling protein 2 and the adenosine nucleotide transporter was demonstrated as a mechanism causing the increased kidney oxygen consumption. Importantly, blood glucose levels, mean arterial blood pressure and oxidative stress levels were not affected by T_3. In conclusion, the present study provides further evidence for increased kidney oxygen consumption causing intrarenal tissue hypoxia, as a causal pathway for development of nephropathy.

Introduction

Diabetes mellitus and hypertension are leading causes for development of nephropathy and subsequent end-stage renal disease (ESRD) [1]. Currently, there is no treatment to reverse already established nephropathy. Despite intense research the detailed mechanisms for development of nephropathy are unclear. However, kidney tissue hypoxia is a common finding in

animal models of diabetes, hypertension and chronic kidney disease [2–7]. Thus, intrarenal hypoxia has been proposed to be a unifying pathway for development of nephropathy [8]. This hypothesis has gained increasing support [9–14] and is now referred to as "the suffocating kidney" [15]. However, when investigating the role of kidney hypoxia confounding factors such as hypertension, hyperglycemia or oxidative stress must be considered.

In most tissues, increased metabolism and oxygen consumption (QO_2) results in increased blood flow to match oxygen supply to oxygen demand. It is therefore uncommon that increased metabolism causes hypoxia. However, the kidney constitutes an exception. Correcting increased kidney QO_2 by increasing the renal blood flow (RBF) may result in increased glomerular filtration rate (GFR), and thus increased tubular electrolyte load. The resulting increase in active tubular transport requires increased QO_2 *per se*, and the kidney is therefore poorly adapted to compensate for increased QO_2 [16]. Consequently, increased kidney QO_2 can result in decreased kidney oxygen tension [17].

Thyroid hormones stimulates increased metabolism throughout the body. Pathologically increased production of thyroid hormones, so called hyperthyroidism, causes clinical symptoms of hyperactivity of multiple organ systems such as tremor, palpitations, dyspnea, anxiety, weight loss (despite increased appetite), heat intolerance and increased sweating [18]. Thyroid stimulating hormone (TSH), secreted from the pituitary gland, stimulates the endocrine function of the thyroid to produce the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). T_4 has only small effects on metabolism and is in most part converted to the more active form T_3 within the thyroid or in peripheral target tissue. The receptors for thyroid hormones are nuclear receptors present in most cells, stimulating proliferation, differentiation and increased energy expenditure [19, 20]. Apart from direct effects on transcription thyroid hormones can also regulate activity of signaling pathways and exhibit direct effects on various organelles in the cell, including the mitochondria [20].

The constant work of reabsorption and secretion in the tubular system requires a high supply of energy, which in the proximal tubule is exclusively produced during oxidative phosphorylation by the mitochondria. The process is oxygen consuming and, therefore, changes in mitochondria metabolism have substantial effects on total kidney QO_2. Under pathological conditions, increased mitochondria leak respiration, i.e. QO_2 unrelated to the production of energy, has been shown to contribute to the development of kidney hypoxia [7, 21, 22].

Hyperthyroidism increases cardiac output by both inotropic and chronotropic mechanisms. An increased cardiac output together with dilation of resistance arterioles increases RBF. This increases the hydrostatic pressure in the glomerulus, thus increasing GFR and the filtered load of electrolytes to the tubular system. Further, hyperthyroidism increase proximal tubule reabsorption by stimulating the activity of several transport proteins, including the Na/K-ATPase, the Na/H-exchanger and Na-P co-transporter [23]. Hyperthyroidism also activates the renin-angiotensin-aldosterone system (RAAS). T_3 increase renin mRNA and stimulate renin release from isolated juxtaglomerular cells [24]. Plasma renin activity, as well as serum levels of angiotensin-converting enzyme (ACE) is increased in rats [25–27] and rabbits [28] by treatment with thyroid hormones. Increased plasma renin activity has also been demonstrated in patients with hyperthyroidism [29]. Activation of RAAS promotes sodium reabsorption, increased plasma volume and vasoconstriction. Taken together, a hyperthyroid state, with increased activity of tubular transport, increased GFR and activation of RAAS substantially increases the metabolic demand of the kidney.

We have previously shown that treatment with thyroid hormones for 10 days induce proteinuria, increase total kidney QO_2 and decrease oxygen availability in cortical tissue [30]. We hypothesized that long-term treatment with T_3 to increase kidney metabolism in healthy rats would result in decreased kidney oxygen tension due to increased QO_2 and ultimately lead to

kidney QO_2 (μmol/min/kidney) was estimated from the arteriovenous difference in oxygen content ($O_2ct = ([Hb]*\text{oxygen saturation}*1.34 + pO_2*0.003))*RBF$. Tubular Na^+ transport (TNa^+, μmol/min/kidney) was calculated as follows: $TNa^+ = [PNa^+]*GFR-UNa^+V$, where $[PNa^+]$ is plasma Na^+ concentration and UNa^+V is the urinary Na^+ excretion. TNa^+ per QO_2 was calculated as TNa^+/QO_2, being a measure of sodium transport efficiency.

Mitochondria isolation procedure

Animals were decapitated and the kidneys rapidly excised and placed in ice-cold isolation buffer (containing in mM: 250 sucrose, 10 (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 ethylene glycol tetraacetic acid (EGTA), 1 mg/ml bovine serum albumin (BSA, further purified fraction V), pH 7.4 and osmolality adjusted to 300 ± 2 mOsm/kg H_2O (Model 3MO, Advanced Instruments, Norwood, MA, USA). Tissue samples were snap frozen in liquid nitrogen for analysis of reactive oxygen species and placed in Carnoy's fixative (methanol:chloroform:acetic acid, 6:3:1) for histological evaluation. Remaining kidney cortex was separated on ice, homogenized using a cooled Potter-Elvehjelm homogeniser rotating at 600–800 rpm and centrifuged at 800xg for 10 min at 4˚C. The supernatant was transferred to new tubes and centrifuged at 8000xg for 10 min at 4˚C. The pellet was washed once, carefully removing the buffy coat, and then stored as a pellet on ice.

Mitochondria respiration measurements

Mitochondria QO_2 was evaluated in an Oroboros O2k (Oroboros Instruments, Innsbrück, Austria) in respiration buffer (containing in mM: 68 sucrose, 198 mannitol, 2 EGTA, 5 $MgCl_2$, 5 KPO_4^- (from a 1M mix of $K_2HPO_4^-$ and $KH_2PO_4^-$), 10 HEPES, 3 mg/ml BSA, pH 7.1, 330 mOsm/kg H_2O) in the presence of 10 mM glutamate to obtain resting state QO_2 and 300 μM ADP (pH 7.4, 0.6 mol $MgCl_2$ per mol ADP) to obtain maximal QO_2 due to oxidative phosphorylation. Mitochondria viability was assessed by the respiratory control ratio (RCR); fold increase in resting state QO_2 in response to ADP and only mitochondria with an RCR above 4 were used in experiments. QO_2 was evaluated in the presence of ATP-synthase inhibitor oligomycin and the effects of uncoupling protein-2 (UCP-2) inhibitor guanosine diphosphate (GDP, 0.5 mM) and adenine nucleotide transporter (ANT) inhibitor carboxyatractylate (CAT 5 μM) were investigated to estimate mitochondria leak respiration. QO_2 was corrected for protein content in a sample obtained from the respiratory chamber at the end of each experiment.

Urine and tissue analysis

Thiobarbituric acid reactive substances (TBARS) was measured in urine and tissue homogenate of kidney cortex by adding 50 μl sample to 42 μl 0.67% thiobarbituric acid. Samples were vortexed and heated to 97˚C for 60 min. After cooling the samples on ice, 50 μl methanol:1 mM NaOH (91:9) was added, the samples vortexed and centrifuged at 3000 rpm for 5 min at room temperature. The supernatant was analyzed for fluorescence using excitation/emission of 532/553 nm and the concentration calculated using a standard curve of malondialdehyde. All values were corrected for protein concentration. Concentration of TBARS in urine samples were multiplied by urine flow to get urinary TBARS excretion. Protein content was determined by DC-Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). Albuminuria was measured by rat albumin ELISA kit, (Bethyl Laboratories, Montgomery, TX, USA) according to manufacturer´s instruction. Protein carbonylation in kidney cortex was determined spectrophotometrically by protein Carbonyl Colorimetric Assay (Cayman Chemicals, MI, USA) and normalized for protein concentration.

Histology

Carnoy-fixed tissue was paraffin-embedded and three micrometer sections of the kidney tissue were stained with the periodic acid-Schiff (PAS) reagent and counterstained with hematoxylin.

Statistical analysis

The data from kidney *in vivo* and mitochondrial *in vitro* measurements was assumed to be normally distributed and statistical comparisons were performed using Student's t-test (two-tailed, equal variance). All values are given as mean±SEM. Histological sections were analysed for the presence or absence of tubulointerstitial fibrosis. Results were analysed using Fisher's exact test (one-tailed). $P < 0.05$ was considered statistically significant for all comparisons.

Results

Treatment with T_3 to induce hyperthyroidism increased kidney QO_2 and decreased the transport efficiency of sodium (TNa^+/QO_2) compared to control animals (Fig 1A and 1B). T_3 decreased tissue oxygen tension in kidney cortex and increased oxygen tension in the medulla (Fig 1C and 1D). Kidney weight, GFR, RBF, urine flow and excretion of TBARS were not affected by T_3. T_3 decreased the filtration fraction and urinary excretion of sodium and potassium (Table 1).

Excretion of protein in urine and development of tissue fibrosis was evaluated as markers of developing nephropathy. Proteinuria and albuminuria were increased by T_3 (Fig 2A and 2B). Treatment with T_3 induced tubulointerstitial fibrosis (Fig 2C).

Kidney cortex mitochondria isolated from rats administered T_3 had increased maximum oxidative phosphorylation capacity (respiratory control ratio, RCR) compared to controls (5.6 ±0.3 vs 4.6±0.2; $P < 0.05$). QO_2 in the presence of the ATP-synthase inhibitor oligomycin detects QO_2 unrelated to ATP-production (leak respiration), i.e. uncoupling of the mitochondrial membrane (regulated leak) or basal leak of protons over the membrane (unregulated leak). There was a two-fold increase in absolute QO_2 during oligomycin incubation in animals treated with T_3 compared to controls (pmol/O_2/min/mg protein; 29.5±2.0 vs 14.1±2.7; $P < 0.05$), an indicator of increased total leak respiration. The change in QO_2 after addition of the UCP inhibitor GDP and the ANT inhibitor CAT detects the degree of uncoupling via UCP-2 and ANT, respectively. T_3 treated animals had increased uncoupling via UCP-2 (Fig 3A) and ANT (Fig 3B). The remaining QO_2 after addition of oligomycin, GDP and CAT detects QO_2 related to unregulated leak of protons over the mitochondrial membrane. This was increased by T3 treatment compared to controls (pmol/O_2/min/mg protein; 17.9±2.4 vs 9.9±1.1; $P < 0.05$), indicating an increased basal leak.

Although treatment with T_3 induced an increased food and water intake the weight gain per week was the same between groups. Systemic parameters like blood glucose, mean arterial blood pressure (MAP), heart weight, plasma sodium and potassium were not affected by T_3 treatment (Table 2).

Discussion

The main finding of the present study is that increased kidney QO_2 results in sustained kidney cortical hypoxia and development of nephropathy, manifested as albuminuria and tubulointerstitial fibrosis. Further, we show one causative mechanism being increased QO_2 unrelated to ATP production by mitochondria. This was independent of alterations in arterial blood pressure, glycaemic status and oxidative stress. These results provide further support for
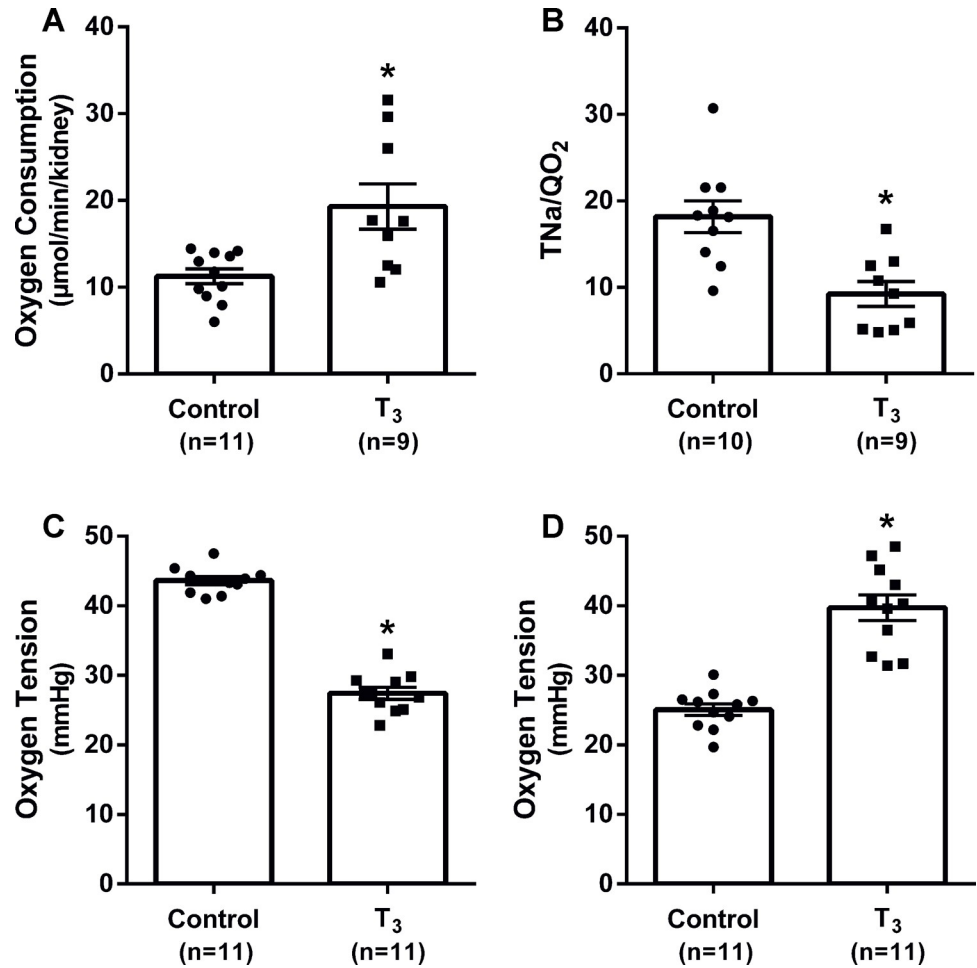
Fig 1. *In vivo* **measurements of kidney function and oxygen metabolism in rats with and without chronic administration of triiodothyronine (T$_3$).** Total kidney oxygen consumption is shown in (A); transported sodium per consumed oxygen (TNa$^+$/QO$_2$) in (B); kidney oxygen tension in kidney cortex in (C); and medulla in (D). * denotes P<0.05 compared to control (two-tailed Student's t-test).

https://doi.org/10.1371/journal.pone.0264524.g001

kidney tissue hypoxia as an independent pathway for development of nephropathy. We have previously shown the short term effects of thyroid hormones on kidney metabolism and development of kidney hypoxia [30]. We now extend this knowledge, demonstrating that the long-

Table 1. *In vivo* kidney function in rats with and without chronic administration of triiodothyronine (T$_3$) for seven weeks.

	KW/BW (mg/g)	GFR (ml/ min/kidney)	RBF (ml/ min/kidney)	FF	UV (μl/min/ kidney)	U-Na$^+$ (μmol/ min/kidney)	U-K$^+$ (μmol/ min/kidney)	U-TBARS (fmol/ min/kidney)	C-TBARS (μmol/mg)	C-Protein carbonyls (nmol/ mg)
Control	3.4±0.1	1.4±0.1	11.1±0.8	0.24 ±0.03	2.9±0.3	0.4±0.01	0.9±0.1	15.7±2.5	1.0±0.2	2.2±0.4
T$_3$	3.2±0.2	1.2±0.1	12.7±1.6	0.16 ±0.02*	3.1±0.3	0.2±0.01*	0.5±0.1*	15.4±1.9	0.8±0.2	1.9±0.3

KW/BW = kidney weight per bodyweight, GFR = glomerular filtration rate, RBF = renal blood flow, FF = filtration fraction, UV = urine flow, U-Na$^+$ = urinary excretion of sodium, U-K$^+$ = urinary excretion of potassium and U/C-TBARS = urinary excretion and cortical tissue concentration of TBARS. All values are mean±SEM.

* denotes P<0.05 compared to control.

https://doi.org/10.1371/journal.pone.0264524.t001
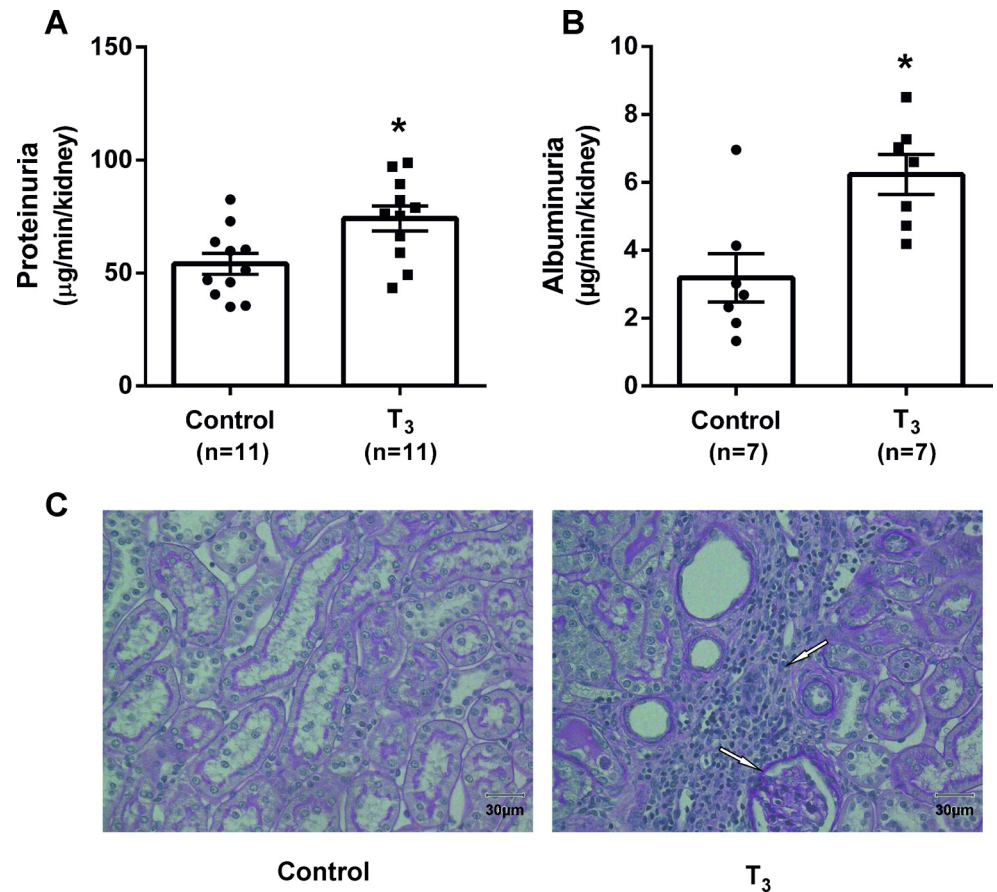
Fig 2. Markers of nephropathy in urine and kidney tissue from rats with and without chronic administration of triiodothyronine (T_3). Urinary excretion of total protein is shown in (A); and urinary excretion albumin in (B). * denotes $P<0.05$ compared to control (Student's t-test). A representative image of histological sections for analysis of fibrosis development are shown in (C). Focal tubulointerstitial fibrosis was present in five out of eight T_3-treated animals whereas fibrosis could not be detected in any of the control animals ($P<0.05$, one-tailed Fisher's exact test).

https://doi.org/10.1371/journal.pone.0264524.g002

term effects of increased kidney oxygen consumption and kidney tissue hypoxia leads to kidney damage.

T_3 induce renin release and activate the renin-angiotensin-aldosterone system (RAAS), resulting in increased levels of angiotensin II signalling, hypertension and oxidative stress [31–33]. To prevent renin induced angiotensin II signalling, animals in the present study were co-treated with the angiotensin II receptor blocker candesartan throughout the study. This allowed us to study the role of kidney tissue hypoxia *per se* without the influences of confounding factors such as hypertension or oxidative stress.

Kidney QO_2 has been demonstrated as one important factor determining intrarenal oxygen availability [17], and we have previously demonstrated a close relationship between QO_2 and oxygen availability [7, 22, 34, 35]. The inability of the kidney to match oxygen demand to delivery originates from the close relationship between RBF and GFR, the tubular electrolyte load requiring energy-demanding tubular transport, and the structural alignment of arteries next to veins allowing for arterio-venous oxygen shunting [36, 37]. Therefore, increased kidney QO_2 is often associated with development of kidney tissue hypoxia.

In this study, long-term treatment with T_3 increased *in vivo* kidney QO_2 and induced cortical hypoxia. The importance of cortical hypoxia has been demonstrated in CKD patients
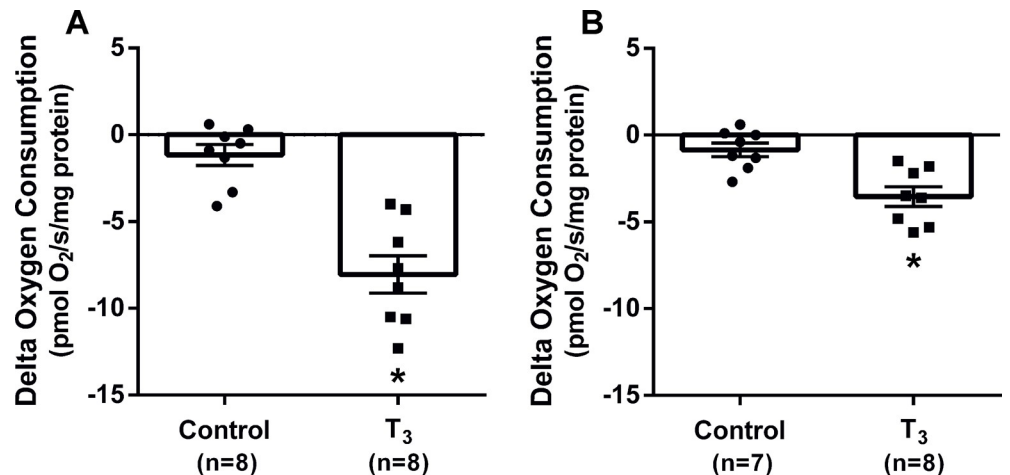
Fig 3. *In vitro* respiration measurements of mitochondria isolated from kidney cortex in rats with and without chronic administration of triiodothyronine (T_3). Absolute delta change in oxygen consumption of mitochondria, after incubation with GDP to inhibit uncoupling protein 2 are shown in (A) and after incubation with CAT to inhibit adenine nucleotide translocator are shown in (B). * denotes $P < 0.05$ compared to control (two-tailed Student's T-test).

where decreased oxygen availability in the cortex, but not medulla, correlated with eGFR decline [38]. Also, the subgroup of CKD patients with the lowest estimated cortical oxygenation had a three times higher risk of major renal adverse events (i.e. initiation of renal replacement therapy or a 30% increase in serum creatinine).

We demonstrate that one contributing factor for the increased kidney QO_2 is increased mitochondria uncoupling via UCP-2 and ANT. Mitochondrial uncoupling has been suggested as a protective mechanism against excessive production of superoxide radicals by decreasing the membrane potential [39–41]. However, this is done at the cost of increased QO_2 to maintain ATP production. Thyroid hormones are known to increase heat production in brown adipose tissue by inducing uncoupling of the mitochondrial membrane via UCP-1. We now extend this knowledge to also include thyroid hormones as an inducer of UCP-2 uncoupling in the rat kidney.

UCP-2 is upregulated and activated in diabetic kidneys, where it uncouples the mitochondrial membrane and increases QO_2 [7, 42, 43]. Furthermore, mitochondria uncoupling is also present in kidneys from hypertensive animals [44], suggesting uncoupling as a pathway to increased kidney QO_2 also in hypertension. Oxidative stress is a known inducer of mitochondrial uncoupling [45, 46]. However, in our study oxidative stress was not increased by T_3 treatment. The uncoupling properties of thyroid hormones could be via direct actions on the mitochondria as T_3-receptors have been localized to the inner mitochondrial membrane [47] and it has been shown that T_3 can directly control UCP-2 expression [48] and induce mitochondria uncoupling within minutes [49]. Thyroid hormones also bind to nuclear receptors

Table 2. *In vivo* systemic parameters in rats with and without chronic administration of triiodothyronine (T_3) for seven weeks.

	Weight gain (g/day)	Blood glucose (mM)	MAP (mmHg)	Plasma Na^+ (mM)	Plasma K^+ (mM)	HW/BW (mg/g)
Control	6.3±0.4	5.1±0.2	104.0±3.5	139.4±0.5	4.3±0.1	2.5±0.1
T_3	6.4±0.3	4.8±0.2	97.4±3.7	138.9±0.4	4.1±0.1	2.7±0.1

MAP = mean arterial pressure, HW/BW = heart weight per body weight. All values are mean±SEM. * denotes $P < 0.05$ compared to control.

that stimulate transcription of mitochondria specific genes [20]. Thus, a genomic effect cannot be excluded.

This study also provides evidence that T_3 increased basal leak of protons over the mitochondrial membrane, which is another process that increases total mitochondria QO_2. The phospholipid composition in the mitochondrial membrane determines the proton conductance over the membrane [50]. Thyroid hormones alters the composition of the mitochondrial membrane by decreasing the content of linoleic acid, a fatty acid with a strong negative correlation with basal proton leak [51]. Taken together, mitochondria uncoupling and increased basal leakage provides a mechanistic explanation for increased QO_2 and decreased efficiency for tubular sodium reabsorption seen *in vivo* in T_3 treated animals.

T_3 increased mitochondria RCR, indicating increased ATP-synthesis which is a known effect of thyroid hormones on mitochondrial biogenesis [20, 52]. Since thyroid hormones increase electrolyte transport in the proximal tubule by inducing the activity of several transport proteins [23], this could indicate an increased ATP demand in the proximal tubule.

A redistribution of electrolyte reabsorption to the proximal tubule could also explain the increased oxygen availability seen in the medulla of the T_3 treated animals. Brezis *et al*. demonstrated that direct inhibition of medullary sodium transport in the thick ascending limb increased the oxygen availability in this region [53]. This was not accompanied by an increased blood flow to the medulla. Thus, increased kidney oxygen tension in the medulla may be secondary to increased proximal sodium reabsorption. This would reduce electrolyte transport in more distal segments and lower energy demand and oxygen consumed. However in our study, medullary blood flow was not assessed and therefore hemodynamic alterations in this part of the kidney cannot be excluded, although unlikely to account for the alterations observed in kidney cortex.

Increased leakage of proteins to the urine is a well-known marker for kidney dysfunction in both humans and animal models. This can be due to damage of the filtration barrier in the glomerulus and/or damage to the tubular cells responsible for protein secretion and reabsorption. Importantly, proteinuria predicts the rate of GFR decline in patients [54]. T_3 treatment increased total proteinuria and also more specifically albuminuria, which is commonly used as a marker of glomerular injury. Early studies of patients with autoimmune hyperthyroidism done by Weetman *et al*. revealed that approximately 30% had proteinuria despite most of them being euthyroid in response to treatment [55]. Persistent proteinuria in patients with hyperthyroidism without increased levels of circulating immune complexes have been reported by others [56, 57], indicating non-reversible kidney damage in some patients. Some studies report increased prevalence of circulating antibody-complexes in patients with hyperthyroidism that can become trapped in the glomeruli and cause glomerulonephritis with resulting nephrotic proteinuria [58–60]. However, this was not confirmed by Weetman *et al*. who neither found a high prevalence of circulating immune complexes, nor an association between immune complexes and prevalence of proteinuria [55].

It was originally hypothesized by Fine *et al*. in 1998 that kidney hypoxia could induce altered kidney function and damage [8]. Since then, the concept of kidney hypoxia as a unifying mechanism for the development of nephropathy has gained further support [9–14] based on the fact that diabetic, hypertensive and animals with chronic kidney disease all present with kidney tissue hypoxia [3, 6, 32, 61–67]. Also, type 2 diabetic patients living at high altitude have increased prevalence of nephropathy compared to a corresponding patient group living at sea level without differences in arterial blood pressure, lipid status or prevalence of retinopathy [68]. Furthermore, using a non-invasive magnetic resonance technique, it has been demonstrated that both non-diabetic chronic kidney disease patients [69] and patients with diabetic nephropathy have reduced kidney oxygen levels [69, 70]. The available literature

provides support for the hypothesis that kidney hypoxia has a central role in the development of nephropathy.

The role of kidney hypoxia for the development of nephropathy has been addressed in a previous study using chronic administration of the mitochondrial uncoupler dinitrophenol (DNP). DNP administered to otherwise healthy animals for 4 weeks increased kidney QO_2 and caused similar kidney hypoxia as in the present study. Importantly, DNP induced proteinuria, damaged tubules and infiltration of immune cells [34] but a potential nephrotoxic effect of DNP *per se* could not be excluded. However, taken together with the results from the present study where T_3 administration induced a similar degree of increased kidney QO_2, tissue hypoxia and nephropathy we have further support for hypoxia as an important factor for development of kidney injury.

Conclusion

Hyperthyroidism in rats for seven weeks increased kidney QO_2 and, consequently, induced kidney tissue hypoxia and development of nephropathy, evident as albuminuria and tubulointerstitial fibrosis. These events occurred despite unaltered glycaemic status, arterial blood pressure or oxidative stress level. The present study therefore provides further support for kidney tissue hypoxia as causal pathway to nephropathy.

Author Contributions

References

1. Collins AJ, Foley RN, Chavers B, Gilbertson D, Herzog C, Ishani A, et al. US Renal Data System 2013 Annual Data Report. Am J Kidney Dis. 2014; 63(1 Suppl):A7. https://doi.org/10.1053/j.ajkd.2013.11.001 PMID: 24360288.

2. Palm F, Cederberg J, Hansell P, Liss P, Carlsson PO. Reactive oxygen species cause diabetes-induced decrease in renal oxygen tension. Diabetologia. 2003; 46(8):1153–60. https://doi.org/10.1007/s00125-003-1155-z PMID: 12879251.

3. Welch WJ, Baumgartl H, Lubbers D, Wilcox CS. Nephron pO2 and renal oxygen usage in the hypertensive rat kidney. Kidney Int. 2001; 59(1):230–7. https://doi.org/10.1046/j.1523-1755.2001.00483.x PMID: 11135075.

4. Persson MF, Welch WJ, Wilcox CS, Palm F. Kidney function after in vivo gene silencing of uncoupling protein-2 in streptozotocin-induced diabetic rats. Adv Exp Med Biol. 2013; 765:217–23. https://doi.org/10.1007/978-1-4614-4989-8_30 PMID: 22879036.

5. Palm F, Onozato M, Welch WJ, Wilcox CS. Blood pressure, blood flow, and oxygenation in the clipped kidney of chronic 2-kidney, 1-clip rats: effects of tempol and Angiotensin blockade. Hypertension. 55 (2):298–304. https://doi.org/10.1161/HYPERTENSIONAHA.109.135426 PMID: 20048199.

6. Bernhardt WM, Wiesener MS, Weidemann A, Schmitt R, Weichert W, Lechler P, et al. Involvement of hypoxia-inducible transcription factors in polycystic kidney disease. Am J Pathol. 2007; 170(3):830–42. https://doi.org/10.2353/ajpath.2007.060455 PMID: 17322369.

7. Sivertsson E, Friederich-Persson M, Öberg CM, Fasching A, Hansell P, Rippe B, et al. Inhibition of mammalian target of rapamycin decreases intrarenal oxygen availability and alters glomerular permeability. Am J Physiol Renal Physiol. 2018; 314(5):F864–F72. Epub 2017/09/27. https://doi.org/10.1152/ajprenal.00033.2017 PMID: 28971989.

8. Fine LG, Orphanides C, Norman JT. Progressive renal disease: the chronic hypoxia hypothesis. Kidney Int Suppl. 1998; 65:S74–8. PMID: 9551436.

9. Palm F, Nordquist L. Renal tubulointerstitial hypoxia: cause and consequence of kidney dysfunction. Clin Exp Pharmacol Physiol. 38(7):424–30. https://doi.org/10.1111/j.1440-1681.2011.05532.x PMID: 21545630.

10. Nangaku M. Hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. Nephron Exp Nephrol. 2004; 98(1):e8–12. https://doi.org/10.1159/000079927 PMID: 15361693.

11. Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. J Am Soc Nephrol. 2006; 17(1):17–25. https://doi.org/10.1681/ASN.2005070757 PMID: 16291837.

12. Singh DK, Winocour P, Farrington K. Mechanisms of disease: the hypoxic tubular hypothesis of diabetic nephropathy. Nat Clin Pract Nephrol. 2008; 4(4):216–26. https://doi.org/10.1038/ncpneph0757 PMID: 18268525.

13. Palm F. Intrarenal oxygen in diabetes and a possible link to diabetic nephropathy. Clin Exp Pharmacol Physiol. 2006; 33(10):997–1001. https://doi.org/10.1111/j.1440-1681.2006.04473.x PMID: 17002679.

14. Eckardt KU, Bernhardt WM, Weidemann A, Warnecke C, Rosenberger C, Wiesener MS, et al. Role of hypoxia in the pathogenesis of renal disease. Kidney Int Suppl. 2005;(99):S46–51. https://doi.org/10.1111/j.1523-1755.2005.09909.x PMID: 16336576.

15. Mimura I, Nangaku M. The suffocating kidney: tubulointerstitial hypoxia in end-stage renal disease. Nat Rev Nephrol. 2010; 6(11):667–78. https://doi.org/10.1038/nrneph.2010.124 PMID: 20877304.

16. Palm F, Nordquist L. Renal oxidative stress, oxygenation, and hypertension. Am J Physiol Regul Integr Comp Physiol. 2011; 301(5):R1229–41. Epub 20110810. https://doi.org/10.1152/ajpregu.00720.2010 PMID: 21832206; PubMed Central PMCID: PMC3213938.

17. Hansell P, Welch WJ, Blantz RC, Palm F. Determinants of kidney oxygen consumption and their relationship to tissue oxygen tension in diabetes and hypertension. Clin Exp Pharmacol Physiol. 2013; 40 (2):123–37. https://doi.org/10.1111/1440-1681.12034 PMID: 23181475.

18. De Leo S, Lee SY, Braverman LE. Hyperthyroidism. Lancet. 2016; 388(10047):906–18. Epub 2016/03/30. https://doi.org/10.1016/S0140-6736(16)00278-6 PMID: 27038492; PubMed Central PMCID: PMC5014602.

19. Pascual A, Aranda A. Thyroid hormone receptors, cell growth and differentiation. Biochim Biophys Acta. 2013; 1830(7):3908–16. Epub 2012/03/29. https://doi.org/10.1016/j.bbagen.2012.03.012 PMID: 22484490.

20. Lanni A, Moreno M, Goglia F. Mitochondrial Actions of Thyroid Hormone. Compr Physiol. 2016; 6 (4):1591–607. Epub 2016/09/15. https://doi.org/10.1002/cphy.c150019 PMID: 27783852.

21. Papazova DA, Friederich-Persson M, Joles JA, Verhaar MC. Renal transplantation induces mitochondrial uncoupling, increased kidney oxygen consumption, and decreased kidney oxygen tension. Am J Physiol Renal Physiol. 2015; 308(1):F22–8. https://doi.org/10.1152/ajprenal.00278.2014 PMID: 25275014.

22. Friederich-Persson M, Persson P, Hansell P, Palm F. Deletion of Uncoupling Protein-2 reduces renal mitochondrial leak respiration, intrarenal hypoxia and proteinuria in a mouse model of type 1 diabetes. Acta Physiol (Oxf). 2018; 223(4):e13058. Epub 2018/03/15. https://doi.org/10.1111/apha.13058 PMID: 29480974.

23. Iglesias P, Bajo MA, Selgas R, Díez JJ. Thyroid dysfunction and kidney disease: An update. Rev Endocr Metab Disord. 2017; 18(1):131–44. https://doi.org/10.1007/s11154-016-9395-7 PMID: 27864708.

24. Ichihara A, Kobori H, Miyashita Y, Hayashi M, Saruta T. Differential effects of thyroid hormone on renin secretion, content, and mRNA in juxtaglomerular cells. Am J Physiol. 1998; 274(2):E224–31. https://doi.org/10.1152/ajpendo.1998.274.2.E224 PMID: 9486151; PubMed Central PMCID: PMC2573038.

25. Kobori H, Ichihara A, Suzuki H, Miyashita Y, Hayashi M, Saruta T. Thyroid hormone stimulates renin synthesis in rats without involving the sympathetic nervous system. Am J Physiol. 1997; 272(2 Pt 1): E227–32. https://doi.org/10.1152/ajpendo.1997.272.2.E227 PMID: 9124327; PubMed Central PMCID: PMC2574496.

26. Dzau VJ, Herrmann HC. Hormonal control of angiotensinogen production. Life Sci. 1982; 30(7–8):577–84. https://doi.org/10.1016/0024-3205(82)90272-7 PMID: 7040893.

27. Michel B, Grima M, Coquard C, Welsch C, Barthelmebs M, Imbs JL. Effects of triiodothyronine and dexamethasone on plasma and tissue angiotensin converting enzyme in the rat. Fundam Clin Pharmacol. 1994; 8(4):366–72. https://doi.org/10.1111/j.1472-8206.1994.tb00814.x PMID: 7851842.

28. Yeğin E, Yiğitoğlu R, Ari Z, Celik I, Akçay F, Süzek H. Serum angiotensin-converting enzyme and plasma atrial natriuretic peptide levels in hyperthyroid and hypothyroid rabbits. Jpn Heart J. 1997; 38 (2):273–9. PMID: 9201114.

29. Baba T, Murabayashi S, Aoyagi K, Kitaoka M, Nakazono M, Ishizaki T, et al. Plasma renin activity, active and inactive renin concentrations, and their responses to beta 1-adrenoceptor blockade with metoprolol in hyperthyroidism. Horm Metab Res. 1986; 18(9):630–4. https://doi.org/10.1055/s-2007-1012391 PMID: 3023226.

30. Friederich-Persson M, Persson P, Fasching A, Hansell P, Nordquist L, Palm F. Increased kidney metabolism as a pathway to kidney tissue hypoxia and damage: effects of triiodothyronine and dinitrophenol in normoglycemic rats. Adv Exp Med Biol. 2013; 789:9–14. https://doi.org/10.1007/978-1-4614-7411-1_2 PMID: 23852470.

31. Rodriguez-Gomez I, Sainz J, Wangensteen R, Moreno JM, Duarte J, Osuna A, et al. Increased pressor sensitivity to chronic nitric oxide deficiency in hyperthyroid rats. Hypertension. 2003; 42(2):220–5. https://doi.org/10.1161/01.HYP.0000081944.47230.69 PMID: 12821601.

32. Welch WJ, Baumgartl H, Lubbers D, Wilcox CS. Renal oxygenation defects in the spontaneously hypertensive rat: role of AT1 receptors. Kidney Int. 2003; 63(1):202–8. https://doi.org/10.1046/j.1523-1755.2003.00729.x PMID: 12472784.

33. Welch WJ, Blau J, Xie H, Chabrashvili T, Wilcox CS. Angiotensin-induced defects in renal oxygenation: role of oxidative stress. Am J Physiol Heart Circ Physiol. 2005; 288(1):H22–8. https://doi.org/10.1152/ajpheart.00626.2004 PMID: 15598867.

34. Friederich-Persson M, Thörn E, Hansell P, Nangaku M, Levin M, Palm F. Kidney hypoxia, attributable to increased oxygen consumption, induces nephropathy independently of hyperglycemia and oxidative stress. Hypertension. 2013; 62(5):914–9. https://doi.org/10.1161/HYPERTENSIONAHA.113.01425 PMID: 24019401; PubMed Central PMCID: PMC3867444.

35. Nordquist L, Friederich-Persson M, Fasching A, Liss P, Shoji K, Nangaku M, et al. Activation of hypoxia-inducible factors prevents diabetic nephropathy. J Am Soc Nephrol. 2015; 26(2):328–38. Epub 20140902. https://doi.org/10.1681/ASN.2013090990 PMID: 25183809; PubMed Central PMCID: PMC4310648.

36. Levy MN, Imperial ES. Oxygen shunting in renal cortical and medullary capillaries. Am J Physiol. 1961; 200:159–62. https://doi.org/10.1152/ajplegacy.1961.200.1.159 PMID: 13761637.

37. Levy MN, Sauceda G. Diffusion of oxygen from arterial to venous segments of renal capillaires. Am J Physiol. 1959; 196(6):1336–9. https://doi.org/10.1152/ajplegacy.1959.196.6.1336 PMID: 13661371.

38. Pruijm M, Milani B, Pivin E, Podhajska A, Vogt B, Stuber M, et al. Reduced cortical oxygenation predicts a progressive decline of renal function in patients with chronic kidney disease. Kidney Int. 2018; 93 (4):932–40. Epub 20180109. https://doi.org/10.1016/j.kint.2017.10.020 PMID: 29325997.

39. Duval C, Negre-Salvayre A, Dogilo A, Salvayre R, Penicaud L, Casteilla L. Increased reactive oxygen species production with antisense oligonucleotides directed against uncoupling protein 2 in murine endothelial cells. Biochem Cell Biol. 2002; 80(6):757–64. https://doi.org/10.1139/o02-158 PMID: 12555808.

40. Miwa S, Brand MD. Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. Biochem Soc Trans. 2003; 31(Pt 6):1300–1. https://doi.org/10.1042/bst0311300 PMID: 14641047.

41. Friederich M, Hansell P, Palm F. Diabetes, oxidative stress, nitric oxide and mitochondria function. Curr Diabetes Rev. 2009; 5(2):120–44. https://doi.org/10.2174/157339909788166800 PMID: 19442097.

42. Friederich M, Fasching A, Hansell P, Nordquist L, Palm F. Diabetes-induced up-regulation of uncoupling protein-2 results in increased mitochondrial uncoupling in kidney proximal tubular cells. Biochim Biophys Acta. 2008; 1777(7–8):935–40. https://doi.org/10.1016/j.bbabio.2008.03.030 PMID: 18439413.

43. Friederich M, Nordquist L, Olerud J, Johansson M, Hansell P, Palm F. Identification and distribution of uncoupling protein isoforms in the normal and diabetic rat kidney. Adv Exp Med Biol. 2009; 645:205–12. https://doi.org/10.1007/978-0-387-85998-9_32 PMID: 19227473.

44. de Cavanagh EM, Toblli JE, Ferder L, Piotrkowski B, Stella I, Inserra F. Renal mitochondrial dysfunction in spontaneously hypertensive rats is attenuated by losartan but not by amlodipine. Am J Physiol Regul Integr Comp Physiol. 2006; 290(6):R1616–25. https://doi.org/10.1152/ajpregu.00615.2005 PMID: 16410402.

45. Echtay KS, Murphy MP, Smith RA, Talbot DA, Brand MD. Superoxide activates mitochondrial uncoupling protein 2 from the matrix side. Studies using targeted antioxidants. J Biol Chem. 2002; 277 (49):47129–35. https://doi.org/10.1074/jbc.M208262200 PMID: 12372827.

46. Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, et al. Superoxide activates mitochondrial uncoupling proteins. Nature. 2002; 415(6867):96–9. https://doi.org/10.1038/415096a PMID: 11780125.

47. Hashizume K, Ichikawa K. Localization of 3,5,3'-L-triiodothyronine receptor in rat kidney mitochondrial membranes. Biochem Biophys Res Commun. 1982; 106(3):920–6. https://doi.org/10.1016/0006-291x (82)91798-3 PMID: 6288037.

48. Lanni A, De Felice M, Lombardi A, Moreno M, Fleury C, Ricquier D, et al. Induction of UCP2 mRNA by thyroid hormones in rat heart. FEBS Lett. 1997; 418(1–2):171–4. https://doi.org/10.1016/s0014-5793 (97)01375-6 PMID: 9414120.

49. O'Reilly I, Murphy MP. Studies on the rapid stimulation of mitochondrial respiration by thyroid hormones. Acta Endocrinol (Copenh). 1992; 127(6):542–6. https://doi.org/10.1530/acta.0.1270542 PMID: 1492538.

50. Fontaine EM, Moussa M, Devin A, Garcia J, Ghisolfi J, Rigoulet M, et al. Effect of polyunsaturated fatty acids deficiency on oxidative phosphorylation in rat liver mitochondria. Biochim Biophys Acta. 1996; 1276(3):181–7. https://doi.org/10.1016/0005-2728(96)00075-8 PMID: 8856103.

51. Harper ME, Seifert EL. Thyroid hormone effects on mitochondrial energetics. Thyroid. 2008; 18(2):145–56. https://doi.org/10.1089/thy.2007.0250 PMID: 18279015.

52. Thomas WE, Crespo-Armas A, Mowbray J. The influence of nanomolar calcium ions and physiological levels of thyroid hormone on oxidative phosphorylation in rat liver mitochondria. A possible signal amplification control mechanism. Biochem J. 1987; 247(2):315–20. https://doi.org/10.1042/bj2470315 PMID: 3426539; PubMed Central PMCID: PMC1148410.

53. Brezis M, Agmon Y, Epstein FH. Determinants of intrarenal oxygenation. I. Effects of diuretics. Am J Physiol. 1994; 267(6 Pt 2):F1059–62. https://doi.org/10.1152/ajprenal.1994.267.6.F1059 PMID: 7810692.

54. Turin TC, James M, Ravani P, Tonelli M, Manns BJ, Quinn R, et al. Proteinuria and rate of change in kidney function in a community-based population. J Am Soc Nephrol. 2013; 24(10):1661–7. https://doi.org/10.1681/ASN.2012111118 PMID: 23833255; PubMed Central PMCID: PMC3785273.

55. Weetman AP, Tomlinson K, Amos N, Lazarus JH, Hall R, McGregor AM. Proteinuria in autoimmune thyroid disease. Acta Endocrinol (Copenh). 1985; 109(3):341–7. https://doi.org/10.1530/acta.0.1090341 PMID: 3839619

56. Agras PI, Kinik ST, Cengiz N, Baskin E, Saatci U. Autoimmune thyroiditis with associated proteinuria: report of two patients. Journal of pediatric endocrinology & metabolism: JPEM. 2005; 18(3):319–22. https://doi.org/10.1515/jpem.2005.18.3.319 PMID: 15813612.

57. Shuper A, Leathem T, Pertzelan A, Eisenstein B, Mimouni M. Familial Hashimoto's thyroiditis with kidney impairment. Archives of disease in childhood. 1987; 62(8):811–4. https://doi.org/10.1136/adc.62.8.811 PMID: 3662585; PubMed Central PMCID: PMC1778483.

58. Iwaoka T, Umeda T, Nakayama M, Shimada T, Fujii Y, Miura F, et al. A case of membranous nephropathy associated with thyroid antigens. Japanese journal of medicine. 1982; 21(1):29–34. https://doi.org/10.2169/internalmedicine1962.21.29 PMID: 7069940.

59. Horvath F Jr., Teague P, Gaffney EF, Mars DR, Fuller TJ. Thyroid antigen associated immune complex glomerulonephritis in Graves' disease. Am J Med. 1979; 67(5):901–4. https://doi.org/10.1016/0002-9343(79)90752-6 PMID: 583094.

60. Jordan SC, Buckingham B, Sakai R, Olson D. Studies of immune-complex glomerulonephritis mediated by human thyroglobulin. N Engl J Med. 1981; 304(20):1212–5. https://doi.org/10.1056/NEJM198105143042006 PMID: 7012624.

61. Edlund J, Hansell P, Fasching A, Liss P, Weis J, Glickson JD, et al. Reduced oxygenation in diabetic rat kidneys measured by T2* weighted magnetic resonance micro-imaging. Adv Exp Med Biol. 2009; 645:199–204. https://doi.org/10.1007/978-0-387-85998-9_31 PMID: 19227472.

62. Haidara MA, Mikhailidis DP, Rateb MA, Ahmed ZA, Yassin HZ, Ibrahim IM, et al. Evaluation of the effect of oxidative stress and vitamin E supplementation on renal function in rats with streptozotocin-induced Type 1 diabetes. J Diabetes Complications. 2009; 23(2):130–6. https://doi.org/10.1016/j.jdiacomp.2008.02.011 PMID: 18436458.

63. Rosenberger C, Khamaisi M, Abassi Z, Shilo V, Weksler-Zangen S, Goldfarb M, et al. Adaptation to hypoxia in the diabetic rat kidney. Kidney Int. 2008; 73(1):34–42. https://doi.org/10.1038/sj.ki.5002567 PMID: 17914354.

64. dos Santos EA, Li LP, Ji L, Prasad PV. Early changes with diabetes in renal medullary hemodynamics as evaluated by fiberoptic probes and BOLD magnetic resonance imaging. Invest Radiol. 2007; 42 (3):157–62. https://doi.org/10.1097/01.rli.0000252492.96709.36 PMID: 17287645.

65. Ries M, Basseau F, Tyndal B, Jones R, Deminiere C, Catargi B, et al. Renal diffusion and BOLD MRI in experimental diabetic nephropathy. Blood oxygen level-dependent. J Magn Reson Imaging. 2003; 17 (1):104–13. https://doi.org/10.1002/jmri.10224 PMID: 12500279.

66. Welch WJ. Intrarenal oxygen and hypertension. Clin Exp Pharmacol Physiol. 2006; 33(10):1002–5. https://doi.org/10.1111/j.1440-1681.2006.04478.x PMID: 17002680.

67. Manotham K, Tanaka T, Matsumoto M, Ohse T, Miyata T, Inagi R, et al. Evidence of tubular hypoxia in the early phase in the remnant kidney model. J Am Soc Nephrol. 2004; 15(5):1277–88. https://doi.org/10.1097/01.asn.0000125614.35046.10 PMID: 15100368.

68. Sayarlioglu H, Erkoc R, Dogan E, Topal C, Algun E, Erem C, et al. Nephropathy and retinopathy in type 2 diabetic patients living at moderately high altitude and sea level. Ren Fail. 2005; 27(1):67–71. PMID: 15717637.

69. Inoue T, Kozawa E, Okada H, Inukai K, Watanabe S, Kikuta T, et al. Noninvasive evaluation of kidney hypoxia and fibrosis using magnetic resonance imaging. J Am Soc Nephrol. 2011; 22(8):1429–34. https://doi.org/10.1681/ASN.2010111143 PMID: 21757771.

70. Yin WJ, Liu F, Li XM, Yang L, Zhao S, Huang ZX, et al. Noninvasive evaluation of renal oxygenation in diabetic nephropathy by BOLD-MRI. European journal of radiology. 2012; 81(7):1426–31. https://doi.org/10.1016/j.ejrad.2011.03.045 PMID: 21470811.