Chapter 2
Increased Kidney Metabolism as a Pathway to Kidney Tissue Hypoxia and Damage: Effects of Triiodothyronine and Dinitrophenol in Normoglycemic Rats

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Abstract Intrarenal tissue hypoxia is an acknowledged common pathway to end-stage renal disease in clinically common conditions associated with development of chronic kidney disease, such as diabetes and hypertension. In diabetic kidneys, increased oxygen metabolism mediated by mitochondrial uncoupling results in decreased kidney oxygen tension (PO_2) and contributes to the development of diabetic nephropathy. The present study investigated whether increased intrarenal oxygen metabolism *per se* can cause intrarenal tissue hypoxia and kidney damage, independently of confounding factors such as hyperglycemia and oxidative stress. Male Sprague–Dawley rats were untreated or treated with either triiodothyronine (T_3, 10 g/kg bw/day, subcutaneously for 10 days) or the mitochondria uncoupler dinitrophenol (DNP, 30 mg/kg bw/day, oral gavage for 14 days), after which in vivo kidney function was evaluated in terms of glomerular filtration rate (GFR, inulin clearance), renal blood flow (RBF, Transonic, PAH clearance), cortical PO_2 (Clark-type electrodes), kidney oxygen consumption (QO_2), and proteinuria. Administration of both T_3 and DNP increased kidney QO_2 and decreased PO_2 which resulted in proteinuria. However, GFR and RBF were unaltered by either treatment. The present study demonstrates that increased kidney metabolism per se can cause intrarenal tissue hypoxia which results in proteinuria. Increased kidney QO_2 and concomitantly reduced PO_2 may therefore be a mechanism for the development of chronic kidney disease and progression to end-stage renal disease.

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2.1 Introduction

Increased kidney oxygen consumption (QO_2) may result in decreased kidney tissue oxygen tension (PO_2), ultimately resulting in kidney damage. Importantly, intrarenal kidney tissue hypoxia is a proposed pathway to chronic kidney disease and end-stage renal disease in conditions such as diabetes and hypertension [1, 2]. To increase kidney QO_2, healthy rats were treated with either dinitrophenol (DNP) or triiodothyronine (T_3). DNP is a chemical uncoupler of mitochondria [3]. By decreasing the mitochondria membrane potential, the process of mitochondria uncoupling reduces formation of oxidative stress but increases mitochondria QO_2 [4]. Through the nuclear receptor superfamily, T_3 controls gene transcription and results in increased QO_2, mainly via mitochondrial pathways [5]. By utilizing DNP and T_3, we create two independent models of increased kidney QO_2 to investigate whether increased QO_2 per se can cause intrarenal tissue hypoxia and kidney damage.

2.2 Materials and Methods

All animal procedures were carried out according to the National Institutes of Health guidelines and approved by the local animal ethics committee. Normoglycemic male Sprague–Dawley rats (Charles River, Sulzfeld, Germany) received either no treatment, DNP administration (30 mg/kg bw dissolved in 1.5 % methyl cellulose) by oral gavage for14 days or T_3 (10 μg/kg bw/day) administered for 10 days by surgically implanted osmotic minipumps (Alzet Company, Cupertino, CA, USA). The animals receiving T_3 were simultaneously treated with candesartan (1 mg/kg bw in drinking water) in order to minimize the influence of thyroid hormone-induced renin release.

Animals were sedated with sodium thiobutabarbital (Inactin, 120 mg/kg bw i.p.) and placed on a servo-rectally controlled heating pad to maintain body temperature at 37 °C. Tracheotomy was performed to facilitate breathing, and polyethylene catheters were placed in the carotid artery and femoral vein to allow monitoring of mean arterial pressure (MAP, Statham P23dB, Statham Laboratories, Los Angeles, CA, USA), blood sampling, and infusion of saline (5 ml/kg bw/h). The left kidney was exposed by a subcostal flank incision and immobilized in a plastic cup. The left ureter and bladder were catheterized to allow for timed urine sampling and urinary drainage, respectively. After surgery, the animal was allowed to recover for 30 min followed by a 40-min experimental period at the end of which a blood sample was carefully collected from the renal vein in order to calculate arteriovenous differences.

Kidney cortex PO_2 was measured using Clark-type oxygen electrodes (Unisense, Aarhus, Denmark). Glomerular filtration rate (GFR) was measured by clearance of ^{3}H-inulin (185 kBq bolus followed by 185 kBq/kg bw/h; American Radiolabeled Chemicals, St Louis, MO, USA). Renal blood flow (RBF) was measured by Transonic flow probe (Transonic Systems Inc., Ithaca, NY, USA) or clearance by

^{14}C-para-aminohippuric acid (PAH, 185 kBq bolus followed by 185 kBq/kg bw/h; American Radiolabeled Chemicals). GFR was calculated as inulin clearance = ([inulin]$_{urine}$*urine flow)/[inulin]$_{plasma}$ and RBF with PAH clearance corrected for PAH extraction and the hematocrit. Total kidney QO_2 (μmol/min) was estimated from the arteriovenous difference in O_2 content (O_2ct = ([hemoglobin]*oxygen saturation*1.34 + PO_2*0.003)) *total RBF. Tubular Na^+ transport (T_{Na+}, μmol/min) was calculated as follows: $T_{Na+} = [P_{Na+}]$*GFR, where $[P_{Na+}]$ is plasma Na^+ concentration. T_{Na+} per Q_{O2} was calculated as T_{Na+}/Q_{O2}.

Statistical comparisons were performed using one-way analysis of variance with Dunnett's post hoc test. $p < 0.05$ was considered significant and all values are presented as mean ± SEM.

2.3 Results

A decreased body weight and increased kidney weight was observed in T_3-treated animals compared to controls. GFR, MAP, RBF, Na^+–excretion, and urinary flow rates were unaffected in both treated groups (Table 2.1). DNP and T_3 administration resulted in increased kidney QO_2 (Fig. 2.1) and decreased intrarenal kidney PO_2 (Fig. 2.2) compared to no treatment. Importantly, the reduced PO_2 resulted in increased proteinuria in both DNP and T_3-treated animals (Fig. 2.3).

Table 2.1 Systemic and kidney-specific parameters in control animals receiving either no treatment, dinitrophenol for 14 days, or triiodothyronine for 10 days

	Body weight (g)	Kidney weight (g)	Mean arterial pressure (mmHg)	Urine flow (μl/min)	Renal blood flow (ml/min)	Glomerular filtration rate (ml/min)	Sodium excretion (μmol/min)
No treatment	399 ± 10	1.4 ± 0.1	107 ± 2	3.2 ± 0.8	11.1 ± 0.7	1.6 ± 0.2	0.1 ± 0.1
Dinitrophenol	399 ± 7	1.4 ± 0.0	108 ± 7	4.1 ± 0.8	11.4 ± 1.1	2.1 ± 0.3	0.2 ± 0.1
Triiodothyronine	350 ± 7*	2.7 ± 0.1*	97 ± 4	4.4 ± 0.6	8.1 ± 1.3	1.8 ± 0.3	0.2 ± 0.2

*Denotes $p < 0.05$ versus control animals

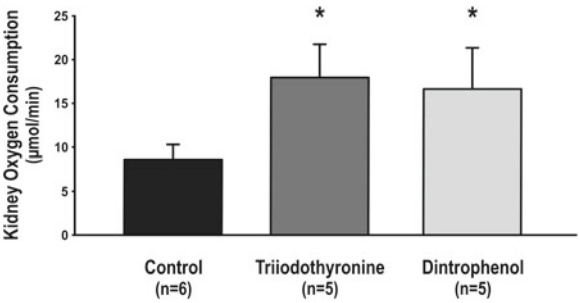

Fig. 2.1 Kidney oxygen consumption in control animals receiving either no treatment, dinitrophenol for 14 days, or triiodothyronine for 10 days. * denotes $p < 0.05$ versus control

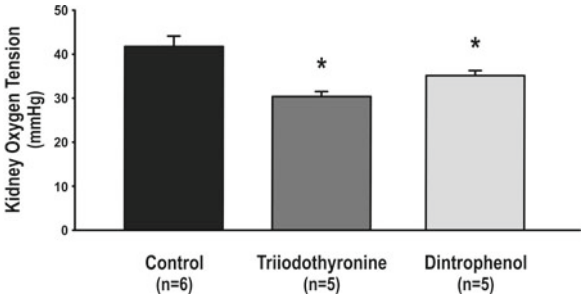

Fig. 2.2 Kidney oxygen tension in control animals receiving either no treatment, dinitrophenol for 14 days, or triiodothyronine for 10 days. * denotes $p < 0.05$ versus control

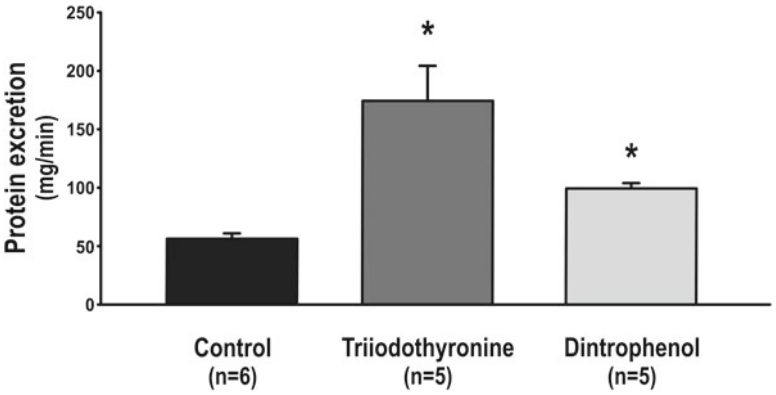

Fig. 2.3 Protein excretion in control animals receiving either no treatment, dinitrophenol for 14 days, or triiodothyronine for 10 days. * denotes $p < 0.05$ versus control

2.4 Discussion

In the present study, administration of DNP and T_3 to normoglycemic rats increased kidney QO_2, decreased kidney PO_2, and increased proteinuria, demonstrating that increased kidney QO_2 is a pathway to kidney damage. Interestingly, treatment with DNP in patients has resulted in proteinuria [6, 7] and patients with untreated Grave's disease also develop proteinuria [8]. It may be argued that DNP is directly nephrotoxic as it is a known environmental toxin. However, this argument cannot apply to T_3 as it is an endogenous hormone. The use of T_3 to establish intrarenal hypoxia verifies that increased kidney QO_2 indeed ultimately results in kidney damage. As T_3 is known to affect the renal angiotensin system and cause hypertension [9], these animals were simultaneously treated with candesartan. It is also unlikely that the observed kidney damage is a result of ATP shortage rather than limited availability of oxygen as the Na^+ excretion is unchanged in both DNP and T_3-treated animals,

a finding supported by others [10]. We propose that the mechanism to kidney damage is decreased kidney PO_2 resulting in intrarenal tissue hypoxia, leading to development of proteinuria.

In 1998, it was originally proposed by Fine et al., that the limitation of intrarenal oxygen availability is the key mechanism initiating kidney damage [11]. Since then, this theory has received considerable support, and intrarenal hypoxia is now an acknowledged pathway to chronic kidney disease and end-stage renal disease [1, 2, 12–14]. The oxygen level in the kidney is important because an increased RBF will increase the workload and, therefore, the kidney QO_2. This makes increased RBF a very inefficient way to increase renal PO_2, and the kidney is therefore vulnerable to alterations in either QO_2 or oxygen availability. This is further supported by studies reporting that Navajo Indians living at high altitude have increased incidence of end-stage renal disease compared to their corresponding control group at sea level [15]. Also, type 2 diabetic patients at high altitude have increased incidence of diabetic nephropathy despite similar blood pressure and metabolic parameters [16].

In the present study, the utilization of two independent models to create intrarenal tissue hypoxia both resulted in kidney damage evident as increased urinary protein leakage. This clearly demonstrated the importance of intrarenal PO_2 in maintaining normal kidney function.

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