The Role of Uric Acid in Renal and Cardiovascular Function in Patients with Uncomplicated Type 1 Diabetes Mellitus

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy

Pharmacology and Toxicology
University of Toronto

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2016

ABSTRACT

Patients with type 1 diabetes (T1D) have a 30% lifetime risk of developing diabetic nephropathy (DN) despite the use of current available therapies. It is of paramount importance to identify agents that protect patients with T1D from the initiation and progression of DN. Unfortunately, recent renal protection trials in patients with T1D have either failed, demonstrated harm or reported effects that are far below expectations based on data from experimental models. Accumulating evidence suggests that plasma uric acid (PUA) is associated with multiple key pathways involved in the pathogenesis of diabetic complications, including metabolic, cardiovascular and renal abnormalities. Our work is focused on elucidating the pathophysiologic role of PUA in renal and cardiovascular function in patients with T1D. We showed that PUA levels are surprisingly lower in adolescents and young adults with T1D compared to healthy controls (HC) due to glycosuria, which stimulates increased PUA excretion, likely through the GLUT9 isoform 2 transporter. Despite having lower PUA levels, adolescents and young adults with exhibit a negative correlation between PUA and glomerular filtration rate (GFR), possibly due to increased afferent renal arteriolar resistance and reduced renal perfusion. In the context of previous experimental work, our observation suggests that PUA may mediate afferent renal arteriolar disease, causing ischemia to the renal microcirculation, thereby potentiating renal injury. Although not observed in T1D adolescents, the association of higher PUA levels with

higher blood pressure emerged in the young adults with T1D. In our final set of experiments, lowering PUA with febuxostat (FBX) for 8 weeks had a modest blood pressure lowering effect in young adults with T1D. FBX enhanced the renal filtration fraction response to clamped hyperglycemia through an increase in efferent renal arteriolar resistance without impacting the RAAS, suggesting that PUA may augment other vasoconstrictor or vasodilatory mechanisms leading to an augmented renal hemodynamic response to hyperglycemia. Longitudinal outcome trials are required to determine whether our physiologic findings impact renal or cardiovascular outcomes in response to PUA lowering therapies in patients with T1D.

Acknowledgments

Words cannot express the enormous gratitude I have to a number of great individuals who guided, supported me and helped me grow as an individual and a researcher throughout the PhD program. My achievements would not be possible without such an incredible group of people that I am very fortunate to have met throughout my graduate degree.

First and foremost, I would like to express immense gratitude for Dr. David Cherney, who was my supervisor for the last 2.5 years of the PhD program. Dr. Cherney's talent and passion for his work and dedication to his students have been incredibly inspiring and continue to be a model towards my future personal and academic development. Dr. Cherney provided an unbelievable number of incredible opportunities, endless support, encouragement and the best experience that I could imagine for a PhD program. I am most sincerely thankful for this opportunity to learn from such a great role model and I hope to continue learning from him throughout my future career path.

I am incredibly thankful to my co-supervisor Dr. Paul Dorian, who consistently provided enormous support and feedback from the beginning of my MSc and right to the PhD defense. To my PhD advisory committee members: Dr. Paul Dorian, Dr. Bruce Perkins and Dr. Susanna Mak, thank you for all the guidance, input, challenges, constructive critiques and lessons on how to defend my research. I was fortunate to have the opportunity to collaborate on projects with Dr. Perkins and his team from Mount Sinai Hospital and am very thankful for the opportunities, support and dedication that Dr. Perkins endlessly provides. I hope to continue such work and to continue learning from such a talented and a dedicated individual.

I would also like to sincerely thank Dr. John Parker for introducing me to human physiology research, contributing to the foundation of my PhD work and for continuing to collaborate on exciting new projects. I am thankful for the help, support and guidance I received from the research team at the Cardiac Catheterization Research Laboratory at the Mount Sinai Hospital and from Dr. Susanna Mak and Dr. Gary Newton.

I am deeply thankful to the nursing staff, students and fellows of the Renal Physiology Laboratory at the Toronto General Hospital. Without their outstanding expertise and dedication, none of the work described in this thesis would be possible. I sincerely thank Vesta Lai for being a caring

friend and a skillful leader of the research activities in the lab, Josephine Tse and Leslie Cham for their unique nursing expertise and diligent work. I also owe sincere thanks to Ronnie Har, Amie Locke, Derek Fong, Dr. Marko Škrtić, Dr. Julie Lovshin, Harindra Rajasekeran, Dr. Genevieve Boulet and Dr. Mohammed Farooqi for their help and company.

The Department of Pharmacology at the University of Toronto has been a source of constant support throughout my graduate studies. I am especially grateful to Dr. Ruth Ross, Dr. Peter McPherson and Dr. Denis Grant for their guidance and efforts to help me succeed in the PhD program. I would also like to express my sincere gratitude to Dr. Michelle Arnot, the undergraduate coordinator at the Department of Pharmacology who continues to provide me with teaching opportunities and opportunities to stay involved with the departmental activities.

Last, but definitely not least, I would like to thank my incredible family and friends. I owe a special thanks to my wonderful family for their unconditional love, support and encouragement. I am forever grateful to my parents and grandparents for teaching me by example that any goal can be achieved through hard work. I also want to thank my brother, Andrew Lytvyn, for being a true friend and always being there for me. I am thankful for my friends for being there through the highs and the lows of this endeavor and sharing many great moments, laughs, delicious meals and fun outings. Thank you, Kelsey McLaughlin, for your constant support and friendship through it all – we did it! To Sue Webber, for always deeply caring for our success. Deepest thanks to Slawko Sekunda, who has always been there for me and has always been a source of encouragement, support and help.

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ABCG2	ATP-binding cassette sub-family G member 2
ACE	Angiotensin converting enzyme
ACEi	Angiotensin converting enzyme inhibitors
ACh	Acetylcholine
ACR	Albumin to creatinine ratio
AdDIT	Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial
AIX	Augmentation index
ANCOVA	Analysis of covariance
Ang II	Angiotensin II
ARBs	Angiotensin II receptor blockers
ATIRMA	Adjunctive-To-Insulin and Renal MechAnistic pilot trial of empagliflozin in T1D Trial
BH_4	Tetrahydrobiopterin
BP	Blood pressure
CACTI	Coronary Artery Calcification in Type 1 Diabetes Study
cGMP	Cyclic guanosine monophosphate
CKD	Chronic kidney disease
C_M	Plasma protein mean concentration
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
DBP	Diastolic blood pressure
DCCT	Diabetes Control Complications Trial
DM	Diabetes mellitus
DN	Diabetic nephropathy
DRI	Direct renin inhibitors
eGFR	Estimated glomerular filtration rate
eNOS	Endothelial nitric oxide synthase
ERFD	Early renal function decline
ERPF	Effective renal plasma flow
FBF	Forearm blood flow

FBX	Febuxostat
FE_{Na}	Fractional sodium excretion
FE_{UA}	Fractional UA excretion
FF	Filtration fraction
FMD	Flow-mediated dilation
FOCUS	Febuxostat Open-Label Clinical Trial of Urate-Lowering Efficacy and Safety
GAGs	Glycosaminoglycans
GFR	Glomerular filtration rate
GLUT9	Glucose transporter 9
HbA1c	Hemoglobin A1c
HC	Healthy controls
HR	Heart rate
IL-6	Interleukin 6
K_{FG}	Gross filtration coefficient
MAP	Mean arterial pressure
MCP-1	Monocyte chemoattractant protein-1
NAD^+	Nicotinamide adenine dinucleotide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
NOS	Nitric oxide synthase
NPT1	Inorganic phosphate transporter 1
NPT4	Inorganic phosphate transporter 4
ORPS	Oxford Regional Prospective Study
PAH	Paraaminohippurate
P_{Bow}	Pressure in Bowman's space and interstitium
P_{Cr}	Plasma creatinine concentration
PERL	Protecting Early Renal Function Loss Study
P_G	Glomerular oncotic pressure
P_{GLO}	Glomerular hydrostatic pressure
P_{Na}	Plasma sodium concentration
PRA	Plasma renin activity
PUA	Plasma uric acid

P_{UA}	Plasma uric acid concentration
PWV	Pulse wave velocity
R_A	Afferent arteriolar resistance
RAAS	Renin angiotensin aldosterone system
RBF	Renal blood flow
R_E	Efferent arteriolar resistance
RENAAL	Reduction of Endpoints in Non-Insulin-Dependent Diabetes Mellitus With the Angiotensin II Antagonist Losartan
ROS	Reactive oxygen species
RVR	Renal vascular resistance
SBP	Systolic blood pressure
SD	Standard deviation
SDNN	Standard deviation of normal-to-normal interval
sGC	Soluble guanylate cyclase
SGLT2	Sodium glucose co-transporter 2
SGLT2i	Sodium glucose transporter 2 inhibitor
T1D	Type 1 diabetes mellitus
T1D-H	T1D patients with hyperfiltration
T1D-N	T1D patients with normofiltration
TGF-b	Transforming growth factor-beta
TGFβ-1	Transforming growth factor β 1
TNF-α	Tumour necrosis factor α
UA	Uric acid
U_{Cr}	Urinary creatinine concentration
U_{Na}	Urinary sodium concentration
URAT1	Urate transporter 1
U_{UA}	Urinary uric acid concentration
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase
ΔP_F	Filtration pressure

List of Thesis Publications

Chapter 1 Lytvyn Y., Perkins BA., Cherney DZI. **(2015)** Uric Acid as a Biomarker and a Therapeutic Target in Diabetes. **Canadian Journal of Diabetes**. 39(3):239-246.

Chapter 2 Lytvyn Y., Mahmud FH., Daneman D., Deda L., Dunger DB., Deanfield J., Dalton N., Elia Y., Har R., Bradley TJ., Slorach C., Hui W., Moineddin R., Reich HN., Scholey JW., Mertens L., Sochett E., Cherney DZI. **(2016)** Association Between Plasma Uric Acid Levels and Cardiorenal Function in Adolescents With Type 1 Diabetes. **Diabetes Care**. 39(4): 611-616.

Chapter 3 Lytvyn Y., Škrtić M.,Yang GK., Yip P., Perkins BA., Cherney DZI. **(2015)** Glycosuria mediated urinary acid excretion in patients with uncomplicated type 1 diabetes mellitus. **American Journal of Physiology – Renal Physiol.** 308(2):F77-83.

Chapter 4 Lytvyn Y., Škrtić M.,Yang GK., Lai V., Scholey JW., Yip P., Perkins BA., Cherney DZI. **(2015)** Plasma Uric Acid Effects on Glomerular Hemodynamic Profile of Patients with Uncomplicated Type 1 Diabetes Mellitus. **Diabetic Medicine**. Epub ahead of print.

Chapter 5 Lytvyn Y., Har R., Lai V., Locke A., Fong D., Advani A., Perkins P.A., Cherney D.Z.I. **(2016)** Renal and Vascular Effects of Uric Acid Lowering in Patients with Uncomplicated Type 1 Diabetes Mellitus. **Manuscript in preparation.**

Chapter 1: Uric Acid as a Biomarker and a Therapeutic Target in Diabetes

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A version of this chapter is published in: Canadian Journal of Diabetes 2015; 39(3): 239-246

1.1 Abstract

Diabetic nephropathy (DN) is a long-standing microvascular complication of diabetes mellitus (DM), and is the leading cause of end stage renal disease in developed countries. Current therapeutic strategies used to prevent or delay DN exert limited clinical protective effects and can have serious adverse effects. Thus, identification of new pharmacologic agents that protect against initiation and progression of diabetic complications is of the utmost importance. Uric acid (UA) recently emerged as an inflammatory factor that increases oxidative stress and promotes activation of the renin angiotensin aldosterone system. As a consequence, higher UA levels are associated with various stages of DN onset and progression, including metabolic, cardiovascular and kidney function abnormalities. If UA lowering drugs, such as the xanthine oxidase inhibitors, block the mechanisms responsible for micro- and macrovascular injury in DM, then these agents could represent a critical step toward preventing the progression of DM. This review will focus on the evidence supporting serum UA levels as a biomarker of renal and cardiovascular risk and as a potential additional therapeutic target in DM.

1.2 Introduction

Diabetic nephropathy (DN) is the most common microvascular complication of diabetes mellitus (DM), which causes more than 40% of end-stage renal disease cases requiring dialysis worldwide. Dialysis patients with DM have a high risk of coronary disease, stroke, and peripheral arterial disease (1). Moreover, even mild chronic kidney disease (CKD) is associated with the development of cardiovascular complications (1).

Despite what is known about the risk associated with DN, the responsible mechanisms are complex and remain incompletely understood. Chronic hyperglycemia increases the risk of DN through the activation of hemodynamic and metabolic pathways, including cytokines, chemokines, growth factors, intracellular signaling cascades, and neurohormonal mechanisms such as the renin angiotensin aldosterone system (RAAS). These changes result in increased intraglomerular pressure, oxidative stress, inflammation and endothelial dysfunction, ultimately leading to structural kidney abnormalities characteristic of DM (1). Many of these abnormalities are clinically silent for many years. As a consequence, DN progresses slowly and by the time DN presents clinically as albuminuria and loss of renal function, significant renal parenchymal damage has already occurred. It is therefore important to identify novel biomarkers of DN risk to better target high-risk patients with earlier therapies prior to the onset of albuminuria or renal function decline.

The current gold-standard therapeutic strategies to decrease the risk of DN progression are intensive glycemic control and RAAS inhibition. The first recognition of the intensive glycemic control benefit stemmed from the Diabetes Control Complications Trial (DCCT) where intensive glycemic control over a period of 6.5 years resulted in a 39% reduction in microalbuminuria and a 54% reduction in macroalbuminuria frequency compared to the conventional glycemic therapy in type 1 DM patients (2). The long-lasting benefits of intensive glycemic control were also shown in type 2 DM patients (3). Unfortunately, the use of glycemic control is limited by side effects, such as hypoglycemia and weight gain. Despite the 2 decades that have elapsed since the DCCT, optimal glycemic control is difficult to achieve and a substantial proportion of DM patients fail to reach the hemoglobin A1C target levels and progress to develop renal and cardiovascular complications (1, 4). RAAS inhibition emerged as an additional protective treatment, but unfortunately, angiotensin converting enzyme inhibitors (ACEi), angiotensin II (Ang II) receptor

blockers (ARBs) and direct renin inhibitors (DRI) lead to incomplete suppression of the RAAS, resulting in persistent efferent renal arteriolar vasoconstriction, high intraglomerular pressure, and renal hyperfiltration (5, 6). From a clinical perspective, RAAS inhibitors have also failed to eliminate renal or cardiovascular complications (1, 7). Moreover, dual RAAS blockade is associated with serious side effects such as the risk of renal dysfunction and hyperkalemia (1, 7). Finally, RAAS blockade has failed as a primary prevention therapy in type 1 DM (7). Normoalbuminuric, normotensive patients with type 1 DM therefore do not benefit clinically from the early institution of RAAS blockade therapies. The identification of new safe pharmacologic agents that protect against the initiation and progression of diabetic complications is therefore of the utmost importance.

More recently, it has been demonstrated that uric acid (UA) exerts deleterious effects on blood pressure and renal function, even when baseline UA levels are within the normal range (8). UA activates the RAAS, increases oxidative stress and promotes inflammation (8). As a consequence, higher UA levels are associated with metabolic abnormalities (insulin resistance, hyperglycemia), cardiovascular disease (hypertension, endothelial dysfunction, arterial stiffness, cardiac diastolic dysfunction) and kidney dysfunction (1, 5, 7). Experimental work has suggested that pharmacologic lowering of UA also blocks the RAAS, suppresses inflammation and promotes renal and cardiovascular protection (9). Thus, there is evidence that UA is involved in the mechanisms of various stages of DN onset and progression. If UA lowering drugs such as the xanthine oxidase (XO) inhibitors, allopurinol and febuxostat, attenuate some of the mechanisms responsible for micro- and macrovascular injury in DM, then these agents could represent a critical step toward preventing the progression of DM. Serum UA levels could therefore serve as an earlier biomarker and an effective therapeutic target to supplement the current hemoglobin A1c, cholesterol and blood pressure targets.

1.3 UA Homeostasis

In humans, UA is a breakdown product of purine nucleotides (Figure 1.1). The exogenous pool of UA varies with dietary intake, including purine rich products, fructose and glucose. Of interest is the suggested association between high fructose consumption and high UA levels. Upon ingestion, fructose gets absorbed into cells, is phosphorylated by fructokinase leading to ATP depletion and subsequently increased AMP production, which results in increased UA levels (10).

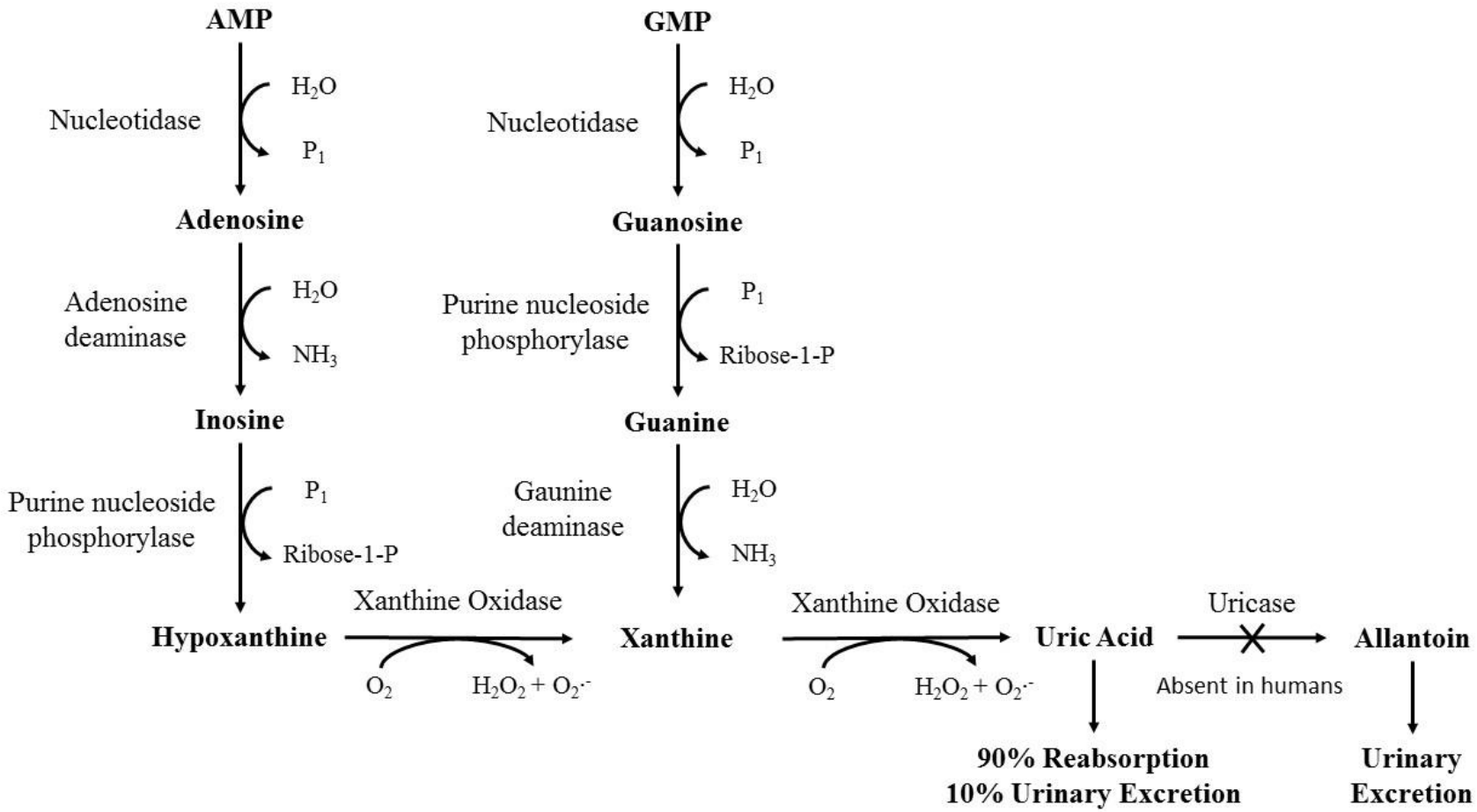

Figure 1.1. Summary of purine metabolism in humans.

Human trial data linking high fructose intake to increased serum UA levels has, however, been mixed, with some data attributing the association to the hypercaloric state rather than fructose directly (10). Given that the increased worldwide intake of fructose in the form of high fructose corn syrup parallels the rise in metabolic syndrome and hyperuricemia, more studies are needed to confirm this association. The endogenous pool of UA is mainly regulated by xanthine oxidoreductase mediated hepatic production, intestinal secretion and renal excretion (5). Since UA is primarily excreted by the kidney, studying the role of UA in kidney disease is difficult as the decline in the glomerular filtration rate is inevitably associated with an increase in serum UA levels. Xanthine oxidoreductase is the main rate-limiting liver enzyme involved in UA biosynthesis and it exists in two functional forms: (1) xanthine dehydrogenase (XDH) and (2) XO. XDH has low reactivity with molecular oxygen, high reactivity with nicotinamide adenine dinucleotide (NAD^+) and can be converted to XO, while XO is oxygen dependent and is responsible for formation of superoxide anion radicals and hydrogen peroxide. When grouped together, the dehydrogenase and oxidase forms catalyze the two final steps in purine metabolism by converting hypoxanthine to xanthine and subsequently xanthine to UA (5). UA in plasma is minimally bound to proteins and is thus easily filtered by glomeruli into the renal tubule. About 90% of filtered UA is reabsorbed by the S1 segment of the proximal convoluted tubule by intracellular anion transporters on the basolateral membrane, such as urate transporter 1 (URAT1) and the more recently discovered glucose transporter 9 (GLUT9) (11). Secretion of UA occurs mostly at the S2 segment of the proximal tubule by urate transporters located on the apical membrane, such as ATP-binding cassette sub-family G member 2 (ABCG2), inorganic phosphate transporter 1 and 4 (NPT1and NPT4) (11). Approximately 10% of filtered UA is excreted (11).

In adult humans, UA levels normally range up to 420 μmol/L (Medical Council of Canada, 2010) compared to levels that do not exceed 30-60 μmol/L in other mammals (12). Humans have higher UA levels due to mutational silencing of the enzyme uricase. As a result, humans cannot convert UA to soluble allantoin, which is freely excreted in the urine in other mammals. UA therefore remains the end product of purine metabolism in humans (12). In addition, the great efficiency with which the human kidney reabsorbs UA may contribute to the higher serum UA levels in humans compared to other species. The evolutionary explanation for the loss of uricase and increased UA levels in humans may relate to the ability of UA to activate the RAAS and induce blood pressure sensitivity to salt. Such an effect may have given our ancestors an evolutionary

advantage through enhanced blood pressure stability under conditions of dietary sodium depletion (12).

Regardless of an evolutionary explanation, humans are clearly predisposed to the development of hyperuricemia, which is defined by the accumulation of UA beyond its soluble point in water (0.404 mmol/L) and develops due to defects in purine metabolism, UA overproduction, undersecretion, or a combination of factors (13). At physiological pH, almost all UA is ionized to urate and has a single negative charge. Due to low solubility, excessive UA can lead to crystallization and accumulation in joints and tissues leading to arthritis and gout. Additionally, UA increases oxidative stress, promotes inflammation and activates RAAS. Consequently, higher UA levels are associated with metabolic, cardiovascular, and renal abnormalities, thereby contributing to DN onset and progression (Figure 1.2).

1.4 UA and Inflammation

Hyperfiltration and hyperglycemia are important triggers for inflammatory cytokine/chemokine production, thereby playing a critical role in the early pathogenesis of DN. Glomerular distention and hyperfiltration promote renal inflammation in animal models, through increased shear stress and wall tension (14). Thus, it is not surprising that urinary excretion of platelet derived growth factor, interferon, tumour necrosis factor α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) is higher in hyperglycemia versus euglycemia (15), and in hyperfiltering type 1 DM patients versus normofiltering type 1 DM patients and healthy controls (14). Increased cytokine production induced by hyperfiltration or hyperglycemia may promote renal injury by activating deleterious pro-inflammatory pathways, such as macrophage extravasation, apoptosis and fibrosis (14) .

In animal and human studies, UA correlates with increased production of many of the same inflammatory factors that are activated by hyperfiltration and hyperglycemia (16). Infusion of UA into mice increases TNF-α levels (13) and activates phospholipase A_2 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (13). UA also induces inflammasome activation in mice, which decreases insulin sensitivity and stimulates immune and inflammatory response (16). UA effects in cell culture are mediated by the mitogen activated protein pathway, which facilitates transforming growth factor β 1 (TGFβ-1) gene expression (17). These tissue culture and animal studies implicating UA as a pro-inflammatory factor are supported by several

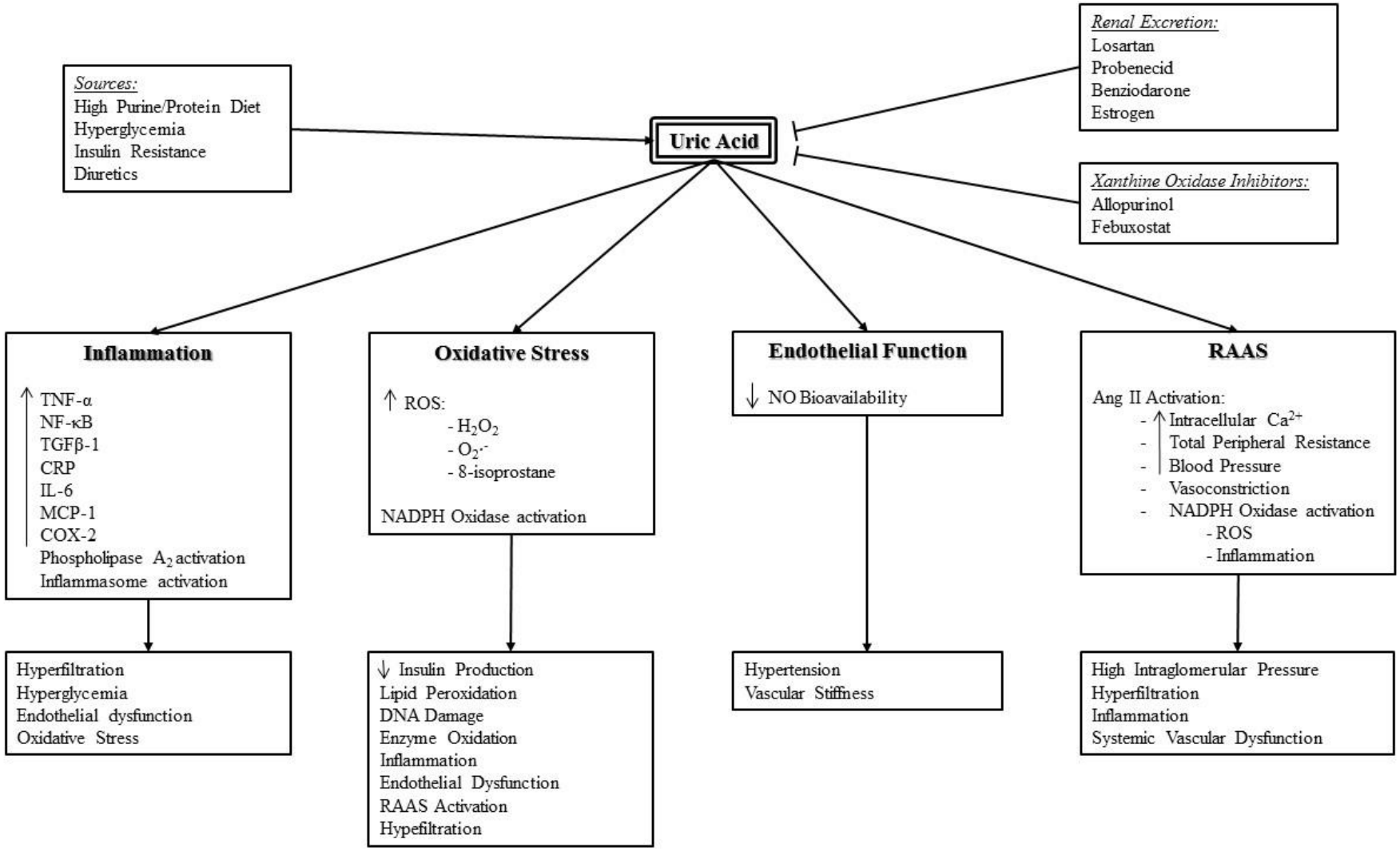

Figure 1.2. Mechanisms by which uric acid could lead to diabetic and vascular complications.

population-based studies in healthy men and women, where serum UA is positively associated with C-reactive protein (CRP) , interleukin 6 (IL-6) and TNF-α (18). Increased UA levels are also associated with increased expression of MCP-1 in the kidney and cyclooxygenase-2 (COX-2) in the vasculature (17). Therefore, there is strong evidence to suggest that UA triggers pro-inflammatory pathways similar to hyperfiltration and hyperglycemia.

From a therapeutic perspective, lowering UA levels with allopurinol in a hyperuricemic mouse model attenuates inflammatory pathways, reduces cytokine expression and improves insulin resistance via increased circulating adiponectin levels (19). Similarly, discontinuation of allopurinol in patients with CKD increases urinary TGFβ-1, suggesting that UA lowering suppresses pathways that promote inflammation and fibrosis (20).

1.5 UA and Oxidative Stress

UA exerts deleterious effects in humans through enhanced production of reactive oxygen species (ROS), which contribute to inflammation and alteration in vascular function. UA is a powerful antioxidant in plasma that can scavenge superoxide and hydroxyl radicals (9). In contrast, UA has more potent pro-oxidant effects in vascular tissue upon entry into the vascular smooth muscle cells through organic acid transporters, leading to increased production of ROS, such as H_2O_2 and 8-isoprostane (9). Oxidative stress can lead to the loss of transcription factors necessary for insulin gene expression leading to decreased insulin production and secretion (21). UA-mediated oxidative stress can also lead to lipid peroxidation, DNA damage, oxidation and inactivation of enzymes, and expression of inflammatory cytokines leading to the disruption of cellular homeostasis and cellular damage (9).

In addition to UA, the XO form of xanthine oxidoreductase is a critical source of ROS production resulting from the oxidation reaction of hypoxanthine to xanthine and xanthine to UA. Inflammatory cytokines/chemokines and hypoxia, conditions often found in DM, induce XDH expression in tissues and vascular endothelial cells and stimulate XDH release into the circulation (22). Once in the circulation, XDH is rapidly converted to XO by reversible oxidation of the sulfhydryl residue or by irreversible proteolysis (23). XO is negatively charged at physiological pH but exhibits cationic amino acid motifs on the surface of the protein resulting in a high affinity for negatively charged glycosaminoglycans (GAGs) on the apical surface of vascular endothelial

cells (23). Sequestration of XO on GAGs produces a microenvironment with amplified XO concentration and alters kinetic properties of XO to further optimize ROS production (23). In the process of hypoxanthine and xanthine catabolism to UA, re-oxidation of one XO molecule generates two H_2O_2 and two O^{2-} species (23). Under normal aerobic conditions H_2O_2 is the major ROS produced (72% at 21% O_2) and under inflammatory condition its production is even higher (86-90% at 1-2% O_2) (23). Thus, inflammatory or hypoxic conditions in DM lead to overproduction and increased immobilization of XO resulting in amplified XO-derived ROS formation. This deleterious oxidative stress action of XO has been reported in various renal and cardiovascular diseases, such as heart failure, chronic obstructive pulmonary disease, pulmonary hypertension, sickle cell disease and DM (23). One of the common pathways that may link levels of oxidative stress due to XO to organ injury relates to increased endothelial dysfunction.

1.6 UA and Endothelial Function

The vascular endothelium senses hemodynamic force changes to maintain vascular tone and homeostasis. One of the prominent endothelial communication lines is established though the L-arginine-NO-cyclic guanosine monophosphate (cGMP) pathway. Endothelial nitric oxide synthase (eNOS) converts L-arginine to L-citrulline and NO in response to receptor-dependent agonists and physicochemical stimuli (24). NO diffuses to the adjacent smooth muscle cells and activates soluble guanylate cyclase (sGC) resulting in increased cGMP production (24). cGMP activates downstream kinases in the pathways that lead to uptake of intracellular Ca^{2+} into the sarcoplasmic reticulum with subsequent vasodilation (24).

Oxidative stress and inflammation play a pivotal role in the pathogenesis of endothelial dysfunction, which is an abnormality in endothelium-dependent vasomotor responses and is implicated in the onset and progression of most vascular diseases. Endothelial dysfunction is often characterized by decreased bioavailability of NO, which can be depleted by UA through several proposed mechanisms: 1) blockade of L-arginine uptake (25); 2) stimulation of L-arginine degradation by arginase (25) or by oxidative stress mediated oxidization of tetrahydrobiopterin (BH_4), the critical co-factor of eNOS; 3) UA can deplete NO by either irreversibly reacting with NO to form 6-aminouracil (25) or by UA-induced ROS reacting with NO to form a highly reactive peroxynitrite intermediate (25). UA-induced NO depletion is supported by hyperuricemic rat studies that display endothelial dysfunction and systemic and glomerular hypertension, which is reversed by lowering UA, L-arginine supplementation or treatment with antioxidants (25).

Similar to renal effects, hyperglycemia induces systemic vascular abnormalities including endothelial dysfunction in humans (26-28). As a result of the effects of hyperglycemia and neurohormonal activation, UA levels are independently associated with endothelial dysfunction in animals and humans, thereby promoting hypertension, even when UA levels are within the normal range (29-31). Additionally, treatment of patient with hyperuricemic with allopurinol improves endothelial dysfunction in humans (32). Thus, UA could act as an important therapeutic target to reduce endothelial dysfunction characteristic of DN. Potential protective effects of UA lowering on endothelial function may be further mediated through effects on other mechanisms that are central to the pathogenesis of DM-related complications, such as hyperglycemia and the RAAS.

1.7 UA, Hyperglycemia and the RAAS

Fundamental to the pathogenesis of diabetic complications, is the development of inflammation, oxidative stress and endothelial dysfunction, which are all closely linked to RAAS activation. The RAAS plays an important role in the homeostasis of arterial pressure, tissue perfusion, extracellular fluid volume and vascular response to injury and inflammation. Renin is an aspartyl protease secreted by the juxtaglomerular cells in the kidney in response to low tubular sodium content, renal perfusion pressure and sympathetic stimulation (33). In the circulation, renin cleaves angiotensinogen from the liver to angiotensin (Ang) I, which in turn gets hydrolyzed by angiotensin converting enzyme (ACE), leading to a bioactive Ang II. Ang II activates its G-protein-coupled Ang II type 1 receptor, leading to increased intracellular Ca^{2+} and vasoconstriction (reducing the vascular capacity and increasing peripheral resistance), increased aldosterone (causing salt retention), increased oxidative stress, promotion of the inflammatory state, increased release of antidiuretic hormone and increased thirst (causing water conservation), increased myocardial contractility (increasing cardiac output) and increased activity of the sympathetic nervous system. Although the RAAS is an important response system to a challenge in intravascular volume depletion, long term Ang II activation can cause structural remodeling of the cardiovascular system as a compensation for prolonged volume contraction and inappropriate activation can cause hypertension, fluid retention and inflammatory effects. For example, Ang II activates NADPH oxidase, thereby promoting ROS production and inflammation (34) resulting in a positive feedback mechanism further intensifying the RAAS activation effect. In the kidney, RAAS mediated efferent arteriolar constriction increases intraglomerular pressure and

hyperfiltration, which has been implicated in the initiation and progression of DN (5, 7). Ang II can also stimulate aldosterone release from the adrenal cortex leading to sodium retention by the kidney. RAAS activation in DM therefore causes maladaptive hemodynamic changes, including inflammation, proliferation, systemic vascular dysfunction, high intraglomerular pressure and renal hyperfiltration leading to cardiovascular and renal complications (35).

Hyperglycemia is a critical determinant of diabetic complications through activation of neurohormonal pathways such as the RAAS (36). Hyperglycemia increases Ang II generation, leading to above described effects on microvascular and macrovascular function (7). Previous animal and human studies examining the pathogenesis of cardiovascular risk have therefore targeted the interaction between hyperglycemia and RAAS activation (26, 37-39). In addition to being activated by hyperglycemia, the RAAS is also stimulated by UA (36). UA-dependent ROS generation promotes Ang II type 1 receptor gene expression and increases plasma Ang II (8, 9). UA also activates the RAAS through increased juxtaglomerular renin production (40). Experimental hyperuricemia in rodent models leads to an afferent renal arteriolopathy and tubulointerstitial fibrosis through RAAS activation (41). Similar to observations in animals, UA is positively associated with plasma renin activity in humans, even when UA levels are within the normal range (9). The association between UA and the RAAS has been strengthened by the observation that UA levels correlate negatively with renal blood flow responses to infusion of Ang II, suggesting a state of intrarenal RAAS activation associated with higher UA levels, leading to blunted responses to exogenous Ang II (42). These observations support work by Messerli et al, which demonstrated a positive correlation between increasing UA levels and intrarenal RAAS activity (43). The effect of UA on the RAAS is inhibited by UA lowering agents such as probenecid (to block UA entry into cells), antioxidants (tempol), or RAAS inhibitors (9). The effect of UA on the RAAS is of greater importance in the context of DM since hyperglycemia increases UA levels *in vitro* and in type 2 DM patients, indicating a positive feedback interaction between hyperglycemia, the RAAS and UA (44).

1.8 UA and Renal Function

1.8.1 UA as a Determinant of Renal Hemodynamic Function

In experimental animal models of DM and in about 50% of patients with early, uncomplicated type 1 DM and ~50% of patients with type 2 DM under the age of 40, glomerular hyperfiltration

is the earliest manifestation of renal microvascular dysfunction (36). Hyperfiltration has been attributed to changes in tubuloglomerular feedback related to increased sodium glucose cotransport-2 activity, and to neurohormonal factors including RAAS activation and the production of ROS, which promote efferent renal arteriolar vasoconstriction, leading to a rise in intraglomerular pressure and single nephron glomerular filtration rate (GFR) (1, 7, 45). Hyperfiltration is associated with hyperglycemia-independent glomerular injury in animals, and normalization of intraglomerular pressure reduces renal injury in experimental DM (7). Consistent with animal studies, hyperfiltration is implicated in the pathogenesis of diabetic renal disease in humans (7, 46-48). In the DM cohort, hyperfiltration is associated with an early decline in GFR and with macrovascular abnormalities, including endothelial dysfunction (46-48). Hyperfiltration also predicts adverse clinical outcomes such as microalbuminuria (7).

Given the UA mediated ROS production and stimulation of RAAS, it is not surprising that UA has been linked with renal functional effects in experimental models (8, 9). Evidence from rodent models suggests an association between high urinary UA levels and hyperfiltration with a subsequent increase in proteinuria and renal failure along with associated glomerular sclerosis and tubulointerstitial fibrosis (1). Similar to the mechanism of gout arthropathy, high UA levels can lower urinary pH favoring UA crystallization and deposition in the collecting duct of the nephron (13). UA crystals then adhere to the renal epithelial cells inducing inflammation, reducing GFR and increasing the risk of kidney stone formation (13). As a consequence, UA lowering reduces hemodynamic injury and inflammation in the high intraglomerular pressure remnant kidney model of CKD (49). More recently, in an animal model of type 1 DM, UA lowering reduced proteinuria, preserved GFR and suppressed renal expression of interleukins (50). In murine models of type 2 DM, UA lowering reduced proteinuria and TGFβ-1 induced renal fibrosis (51). UA therefore causes neurohormonal activation and impaired autoregulation in experimental CKD, leading to tubulointerstitial fibrosis, glomerular hypertension and proteinuria, while UA lowering is renoprotective (40, 41).

Consistent with observations showing correlations between increasing UA levels and intrarenal RAAS activity, epidemiologic and clinical studies have suggested that UA is an independent risk factor for renal dysfunction in the normal population, in patients with diabetes and in those with CKD (42, 43, 48). A 7 year follow up study of 21,475 healthy participants found that slightly elevated UA levels (415 µmol/L to 530 µmol/L) were associated with a two-fold risk of kidney

disease and further elevated UA levels (>535 μmol/L) were associated with more than 3 times the risk of CKD (52). The association between UA levels and progression to DN was first demonstrated in an 18 year prospective observational study by Hovind et al.in 2009 (53). Although the association between UA and microalbuminuria was not observed in the 263 type 1 DM patients, UA levels 3 years after DM onset were predictive of persistent macroalbuminuria. UA is associated with impaired renal function in type 1 DM patients and the general population even when UA levels are in the normal range (1, 53, 54). Data from 355 type 1 DM participants in the second Joslin Study on the Natural History of Microalbuminuria showed a significant association of baseline UA (within the normal range) with early GFR loss of more than 3.3% per year over a 6 year follow up period (55). The risk of renal function decline is low in the normoalbuminuria stage and begins to progress in a large proportion of patients with microalbuminuria despite having normal or elevated baseline renal function (56). Once the renal decline process begins, it progresses linearly and rapidly until it reaches end stage renal disease (56, 57). Thus early renal function decline (ERFD) is relevant for disease prevention. The mechanisms involved in initiating and sustaining the ERFD are unknown, thus it is important to further study the role of UA in the ERFD process to aid in identifying predictors and targets for intervention to protect renal function while it is still normal or even elevated.

UA also increases the risk of developing proteinuria (58). For example, in 652 normoalbuminuric type 1 DM patients recruited into the Coronary Artery Calcification in Type 1 Diabetes Study (CACTI), each 60 μmol/L increment in UA from baseline increased the risk of micro- or macro-albuminuria by 80% after a 6 year follow up period (58). In type 2 DM patients with preserved GFR, hyperuricemia independently increases the risk of proteinuria or GFR <60 ml/min/1.73m^2 by two-fold (59). Interpretation of observational studies is, however, confounded by the increase in UA levels associated with a variety of clinical factors, such as impaired renal function or diuretic use. Additionally, UA as a product of xanthine oxidase could act as a biomarker of oxidative stress in these studies. Though observational associations between higher UA levels and important renal outcomes show consistency among independent cohorts, causal inferences cannot be made with confidence and UA can be a mere biomarker of kidney function due to reduced renal excretion.

1.8.2 The Effect of Pharmacologic UA Lowering on Renal Hemodynamic Function

Evidence is emerging that lowering UA levels could be an important strategy to reduce renal disease progression and diabetic complications. UA lowering can be achieved with XO inhibitors, such as allopurinol and febuxostat, or with uricosuric agents. While the association between UA and renal dysfunction or injury is compelling, only a few human trials with mixed results to date evaluated whether pharmacologic lowering of UA provides renal protection.

In 2006, Siu et al randomized 54 hyperuricemic patients with mild to moderate CKD to receive a 12 months treatment with allopurinol or to continue their usual therapy. A significantly greater number of patients allocated to usual treatment (46% versus 16%) reached a combined endpoint of an increased serum creatinine of greater than 40% or dialysis (60). Although the study by Siu et al suggests that UA lowering would benefit CKD patients, the results should be interpreted with caution due to the extremely high serum UA levels of the enrolled patients, the unrestricted use of antihypertensive agents and the lack of a placebo group. In a prospective study involving 113 CKD patients, Goicoechea et al showed a slower progression of renal disease in patients treated for 2 years with allopurinol versus those continuing regular therapy (GFR change of +1.3 versus -3.3 mL/min/1.73m^2 respectively), independent of age, gender, diabetes, C-reactive protein, albuminuria, and RAAS blockade (61). This clinical trial was also limited by the lack of placebo arm, concomitant mediations use, older age of the patient cohort, and the small proportion of subjects with DN. Similarly, a *post hoc* analysis of the Febuxostat Open-Label Clinical Trial of Urate-Lowering Efficacy and Safety Study (FOCUS) in 116 hyperuricemic patients with gout treated with febuxostat for 5 years showed a similar inverse correlation between UA reduction and the rate of eGFR decline, where every 1 mg/dL decrease in serum UA translated into a 1 mL/min/1.73m^2 improvement in GFR (62). Another *post hoc* analysis of interest stems from the Reduction of Endpoints in Non-Insulin-Dependent Diabetes Mellitus With the Angiotensin II Antagonist Losartan (RENAAL) trial (63). The renoprotective effect of Losartan, an antihypertensive ARB, was attenuated from 22% to 17% when adjusted for serum UA levels suggesting that about one fifth of Losartan's protective effect could be attributed to the serum UA lowering. The renal endpoints in the analysis were defined as a doubling of serum creatinine or end-stage renal disease. Lowering UA with allopurinol in DN patients was also shown to reduce proteinuria by 42% after just 4 months of treatment (64), but the study was limited by the short

follow up period, a sample size of 40 patients and the use concomitant medications. Finally, withdrawal of allopurinol after treatment for 12 months in 50 CKD patients resulted in an increase in UA levels, a significant acceleration in the rate of kidney function loss and a significant increase in urinary TGF-β excretion (20). A recent meta-analysis evaluated the evidence of the benefits and risks of UA lowering with allopurinol on renal outcomes. The 8 evaluated clinical studies had substantial heterogeneity in baseline kidney function data and follow up duration. Five out of the 8 evaluated trials did not show a change in GFR and 3 trials showed an attenuated creatinine rise with allopurinol treatment (65). From the limited data available from UA lowering studies in humans, UA lowering may slow GFR decline and reduces proteinuria. Despite the promising results of these studies, more adequately powered, placebo-controlled studies need to be conducted to determine if lowering plasma UA indeed protects the kidney from injury in patients with DN or if UA is a mere biomarker of reduced kidney function. Given that traditional RAAS blockade provides only partial protection from the development of renal disease, it is of utmost importance to determine if UA lowering therapy can block pathogenic mechanisms promoting DN. The effect of UA lowering on renal protection in type 1 DM is being examined as part of the NIH funded Protecting Early Renal Function Loss or "PERL" study (NCT02017171)(66).

1.9 UA and Cardiovascular Function

1.9.1 UA as a Determinant of Cardiovascular Function

In addition to effects on renal function, UA is associated with changes in cardiovascular function. Studies suggest that UA could have a role in initiating the development of hypertension, which shifts to a salt sensitive kidney dependent hypertensive state over time (67). Acute elevation of UA in murine models by uricase inhibition result in a prompt rise in blood pressure, which can be chronically maintained to induce vascular damage and glomerular changes such as thickening and vasoconstriction of the afferent arteriole, reduction in blood flow, tubulointerstitial injury and inflammation (40, 41). The correlation between UA and blood pressure is also supported by human studies, where UA is independently associated with systemic hypertension, including primary hypertension in children, and higher UA levels in children increase the subsequent risk of developing hypertension in early adulthood (16). UA levels also correlate with blood pressure in 90% of hypertensive pediatric cases (16) and predict the development of hypertension in observational studies involving adults (16). Hyperuricemia is observed in 25-40% of untreated

hypertensive subjects and 75% of malignant hypertensive subjects (68). The association of UA with blood pressure decreases in the elderly and is diminished in patients above 90 years old (69). Interestingly, a recent study by Bjornstad et al. found that over a 6 year follow-up period a positive relationship was observed between UA and systolic blood pressure in non-diabetic individuals, but a negative association was observed in type 1 DM patients (70). A possible explanation is the decline in UA levels due to glycosuria induced proximal tubular dysfunction and uricosuria, which is also supported by higher UA levels in patients with better diabetes control (70). Thus, despite high intracellular levels of UA, patient with poor glycemic control could have reduced UA levels, thereby altering the expected association between serum UA and blood pressure. Accumulating evidence suggests that increased serum UA levels may be associated with intimal medial thickness, endothelial dysfunction and vascular stiffness (35), which all play an important role in the pathogenesis of hypertension, cardiovascular disease and CKD (1, 7). Hyperuricemia has also been associated with other cardiovascular events related to hypertension, such as a non-dipping circadian blood pressure pattern (16), stroke, incidence of heart failure and mortality (16). It is important to note, however, that since UA is associated with both increased cardiovascular risk and the insulin resistance syndrome, insulin resistance may act as a confounder, and at least in part account for the relationship between UA and a higher cardiovascular risk profile (71).

In patients with DM, the deleterious effect of UA on cardiovascular function may be further worsened by the hypertensive effect of hyperglycemia (26, 27, 72). Similar to renal effects, hyperglycemia induces systemic vascular abnormalities including endothelial dysfunction in humans (26-28). As a result of the effects of hyperglycemia and neurohormonal activation, UA levels are independently associated with endothelial dysfunction in animals and humans, thereby promoting hypertension, even when UA levels are within the normal range (29-31). The independent association between UA and blood pressure, including the rate of incident hypertension, has been reported in a variety of study cohorts, including a subset of the Framingham Heart Study (30, 73). In 1993, Rathman et al reported a positive correlation between serum UA levels and CAD in women with type 1 and type 2 DM (17). More recently, baseline serum UA levels were shown to be predictive of coronary calcification in patients with type 1 DM (17). The correlation between UA and cardiovascular function has been attributed to the same mechanisms leading to renal dysfunction, including activation of RAAS and ROS pathways, NOS inhibition and autonomic dysfunction (9, 25, 40).

1.9.2 The Effect of Pharmacologic UA Lowering on Cardiovascular Function

As expected based on compelling associations between UA and macrovascular dysfunction, and increased blood pressure, recent interest has focused on vascular and antihypertensive impact of pharmacological UA lowering. Flow-mediated dilation (FMD) and forearm blood flow (FBF) responses to acetylcholine (ACh) and L-NMMA infusions are common techniques used to assess endothelium-dependent NO induced vasodilation. In type 2 DM adults with and without CKD, UA lowering improves endothelial function (74, 75). A recent meta-analysis showed that treatment with allopurinol increases FMD by 2.5%, FBF responses to ACh infusion increases by 68.8% and a significant decrease in plasma oxidative stress markers (76). It is noteworthy that intravenous uricase (short-term UA lowering) has no effect on FBF response to ACh and L-NMMA (77). More importantly, allopurinol reduces systolic blood pressure by approximately 6 mmHg in pediatric cohorts, thereby normalizing blood pressure in two-thirds of treated patients (16, 73). Similar to results from pediatric studies, UA lowering reduces systolic blood pressure by 4-10 mmHg in adult patients with normal renal function (78). A recent randomized, double-blind placebo-controlled clinical study resulted in a 10 mmHg systolic and 9 mmHg diastolic blood pressure reductions respectively when pre-hypertensive obese adolescents were treated with either allopurinol or probenecid (uricosuric drug) (17). Thus, the blood pressure lowering effect is likely due to the UA lowering and not due to direct effects on XO inhibition. The effects of medications such as allopurinol on endothelial function and blood pressure occur after 4-12 weeks of treatment and have been attributed in part to UA lowering-independent effects, including reduced ROS production and RAAS suppression (75). A *post hoc* analysis from The Losartan Intervention For Endpoint Reduction in Hypertension trial revealed that an increase in serum UA over 4.8 years was attenuated by losartan treatment, which accounted for 29% of the cardiovascular treatment effect with the primary outcomes defined as a cardiovascular death, fatal or nonfatal myocardial infarction, fatal or nonfatal stroke (79). Similar to the renal studies, these cardiovascular trials contain limitations of power and the lack of placebo groups. Despite these significant effects on blood pressure, further work is now required to determine if blood pressure declines with UA lowering therapies translate into improved clinical outcomes.

1.10 Conclusion

Current data strongly suggests that UA is related to the development of hypertension and nephropathy in patients with DM. Furthermore, pharmacological lowering of UA is associated with renal and vascular protective effects in animals. In humans, lowering UA exerts anti-proteinuric and antihypertensive effects, and may prevent renal function loss and provide vascular protection. Nevertheless, large prospective randomized, controlled trials are required to determine causation between UA lowering and long term clinical outcomes.

1.11 Scope and Hypotheses of Thesis

As discussed in previous sections, accumulating evidence in patients with hypertension and DM with CKD strongly suggests that UA plays an important role in the pathogenesis of micro- and macro-vascular complications of DM, while pharmacologic lowering of UA in these cohorts leads to cardiovascular and renal protection. However, very limited information is available around the physiologic role of uric acid in earlier stages of DM, such as in normotensive, normoalbuminuric patients with type 1 DM (T1D). Furthermore, no studies to date examined whether lowering plasma UA (PUA) in patients with uncomplicated T1D would lead to renal and vascular protection. Given the limited clinical protective effects of current therapeutic strategies used to prevent or delay DN, it is of utmost important to examine PUA as a risk biomarker and as a potential therapeutic target in uncomplicated T1D. The major objective of this thesis is to examine the role of baseline PUA in renal and cardiovascular function and to determine the effects of pharmacologic lowering of PUA in young patients with uncomplicated T1D.

1.11.1 Preview and Hypotheses of Chapter 2

The first study of this thesis was designed to examine (1) the baseline levels of PUA and (2) the relationship between PUA and renal and cardiovascular parameters in the earliest subclinical stages - adolescents with T1D. Specifically, the aim of this exploratory analysis was to study the association between PUA and estimated glomerular filtration rate (eGFR), urinary albumin to creatinine ratio (ACR), blood pressure, endothelial function and arterial stiffness in T1D adolescents compared to a well matched group of healthy controls (HC) from the Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT). Based on previous evidence in DM patients with CKD, we hypothesized that even within the normal range PUA levels will be higher in T1D

adolescence compared to HC and higher PUA will be associated with lower GFR, ACR and endothelial function and increased blood pressure and arterial stiffness. The results of this study would allow us to determine whether PUA is associated with cardiovascular and renal pathophysiology at a very early T1D stage.

1.11.2 Preview and Hypotheses of Chapter 3

In chapter 3 we extended our findings to a later, but still uncomplicated cohort of patients with T1D– young adults. Our aims were to examine (1) the baseline levels of PUA, (2) the relationship between PUA and hemodynamic parameters and (3) the effect of clamped hyperglycemia and glycosuria, mediated by inhibition of the sodium glucose cotransporter-2 (SGLT2), on baseline PUA levels in this cohort. We hypothesized that even within the normal range PUA levels will be higher in young T1D adults compared to HC and that higher PUA levels will be associated with higher blood pressure and lower renal function, measured by GFR and effective renal plasma flow (ERPF). Furthermore, we hypothesized that the acute hyperglycemic stimulus, which promotes negative hemodynamic effects, will further increase baseline PUA levels.

1.11.3 Preview and Hypotheses of Chapter 4

The study described in chapter 4 stemmed from the findings in chapter 3 and aimed to elucidate whether the observed association between PUA and renal hemodynamic function in young adults with T1D is driven by effects at the afferent or the efferent renal arterioles. Estimates of afferent and efferent arteriolar resistance and glomerular hydrostatic pressure were indirectly calculated using Gomez equations. Based on previous experimental data, we hypothesized that even within the normal range higher PUA would be associated with higher resistance at the afferent arteriole, but not the efferent arteriole, in young T1D adults while such associations would not be observed in HC.

1.11.4 Preview and Hypotheses of Chapter 5

Our observations in chapters 2-4 did not determine whether PUA is a cause or a consequence of reduced renal and cardiovascular function. Thus, for the final experimental chapter of this thesis we conducted a hypothesis generating, physiological study intended to determine the impact of PUA lowering on renal and vascular function in young uncomplicated T1D adults. The main aims of this study were to investigate if PUA lowering with febuxostat (FBX) treatment for 8 weeks

modifies (1) the effect of hyperglycemia and infused angiotensin II on renal hemodynamic function, (2) systemic blood pressure and (3) arterial stiffness during clamped euglycemia and hyperglycemia in young uncomplicated T1D adults compared to HC. It was hypothesized that PUA lowering will ameliorate early hemodynamic abnormalities characteristic of T1D, including renal hyperfiltration, systemic hypertensive responses to hyperglycemia and arterial stiffness.

Chapter 2: The Association between Plasma Uric Acid Levels and Cardiorenal Function in Adolescents with Type 1 Diabetes

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A version of this chapter is published in: Diabetes Care 2016; 39(4): 611-616.

2.1 Abstract

Objective: The relationship between plasma uric acid (PUA) and renal and cardiovascular parameters in adolescents with T1D is not well understood. Our aims in this exploratory analysis were to study the association between PUA and estimated glomerular filtration rate (eGFR), urinary albumin to creatinine ratio (ACR), blood pressure, endothelial function and arterial stiffness in T1D adolescents. These associations were also studied in healthy controls (HC).

Methods: We studied 188 T1D recruited to the Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT) and 65 HC. Baseline PUA, $eGFR_{cystatin\ C}$, ACR, blood pressure, flow mediated dilation (FMD) and carotid-femoral pulse wave velocity (PWV) were measured.

Results: PUA was lower in T1D vs. HC (242±55 vs. 306±74μmol/L, $p<0.0001$). Higher PUA was inversely associated with eGFR in T1D ($r=-0.48$, $p<0.0001$) even after correcting for baseline clinical demographic characteristics. PUA was not associated with ACR in T1D after adjustment for potential confounders such as eGFR. For cardiovascular parameters, PUA levels did not associate with SBP, FMD or PWV in T1D or HC.

Conclusions: Even within the physiological range, PUA levels were significantly lower in T1D adolescent patients compared to HC. There was an inverse relationship between PUA and eGFR in T1D, likely reflecting an increase in clearance. There were no associations observed with ACR, blood pressure, arterial stiffness or endothelial function. Thus, in contrast with adults, in adolescents with T1D PUA may not yet be associated with cardiorenal abnormalities.

2.2 Introduction

Recent evidence from animal and human models suggests that plasma uric acid (PUA) levels are associated with multiple key pathways implicated in the pathogenesis of T1D complications, such as metabolic abnormalities (insulin resistance and hyperglycemia), cardiovascular disease (hypertension, endothelial dysfunction, arterial stiffness and cardiac diastolic dysfunction) and kidney dysfunction (80). In healthy adult men and women, PUA is positively associated with activation of pro-inflammatory pathways and activation of the renin angiotensin aldosterone system (RAAS) (80). Perhaps as a consequence of PUA-mediated inflammation and RAAS activation, PUA levels - even within the normal range - are independently associated with endothelial dysfunction, arterial stiffness, impaired renal function and albuminuria in adults with T1D and in the general adult population (80). Consequently, the potential renal and vascular protective effects of PUA lowering are being investigated in a T1D population with microalbuminuria in the Protecting Early Renal Function Loss (PERL) Study (NCT02017171) (66).

In adolescents, elevated PUA levels have also been linked with metabolic syndrome (81, 82), obesity, cardiovascular risk (83), inflammation (84), pediatric hypertension and the subsequent development of hypertension in adulthood (80). Similar to observations by others, we also recently showed that PUA levels are lower in otherwise healthy adult T1D patients between 18 and 35 years of age compared to healthy controls (85). This may be due to a decrease in uric acid reabsorption mediated by high concentrations of glucose in the tubular lumen (85). Despite lower PUA levels in adults with T1D, PUA negatively correlates with eGFR and effective renal plasma flow (85, 86). The association between higher PUA with lower GFR and effective renal plasma flow may be on the basis of PUA-mediated renal vasoconstriction (85, 86). PUA levels have not yet been characterized in an even earlier, subclinical disease population, such as in adolescent patients with T1D. Establishing the relationship between PUA and early markers of renal and cardiovascular risk in T1D patients is important to potentially identify predictors of future complications and to target new interventions aimed at improving long-term prognosis.

Accordingly, the goal of this exploratory analysis was to assess the relationship between PUA and important physiologic parameters in patients with early T1D (87). The associations between PUA, eGFR, flow mediated dilation (FMD – measure of endothelial function) and vascular stiffness measures were assessed in T1D and compared to healthy control (HC) adolescents. Based on the

above observations in adults, we hypothesized that the T1D adolescent cohort would exhibit lower PUA overall, and that higher PUA, even within the normal range, would be associated with higher blood pressure, lower eGFR, higher urinary albumin excretion, impaired FMD and increased arterial stiffness measures in the adolescent T1D cohort, but not in HC.

2.3 Research Design and Methods

2.3.1 Subject Inclusion Criteria and Study Population

All studies were approved by the Hospital for Sick Children Research Ethics Board (Toronto, ON, Canada). All study participants provided assent and parents signed the informed consent documents. This analysis was conducted using the blood and urine samples collected from 188 T1D from the AdDIT trial (87) and 65 HC. We included: 11 to 16 years old adolescents, who achieved a minimum of Tanner stage 2 for puberty and were not taking medication that could interfere with the RAAS. The albumin:creatinine ratio (ACR) measures were obtained by taking two sets of three consecutive early-morning urine samples on two separate visits and the average ACR calculated and adjusted on a log ACR scale using age, diabetes duration, sex and the coefficients from the Oxford Regional Prospective Study (ORPS) linear regression model (87-89). In the AdDIT trial, the T1D participants were divided into the following ACR tertiles: (1) 64 patients in low ACR tertile (<0.8mg/mmol), (2) 74 patients in the middle ACR tertile (0.8-1.2 mg/mmol), and (3) 50 patients in the high ACR tertile (>1.2mg/mmol). The tertile boundaries were determined based on preliminary data from the ORPS cohort which predicted the risk for development of microalbuminuria(88).

2.3.2 Renal Assessments

All urine and blood samples were obtained during the screening phase of the study. eGFR was calculated using the Larsson's formula eGFR = 77.24 x Cys $C^{-1.2623}$, where cystatin C was measured by laser immunonephelometry (Dade Behring) (90, 91). As in our previous work, T1D adolescent participants were also subdivided into a normofiltration and a hyperfiltration group, where hyperfiltration was defined as eGFR ≥135 mL/min/1.73m^2 (14, 46, 92, 93).

2.3.3 Vascular Assessments, Sample Collection and Analytical Methods

Arterial stiffness was measured using a SphygmoCor device (SphygmoCor, AtCor Medical Systems Inc., Sydney, Australia). A high-fidelity micromanometer was used to record right radial, carotid and femoral artery pulse pressure waveforms. The corresponding central aortic pressure waveform was generated using a validated transfer function. Mean arterial pressure (MAP) and heart rate (HR) was determined by the analysis software. The distance between the carotid-femoral pulse points was measured and pulse time delay was calculated to obtain pulse wave velocity (PWV).

Endothelial function of the brachial artery was determined by flow mediated dilation (FMD). A pneumatic cuff was placed distal of the antecubital fossa. Reactive hyperemia was stimulated by a 5 minute inflation of the cuff followed by deflation. A high resolution B-mode ultrasound was used to capture longitudinal ECG-gated end-distolic images of the brachial artery pre- and post-cuff inflation. Diameter was determined using an automated edge-detection algorithm and blood flow was measured from the velocity-time integral of the Doppler signal. FMD was defined as the maximal percentage change in vessel diameter after reactive hyperemia.

Plasma samples were used to measure PUA on the Architect c8000 Clinical Chemistry System using the manufacturer's reagents (Abbott Diagnostics, Abbott Park, Illinois, USA).

2.3.4 Statistical Analysis

Continuous data are presented as mean ± standard deviation (SD). To assess for *between-group* differences analysis of covariance (ANCOVA) was used. Pearson correlation analyses were used to assess the relationship between renal parameters, urinary/plasma markers and PUA levels. Regression analysis was used to assess the impact of covariates on continuous outcomes. Based on known factors that influence PUA levels, potentially relevant clinical characteristics that were included as the covariates in regression analysis were systolic blood pressure (SBP), gender, HbA1c, BMI, age, T1D duration, and plasma HDL cholesterol. $p<0.01$ was considered statistically significant to account for multiple comparison. All statistical analyses were performed using SAS v9.1.3 and GraphPad Prism software (version 5.0).

2.4 Results

2.4.1 Baseline Demographic Characteristics and PUA Levels

The adolescent participants were normotensive and normoalbuminuric. Baseline parameters, such as gender distribution, age, blood pressure, eGFR and ACR were similar between HC and T1D adolescents (Table 2.1). T1D patients had a higher BMI compared to HC. Out of the 188 T1D patients, 133 exhibited normofiltration (71%) and 55 hyperfiltration (29%). HbA1c, plasma glucose and plasma HDL cholesterol were higher in T1D compared to HC. PUA was lower in T1D adolescents than in HC (Figure 2.1, 242±55 vs. 306±74μmol/L, $p<0.0001$). In the T1D cohort, insulin doses did not correlate with PUA levels, and also did not correlate with clinical parameters including SBP, eGFR or log ACR.

No significant differences were observed in PUA levels between tertiles of spot check blood glucose levels or tertiles of HbA1c in our T1D adolescent cohort (Table 2.2, Figures 2.2 A and B).

2.4.2 Association between PUA and Renal Function

Higher PUA correlated with lower eGFR in the HC and T1D cohorts (Figure 2.3: $r= -0.39$, $p=0.001$ for HC and $r= -0.48$ and $p<0.0001$ in T1D). Using Fisher's z-transformation technique, these correlations did not differ between the two cohorts ($p=0.4$). Within patients with T1D, PUA levels were significantly higher in normofiltration subjects compared to those with hyperfiltration (Figure 2.2 C, $p<0.0001$). The association between PUA and eGFR remained significant after correcting for SBP z-score, gender, HbA1c, BMI z-score, age, T1D duration and blood HDL cholesterol in the regression analysis of patients with T1D ($\beta = -0.78$, $p<0.0001$). This observation was also present in T1D adolescents when patients with hyperfiltration were removed from the analysis ($\beta=-1.08$, $p=0.003$). In contrast, in HC, after controlling for SBP z-score, gender, HbA1c, BMI z-score, age and blood HDL cholesterol, the association between PUA and eGFR was not significant ($\beta = -1.29$, $p>0.01$). These relationships remained the same when plasma glucose rather than HbA1c was used in the regression model.

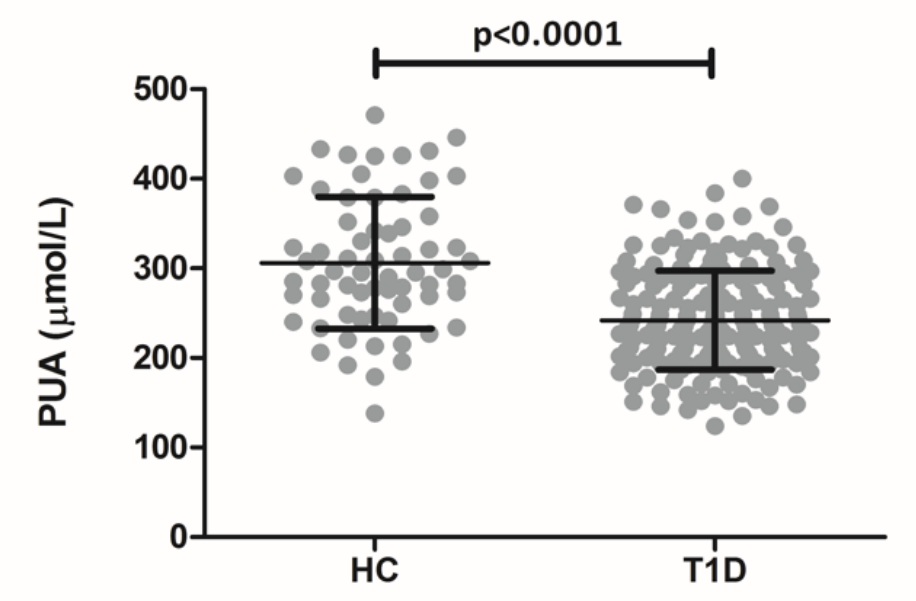
In the T1D cohort, higher PUA levels correlated with lower ACR ($r = -0.20$, $p=0.005$ using log-transformed ACR) an association that was no longer significant after adjusting for eGFR, gender,

Table 2.1. HC and T1D subject characteristics at baseline (mean ± standard deviation).

Parameter	HC (n=65)	T1D (n=188)
Baseline demographic parameters		
Males	28 (43%)	93 (49%)
Age (years)	14.0±2.0	14.4±1.7
Diabetes duration (years)	-	7.2±3.2
Body mass index (z-score)	0.11±1.15	0.65±0.91*
Baseline biochemistry		
Hemoglobin A1c - %	5.4±0.2	8.5±1.3*
(mmol/mol)	(35.3±2.7)	(69.3±13.8)
Plasma uric acid (µmol/L)	306±74	242±55*
Plasma Glucose (mmol/L)	4.7±0.7	9.7±4.3*
Cholesterol (mmol/L)	4.2±0.8	4.3±0.9
HDL Cholesterol (mmol/L)	1.5±0.3	1.6±0.4*
LDL Cholesterol (mmol/L)	2.4±0.7	2.3±0.7
Triglyceride (mmol/L)	0.9±0.4	0.8±0.4
Renal function assessments		
eGFR (mL/min)	121±22	127±29
ACR	1.1±1.6	1.0±1.5
Vascular function assessments		
HR (beats per minute)	67±10	67±8
SBP (mmHg)	111±8	113±9
DBP (mmHg)	62±7	63±6
SBP (z-score)	0.06±0.72	0.19±0.80
DBP (z-score)	-0.18±0.58	-0.16±0.61
FMD (%)	7.6±3.3	6.5±3.1
Carotid Femoral PWV (m/s)	5.2±0.8	5.2±0.7
Insulin Therapy		
Insulin Pump Users (%)	-	116 (62%)
Insulin Injection Users (%)	-	72 (38%)
Insulin Dose (Units/kg)	-	1.00±0.29

Values are mean ± standard deviation. n, number of participants. *p<0.05 vs. HC; HC: healthy controls; T1D: patients with type 1 diabetes; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: glomerular filtration rate, PWV: Pulse Wave Velocity.

Figure 2.1: Plasma uric acid (PUA) levels in healthy controls (HC, n=65) and patients with type 1 diabetes (T1D, n=188).



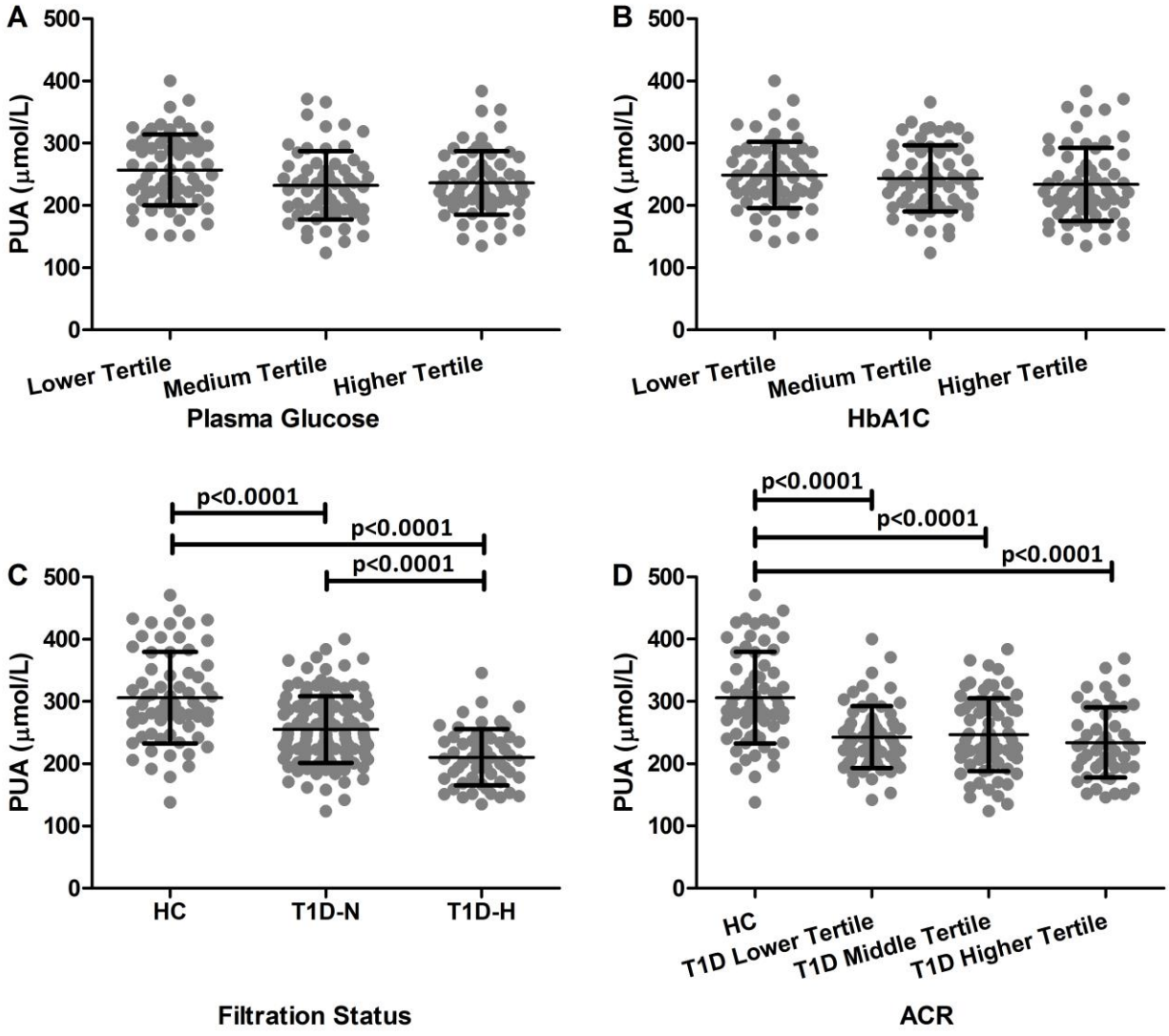
Data are presented as mean ± SD.

Table 2.2. HC and T1D subject characteristics at baseline (mean ± standard deviation). T1D data is divided into tertiles: low ACR tertile (<0.8mg/mmol, n=64), middle ACR tertile (0.8-1.2mg/mmol, n=74) and high ACR tertile (>1.2mg/mmol, n=50).

Parameter	HC (n=65)	T1D (n=188)		
		Lower Tertile (n=64)	Middle Tertile (n=74)	Higher Tertile (n=50)
Baseline demographic parameters				
Males	28 (43%)	26 (41%)	43 (58%)	24 (48%)
Age (years)	14.0±2.0	14.6±1.6	13.9±1.7†	14.8±1.6*‡
Diabetes duration (years)	-	8.1±3.4	6.9±3.0†	6.6±2.9†
Body mass index (z-score)	0.11±1.15	0.78±0.89*	0.63±0.96*	0.53±0.85*
Baseline biochemistry				
Hemoglobin A1c - % (mmol/mol)	5.4±0.2 (35.3±2.7)	8.3±1.2* (67.7±12.9)	8.5±1.3* (69.8±14.3)	8.6±1.3* (70.8±14.1)
Plasma uric acid (µmol/L)	306±74	243±50	247±58	234±56
Plasma Glucose (mmol/L)	4.7±0.7	8.6±3.5*	10.5±4.8*†	9.8±4.3*
Cholesterol (mmol/L)	4.2±0.8	4.4±0.8	4.3±1.0	4.3±0.8
HDL Cholesterol (mmol/L)	1.5±0.3	1.7±0.4*	1.6±0.3*	1.7±0.3*
LDL Cholesterol (mmol/L)	2.4±0.7	2.3±0.7	2.3±0.8	2.2±0.6
Triglyceride (mmol/L)	0.9±0.4	0.8±0.4	0.9±0.4	0.9±0.3
Renal function assessments				
GFR (mL/min)	121±22	124±28	121±23	141±33*†‡
ACR	1.1±1.6	0.67±0.72	0.93±1.31	1.5±2.3†‡
Vascular function assessments				
HR (beats per minute)	67±10	67±9	65±9	65±9
SBP (mmHg)	111±8	113±9	113±11	114±9
DBP (mmHg)	62±7	62±6	62±7	62±7
SBP (z-score)	0.06±0.72	0.21±0.73	0.19±0.89	0.17±0.75
DBP (z-score)	-0.18±0.58	-0.20±0.55	-0.13±0.68	-0.16±0.57
FMD (%)	7.6±3.3	7.0±3.4	6.2±3.0*	6.1±2.9*
Carotid Femoral PWV (m/s)	5.2±0.8	5.2±0.8	5.3±0.7	5.2±0.7
Insulin Therapy				
Insulin Pump Users (%)	-	42 (66%)	46 (62%)	32 (64%)
Insulin Injection Users (%)	-	22 (34%)	28 (38%)	18 (36%)
Insulin Dose (Units/kg)	-	1.00±0.28	0.97±0.31	1.03±0.24

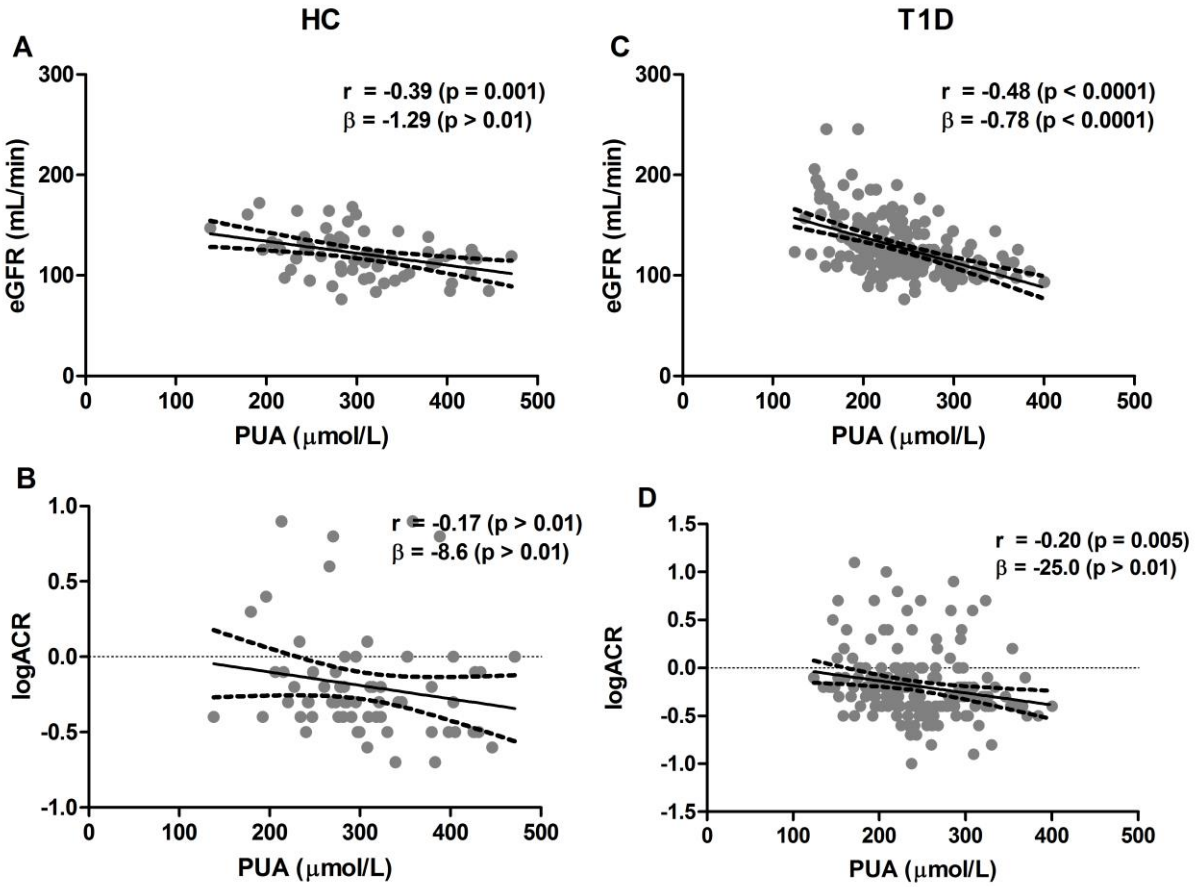
Values are mean ± standard deviation. n, number of participants. *p<0.05 vs. HC; †p<0.05 vs. T1D Lower Tertile; ‡p<0.05 vs. T1D Middle Tertile. HC: healthy controls; T1D: type 1 diabetic patients; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; GFR: glomerular filtration rate, PWV: Pulse Wave Velocity.

Figure 2.2: Plasma uric acid (PUA) levels in patients with type 1 diabetes (T1D, n=188) divided into tertiles according to (A) plasma glucose and (B) HbA1C. Figure (C) represents PUA levels in HC (n=65) compared to T1D patients with normofiltration (T1D-N, n=133) and hyperfiltration (T1D-H, n=55). Figure (D) represents PUA levels in healthy controls (HC, n=65) and T1D in the low ACR tertile (<0.8mg/mmol, n=64), middle ACR tertile (0.8-1.2mg/mmol, n=74) and high ACR tertile (>1.2mg/mmol, n=50).



Data are presented as mean ± SD. When testing for significance, relevant clinical characteristics that were included as the covariates were GFR, systolic blood pressure (SBP), gender, HbA1c, BMI, age, T1D duration, and plasma HDL cholesterol. ACR group tertiles defined according to the ORPS definition criteria (87-89).

Figure 2.3: The relationship between eGFR, log transformation of albumin to creatinine ratio and plasma uric acid (PUA) in HC (panel A and B, n=65) and patients with type 1 diabetes (panel C and D, T1D; n=188).



Pearson correlation analysis was used to obtain r and its associated p value. The β coefficient with the associated p value were obtained from the regression analysis. In the HC PUA and eGFR regression analysis, the covariates were SBP z-score, gender, HbA1C, BMI z-score, age and plasma HDL cholesterol. In the T1D PUA and eGFR regression analysis, the covariates were SBP z-score, gender, HbA1c, BMI z-score, age, diabetes duration and plasma HDL cholesterol. In the HC PUA and log ACR regression analysis, the covariates were eGFR, gender, HbA1c, BMI z-score, age and plasma HDL cholesterol. In the T1D PUA and log ACR regression analysis, the covariates were eGFR, gender, HbA1C, BMI z-score, age, diabetes duration and plasma HDL cholesterol.

HbA1c, BMI z-score, age, T1D duration and blood HDL cholesterol and correcting for multiple comparisons (β = -25.0, $p>0.01$). When plasma glucose rather than HbA1c was used in the model, the association did not change and was still not significant (β=-20.4, $p>0.01$). The association between PUA and log ACR in HC participants was not observed (r = -0.17, $p>0.01$). PUA levels were lower in each of the ACR tertile groups compared to HC. There were no differences in PUA levels observed between the 3 T1D ACR tertile groups studied in the cohort (Figure 2.2 D).

2.4.3 The Association between PUA and Vascular Function

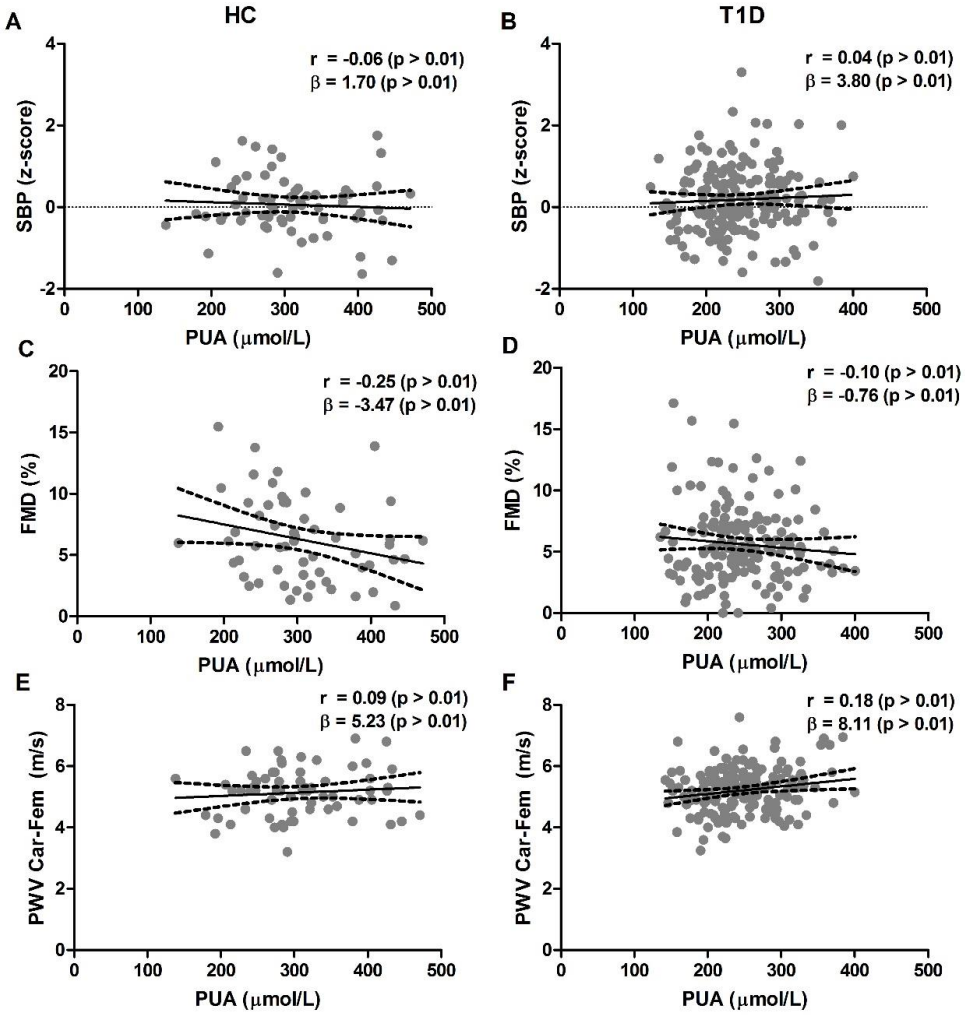
PUA did not correlate with SBP z-score in HC (Figure 2.4, r=-0.06, $p>0.01$) or T1D (r=0.04, $p>0.01$). As shown in Figure 2.4, there was also no association observed after correcting for clinical covariates in HC (β=1.7, $p>0.01$) or T1D (β=3.8, $p>0.01$). PUA did not correlate with PWV, DBP z-score or FMD in either group (Figure 2.4).

2.5 Discussion

Although T1D complications are rarely evident during childhood, pathogenic processes leading to end organ injury begin soon after diagnosis and may accelerate during puberty (94, 95). It is therefore important to study potential preclinical mechanisms leading to early disease pathogenesis to facilitate the identification of high-risk patients and thereby implement early therapeutic prevention strategies. PUA levels are consistently associated with renal and cardiovascular complications in adults with T1D, and predict incident albuminuria, rapid eGFR decline, diabetic retinopathy and coronary artery calcification (96, 97). The influence of PUA levels on renal and cardiovascular function has not yet been carefully characterized in adolescent patients with uncomplicated T1D prior to the onset of clinical complications.

In the current cohort, significantly higher PUA levels were observed in adolescent HC compared to T1D, which is consistent with previous observations in young adults and in adolescents (85, 86, 98). Increased urinary glucose excretion is thought to be the key mechanism responsible for PUA lowering in patients with diabetes due to a stimulatory effect of urinary glucose on the GLUT 9 isoform 2 transporter on the apical membrane of the proximal tubule, which increases UA excretion in *in vitro* studies (99). Importantly, we have previously demonstrated that impairing proximal tubule glucose reabsorption increases fractional excretion of UA in adults with T1D (85). Interestingly, and in contrast with our previous observations in young adults with T1D,

Figure 2.4: The relationship between plasma uric acid (PUA) and systolic blood pressure (SBP – A and B), flow mediated dilation (FMD – C and D), pulse wave velocity (PWV = E and F) in healthy controls (HC – A, C, E, n=65) and patients with type 1 diabetes (T1D – B, D, F, n=188).



Pearson correlation analysis was used to obtain r and its associated p value. The β coefficient with the associated p value were obtained from the regression analysis. In the HC PUA, SBP z-score and PWV regression analysis, the covariates were gender, HbA1C, BMI, age and plasma HDL cholesterol. In the T1D PUA, SBP z-score and PWV regression analysis, the covariates were gender, HbA1c, BMI, age, diabetes duration and plasma HDL cholesterol. In the HC PUA and FMD regression analysis, the covariates were gender, SBP, HbA1c, BMI, age and plasma HDL cholesterol. In the T1D PUA and FMD regression analysis, the covariates were gender, SBP, HbA1C, BMI, age, diabetes duration and plasma HDL cholesterol.

neither HbA1c nor plasma glucose levels significantly influenced PUA in our T1D adolescent cohort. It is thus possible that glycosuria does not modify PUA excretion in adolescents with T1D by the same mechanisms as in adults, and that a longer T1D duration is required for higher glucose levels to influence PUA. It will therefore be important to confirm our observation in a larger cohort of adolescents over a longer period of time.

Previous longitudinal studies have shown a relationship between PUA and renal function decline. For example, the second Joslin Study on the Natural History of Microalbuminuria showed a significant correlation between baseline PUA levels with early eGFR loss over a 6-year time period in older patients with T1D (55). Even in normoalbuminuric patients with T1D, Krolewski et al reported that mildly elevated PUA is an independent predictor of early eGFR loss (100). We also recently showed that higher PUA levels within the normal range are associated with lower GFR and effective renal plasma flow and higher renal vascular resistance in an adult T1D cohort without any complications (85, 86). Consistent with the previous body of work in adults, the association between higher PUA and lower eGFR was present in our even earlier, adolescent cohort of otherwise healthy T1D patients. This association persisted even after correcting for SBP z-score, gender, HbA1c, BMI z-score, age, T1D duration and blood HDL cholesterol. Although we were not able to elucidate the mechanisms, this association is most likely on the basis of increased renal clearance, leading to lower PUA levels. However, the persistent association between higher PUA and lower eGFR in T1D adolescents with normofiltration suggests that hyperfiltration might not be the only factor driving this association. Thus, higher PUA may be linked with lower eGFR through renal vasoconstriction resulting in ischemia (41, 86), but this seems unlikley in a pediatric cohort.

Microalbuminuria is a risk factor for progressive renal function decline (57) and is one of the first clinical markers of nephropathy in adolescents with T1D (89, 101). *Post hoc* analyses of the Coronary Artery Calcification in Type 1 Diabetes study (CACTI) reported that over a 6 year follow up period, each 60 μmol/L increase in PUA from baseline elevated the risk of micro- or macro-albuminuria by 80% in 652 normoalbuminuric patients (58). Similarly, Hovind et al. reported that baseline PUA levels predict the development of macroalbuminuria over 18 years of follow up in patients with T1D (53). In our cohort, in the univariate analysis, higher PUA was modestly associated with lower ACR. However, this interaction was no longer significant after adjustment for clinical characteristics, suggesting the predominant role of other pathways,

including the interaction between PUA and eGFR, that mediate changes in albumin excretion. Furthermore, there was no difference in baseline PUA levels in the low-, middle-, and high-risk within normal range ACR tertiles in the T1D adolescent patients. Overall, our data suggests that baseline PUA levels in this cohort are not associated with ACR, at this early stage of T1D.

Accumulating evidence in patients with hypertension, atherosclerosis, type 2 diabetes, and also in HCs, suggests that increased PUA levels, even within the normal range, may be associated with endothelial dysfunction and vascular stiffness (80), thereby promoting cardiovascular disease. A National Health and Nutrition Examination Survey (1999-2006) found that PUA of >327 μmol/L in 6,036 adolescents carrried a 2-fold risk of developing hypertension (102). We also recently reported an association between higher PUA and higher blood pressure within the normal range in young adults with uncomplicated T1D (70, 85). In our cohort of adolescents with uncomplicated T1D or in HC, PUA did not correlate with SBP z-score, PWV or FMD (a measure of endothelial function) after correcting for age, gender, HbA1c, BMI z-score, T1D duration and HDL. It is therefore possible that the relationship between PUA and blood pressure could be altered over time according to duration of the disease or age in patients with T1D (85).

Our study has limitations. Although CysC based eGFR measurements may better identify acute changes in kidney function comapred to the most widely used serum cretainine method (103), cystatin C still tends to underestimate GFR in the higher range compared to the gold standard inulin clearance based GFR measurement technique (104). Additionally, the patient study cohort consisted of a carefully selected group with no complications in a subset of patients from the AdDIT trial. Thus, our data may only be relevant to adolescent patients with uncomplicated T1D and cannot necessarily be extended to other conditions. Data on urinary glucose levels was not available and thus the association between PUA and glycosuria could not be studied. Dietary consumption of UA-rich foods, such as purine-containing products, was also not recorded and thus could not be taken into account in the analysis. We also recognize that this analysis included a subset of participants in the AdDIT Observation Cohort. As such, our observations should be considered exploratory in nature, and ultimately require confirmation using a larger sample size of patients. Finally, future analyses should examine if PUA levels are influenced by puberty stage – an interaction that was not examined in this cohort.

In conclusion, even within the physiological range, PUA levels were significantly lower in T1D adolescent patients compared to HC. There was an inverse relationship between PUA and eGFR

in T1D and no associations were observed with blood pressure, arterial stiffness or endothelial function. Thus, in contrast with adults, in adolescents with T1D PUA may not yet be associated with cardiorenal abnormalities, highlighting the need to determine if the effect of PUA on renal risk is modified over time.

Chapter 3: Glycosuria-mediated Urinary Uric Acid Excretion in Patients with Uncomplicated Type 1 Diabetes Mellitus

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A version of this chapter is published in: American Journal of Physiology – Renal Physiology (2015), 308(2):F77-83.

3.1 Abstract

Objective: Plasma uric acid (PUA) is associated with metabolic, cardiovascular and renal abnormalities in patients with type 2 diabetes, but is less well understood in type 1 diabetes (T1D). Our aim was to compare PUA levels and fractional UA excretion (FE_{UA}) in patients with T1D vs. healthy controls (HC) during euglycemia and hyperglycemia.

Methods: PUA, FE_{UA}, blood pressure (BP), glomerular filtration rate (GFR-inulin) and effective renal plasma flow (ERPF – paraaminohippurate) were evaluated in patients with T1D (n=66) during clamped euglycemia (glucose 4-6 mmol/L) and hyperglycemia (9-11 mmol/L), and in HC (n=41) during euglycemia. To separate the effects of hyperglycemia vs. increased glycosuria, parameters were evaluated during clamped euglycemia in a subset of T1D patients before and after SGLT2 inhibition for 8 weeks.

Results: PUA was lower in T1D vs. HC (228±62 µmol/L vs. 305±75 µmol/L, $p<0.0001$). In T1D, hyperglycemia further decreased PUA (228±62 µmol/L to 199±65 µmol/L, $p<0.0001$), which was accompanied by an increase in FE_{UA} (7.3±3.8 to 11.6±6.7, $p<0.0001$). In T1D, PUA levels correlated positively with SBP ($p=0.029$) and negatively with ERPF ($p=0.031$) and GFR ($p=0.028$). After induction of glycosuria with SGLT2 inhibition while maintaining clamped euglycemia, PUA decreased ($p<0.0001$) and FE_{UA} increased ($p<0.0001$).

Conclusions: PUA is lower in T1D vs. HC, and positively correlates with SBP and negatively with GFR and ERPF in T1D. Glycosuria rather than hyperglycemia increases uricosuria in T1D. Future studies examining the effect of UA lowering therapies should account for the impact of ambient glycemia, which causes an important uricosuric effect.

3.2 Introduction

Humans have higher uric acid (UA) levels in comparison to other mammals due to mutational silencing of the enzyme uricase, which results in UA remaining the end product of purine metabolism (80). Additionally, about 90% of filtered UA is reabsorbed by the S1 segment of the proximal convoluted tubule, in a process regulated by intracellular anion transporters on the basolateral membrane, such as urate transporter 1 (URAT1) and the more recently discovered glucose transporter 9 (GLUT9) (80). The lack of uricase, combined with the high reabsorptive capacity in the kidney, predisposes humans to the development of hyperuricemia.

UA has recently emerged as an inflammatory factor that increases oxidative stress and promotes activation of the renin angiotensin aldosterone system (RAAS) (80). From a clinical perspective, higher UA levels are associated with metabolic abnormalities (insulin resistance, hyperglycemia), cardiovascular disease (hypertension, endothelial dysfunction, arterial stiffness, cardiac diastolic dysfunction) and kidney injury (1, 80) and thus could be involved in the onset and progression of diabetic nephropathy, a common microvascular complication of diabetes mellitus (DM). Plasma UA (PUA) levels could therefore serve as a biomarker and an effective therapeutic target to supplement current clinical targets such as hemoglobin A1c (HbA1c), cholesterol and blood pressure.

Evidence from rodent models suggests an association between high UA levels and markers of high intraglomerular pressure such as hyperfiltration, and with subsequent increases in proteinuria, glomerular sclerosis and tubulointerstitial fibrosis, leading to chronic kidney disease (1). More recently, in an animal model of type 1 DM (T1D), UA lowering reduced proteinuria, preserved GFR and suppressed renal expression of inflammatory interleukins (50). In patients with T1D, UA is associated with impaired renal function, even when UA levels are in the normal range (1, 54). For example, in 355 T1D participants from the second Joslin Study on the Natural History of Microalbuminuria, baseline UA (within the normal range) showed a significant association with early GFR loss of more than 3.3% per year over a 6-year follow-up period (55). UA also increases the risk of developing proteinuria in T1D patients (58). For example, in 652 normoalbuminuric type 1 DM patients recruited into the Coronary Artery Calcification in Type 1 Diabetes Study (CACTI), each 60 μmol/L increment in UA from baseline increased the risk of micro- or macro-albuminuria by 80% after a 6-year follow-up period (58). Though observational

associations between higher UA levels and renal outcomes show consistency among independent cohorts (80), UA levels are not clearly defined in the T1D populations.

Accordingly, the first goal of this study was to compare PUA levels in healthy control patients (HC) with patients with T1D. It was hypothesized that even within the normal range, PUA levels would be higher in the T1D cohort and that higher PUA levels will be associated with deleterious hemodynamic profiles such as higher blood pressure and changes in renal hemodynamic function. The second goal was to examine the relationship between clamped hyperglycemia, hemodynamic parameters and PUA levels to determine if this acute physiological stimulus, which promotes deleterious hemodynamic effects such as increased blood pressure, influences PUA levels.

3.3 Research Design and Methods

3.3.1 Subject Inclusion Criteria and Study Preparation

Forty-one HC and 66 T1D patients underwent detailed physiological examinations. In brief, inclusion criteria were: 18-40 years of age, blood pressure <140/90, normoalbuminuria on a 24-hour urine collection, diabetes duration >1 year, no history of renal or cardiovascular complications and no intake of concomitant medications that would alter blood pressure or cardiovascular outcomes. Study visits were performed after a controlled diet for 7 days consisting of ≥150 mmol/day sodium and ≤1.5 g/kg/day protein. The sodium-replete diet was used to avoid circulating volume contraction, RAAS activation and *between-subject* heterogeneity. Pre-study protein intake was modest to avoid the hyperfiltration effect of high protein diets (105). All the studies were approved by the University Health Network Research Ethics Board and all subjects gave written informed consent.

3.3.2 Experimental Procedures

Patients with T1D were studied on 2 consecutive days during euglycemia and hyperglycemia. Euglycemic (4-6 mmol/L) and hyperglycemic (9-11 mmol/L) conditions in T1D were maintained using a modified glucose clamp technique as previously described (106). Blood glucose levels were stable for at least 2 hours prior to the measurement of the study end points and were maintained 3 to 5 hours for the rest of the study day. HC were studied during normoglycemic conditions at the Renal Physiology Laboratory at the Toronto General Hospital. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were estimated using inulin and paraaminohippurate (PAH) steady state infusion clearance techniques (107), respectively, using

previously described methods (108). The results of the 2 clearance periods were averaged. Brachial artery blood pressure measurements were obtained at 30-minute intervals throughout the study days (Critikon, Tampa, Florida, USA).

In a *post-hoc* analysis undertaken to understand the relative effects of hyperglycemia vs. increased glycosuria, the effect of sodium glucose co-transporter 2 (SGLT2) inhibition on PUA and urinary UA was examined using frozen, archived samples. The aim of this analysis was to induce glycosuria while maintaining euglycemia to determine whether effects on PUA were due to increased urinary UA excretion. For this analysis, we analyzed urine and plasma samples (n=40) obtained during baseline clamped euglycemic conditions and at follow-up after treatment with empagliflozin 25mg QD for 8 weeks in the Adjunctive-To-Insulin and Renal MechAnistic pilot trial of empagliflozin in T1D (ATIRMA trial, ClinicalTrials.gov NCT01392560). The primary and secondary outcomes from this trial have been published (108).

3.3.3 Sample Collection and Analytical Methods

After clamped euglycemia was achieved for at least 2 hours, blood was collected for measurements of inulin, PAH, sodium, PUA and RAAS mediators (angiotensinogen, plasma renin activity [PRA], aldosterone and angiotensin II) and urine samples were collected for UA, sodium, glucose and creatinine measurements.

The blood samples were immediately centrifuged at 3000 rpm at 4ºC for 10 minutes. Plasma was extracted, placed on ice and stored at -70ºC. Inulin and PAH were measured in serum by colorimetric assays using anthrone and N- (1-naphthyl) ethylene-diamine respectively (109). All hemodynamic measurements were adjusted for body surface area. Filtration fraction (FF) represented the ratio of GFR to ERPF. Renal blood flow (RBF) was derived as ERPF / (1-hematocrit). Renal vascular resistance (RVR) was derived by dividing mean arterial pressure (MAP) by the RBF.

Plasma and urine samples were measured for UA, sodium, creatinine, glucose and urea on the Architect c8000 Clinical Chemistry System using the manufacturer's reagents (Abbott Diagnostics, Abbott Park, Illinois, USA). In addition, UA excretion was expressed as fractional excretion (FE_{UA}), derived using $(U_{UA}\times P_{Cr})/(U_{Cr}\times P_{UA})\times 100$ where U_{UA}, P_{Cr}, U_{Cr} and P_{UA} are urinary UA, plasma creatinine, urinary creatinine and plasma UA concentrations respectively. Similarly, sodium excretion was expressed as fractional excretion (FE_{Na}), derived using

$(U_{Na}\times P_{Cr})/(U_{Cr}\times P_{Na})\times 100$ where U_{Na} and P_{Na} are urinary sodium and plasma sodium concentrations, respectively.

Aldosterone was measured using a Coat-A-Count radioimmunoassay system. PRA was measured using a radioimmunoassay kit (Diasorin, Stillwater, Minnesota, USA). HbA1c was measured by high-performance liquid chromatography with the Variant II system (Bio-Rad Laboratories, Hercules, California, USA).

3.3.4 Statistical Analysis

Data are presented as mean ± standard deviation (SD). To assess for *between-group* differences, analysis of variance with *post-hoc* Tukey's test was used. To compare *within-group* differences (responses to hyperglycemia or SGLT2 inhibition) a paired student's t-test was used. Linear regression analysis was used to determine correlations between responses and PUA levels. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using SAS v9.1.3 and GraphPad Prism software (version 6.0).

3.4 Results

3.4.1 Baseline Characteristics

Baseline parameters were similar between HC and T1D patients (Table 3.1). Participants were young, normotensive, normoalbuminuric and the two groups were similar in age and BMI.

During euglycemia, heart rate was significantly higher, but still within the normal range, in the T1D versus HC and no significant differences in SBP or DBP were observed. During hyperglycemic conditions, SBP significantly increased and HR decreased compared to euglycemia in the T1D group. As expected, T1D participants had significantly lower levels of circulating RAAS mediators compared to HC (aldosterone, PRA and angiotensin II) (110). During hyperglycemia, aldosterone and PRA levels further decreased.

As expected, T1D subjects exhibited higher GFR, ERPF, RBF and lower RVR compared to HC ($p<0.0001$ for all comparisons). Out of the 66 T1D patients, 29 exhibited normofiltration (44%) and 37 hyperfiltration (56%), where hyperfiltration was defined as GFR $\geq$135 mL/min/1.73m^2. In

Table 3.1. Baseline subject characteristics and UA, sodium, glucose handling in HC and patients with T1D during euglycemia and hyperglycemia (mean ± standard deviation).

Parameter	HC (n=41)	T1D (n=66)	
		Euglycemia	Hyperglycemia
Baseline parameters			
Males	19 (43%)	35 (53%)	-
Age (years)	28.4±7.1	25.0±6.0	-
Diabetes duration (years)	-	17.0±6.6	-
Weight (kg)	70.4±11.8	73.9±13.7	-
Height (m)	1.74±0.09	1.73±0.09	-
Body mass index (kg/m^2)	23.3±3.0	24.8±3.9	-
Hemoglobin A1c - mmol/mol (%)	34.8±3.6 (5.3±0.3)	66.7±16.1 (8.2±1.5)*	-
24 hour urine sodium (mmol/day)	177±61	169±85	-
24 hour protein intake (g/kg/day)	1.1±0.3	1.0±0.3	-
Sodium, glucose, uric acid handling			
Plasma uric acid (µmol/L)	305±75	228±62*	199±65†
Urine uric acid/creatinine ratio	248±170	257±121	339±161 †
FE_{UA} (%)	6.1±4.1	7.3±3.8	11.6±6.7 †
FE_{Na} (%)	0.84±0.60	0.87±0.56	1.63±0.89†
Urine glucose/creatinine ratio	0.02±0.03	1.4±3.2*	9.8±10.4 †
Systemic hemodynamic function			
HR (beats per minute)	60±9	74±13*	72±11†
SBP (mmHg)	112±12	115±10	117±11†
DBP (mmHg)	67±8	66±6	66±8
Renal hemodynamic function			
ERPF (mL/min/1.73m^2)	653±157	824±276*	853±253
GFR (mL/min/1.73m^2)	116±12	147±40*	159±39†
Filtration fraction	0.19±0.04	0.19±0.06	0.19±0.05
RBF (mL/min/1.73m^2)	1063±259	1310±434*	1305±419
RVR (mmHg/L/min)	0.081±0.020	0.069±0.021*	0.055±0.016†
Circulating neurohormones			
Aldosterone (ng/dL)	245±254	45±31*	31±10 †
PRA (ng/mL/h)	1.34±1.14	0.53±0.43*	0.35±0.27†
Angiotensinogen (ng/mL)	1264±1000	1092±722	1076±742
Angiotensin II	11.6±8.4	3.1±3.5*	2.2±2.2

24 hour protein intake: estimated by the formula ([urine urea X 0.18] + 14) / weight in kg. Values are mean ± standard deviation. *$p<0.05$ for HC vs. T1D. †$p<0.05$ when comparing parameters of T1D between hyperglycemia and euglycemia states. FE_{UA}: fractional excretion of UA; FE_{Na}: fractional excretion of sodium; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; ERPF: effective renal plasma flow; GFR: glomerular filtration rate; RBF: renal blood

flow; RVR: renal vascular resistance; PRA: plasma renin activity; HC: healthy controls; T1D: type 1 diabetic patients.

response to clamped hyperglycemia, GFR tended to increase in T1D (147±40 to 159±39 mL/min/1.73m^2, p=0.064) and RVR decreased (0.069±0.021 to 0.055±0.016 mmHg/L/min, p<0.0001). No significant changes to ERPF, FF or RBF were observed in response to hyperglycemic conditions.

3.4.2 Sodium, Glucose and UA Handling at Baseline

During clamped euglycemic conditions, PUA levels were lower in the T1D group vs. HC (228±62 vs 305±75 µmol/L, p<0.0001) (Table 3.1, Figure 3.1). PUA negatively correlated with FE_{UA} in T1D patients (r=-0.60, p<0.0001). $U_{glucose}$ excretion levels were also greater in T1D vs. HC during clamped euglycemia, but there was no significant difference in urine uric acid/creatinine ratio, FE_{Na} or FE_{UA} between HC and T1D.

Compared to levels during clamped euglycemia, PUA decreased further in response to clamped hyperglycemia (228±62 µmol/L to 199±65 µmol/L, p<0.0001) (Table 3.1, Figure 3.1). The decline in PUA levels in T1D patients during hyperglycemia was accompanied by significant increases in urine UA levels (257±121 to 339±161 umol/L, p=0.0007) and FE_{UA} (7.3±3.8 to 11.6±6.7, p<0.0001). PUA was negatively correlated with FE_{UA} during clamped hyperglycemia (r=-0.50, p<0.0001). The increase in UA excretion during hyperglycemia was accompanied by significant increases in $U_{glucose}$ (1.4±3.2 to 9.8±10.4 mmol/L, p<0.0001) and FE_{Na} (0.87±0.56 to 1.63±0.89,p<0.001).

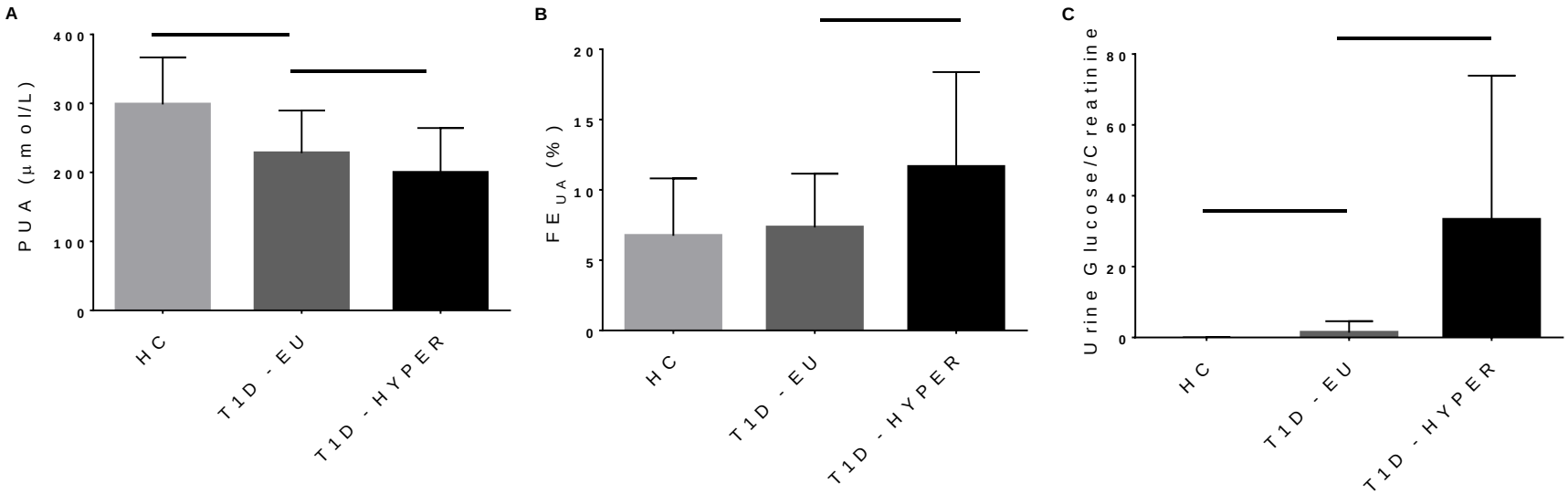
3.4.3 UA Correlations with Hemodynamic Parameters

PUA levels were positively correlated with SBP in T1D (r=0.27, p=0.029) under euglycemic conditions, but not during hyperglycemia (Figure 3.2). During euglycemia, PUA levels negatively correlated with ERPF (r=-0.27, p=0.031), and FE_{UA} positively correlated with ERPF (r=0.30, p=0.017) in T1D patients. During hyperglycemia, PUA negatively correlated with GFR in T1D (r=-0.27, p=0.028).

3.4.4 Sodium, Glucose and Uric Acid Handling Upon Empagliflozin SGLT2 Inhibition

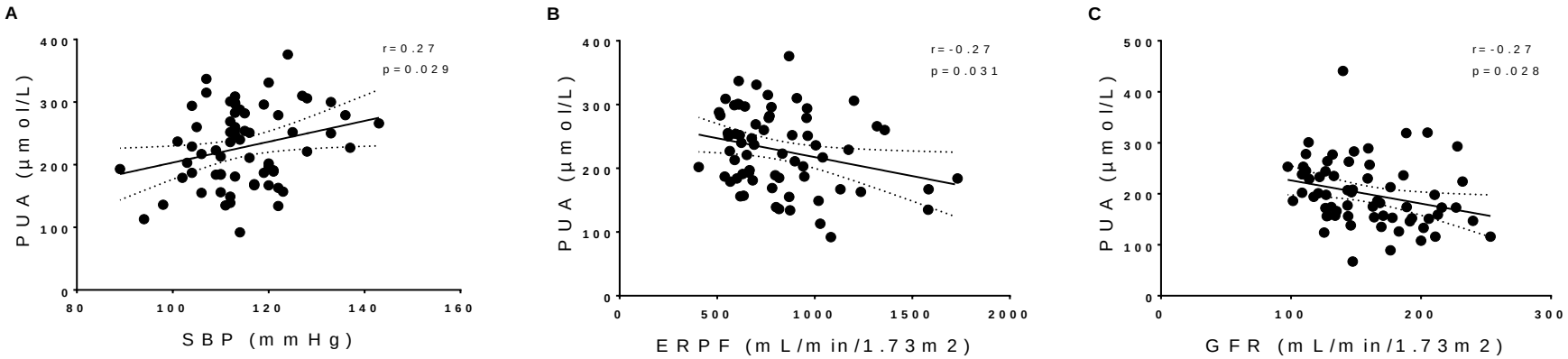
SGLT2 inhibitors are a new class of agents for the treatment of T2D that block proximal renal tubular glucose reabsorption, leading to increased glucose excretion. Therapeutically, this

Figure 3.1. PUA (A), FE_{UA} (B) and urine glucose/creatinine (C) levels in HC (n=41), and T1D (n=66) during euglycemic (EU) and hyperglycemic (HYP) conditions.



The bars above cohorts in each figure represent significance levels of $p<0.05$

Figure 3.2. Linear regression analysis of PUA with SBP in T1D during euglycemia (A), with ERPF in T1D during euglycemia (B), with GFR in T1D during hyperglycemia (C).



T1D n=40; SBP: systolic blood pressure; ERPF: effective renal plasma flow; GFR: glomerular filtration rate.

translates into important plasma glucose lowering effects (111). Trials with SGLT2 inhibitors in patients with T2D have reported consistent and clinically relevant decreases in PUA levels (112, 113); however the mechanisms responsible were never clearly elucidated. Accordingly, to better understand whether PUA lowering with hyperglycemia is due to systemic effects leading to decreased production or renal effects causing increased uricosuria, plasma and urine UA levels were measured before and after SGTL2 inhibition while maintaining clamped euglycemia in 40 T1D patients.

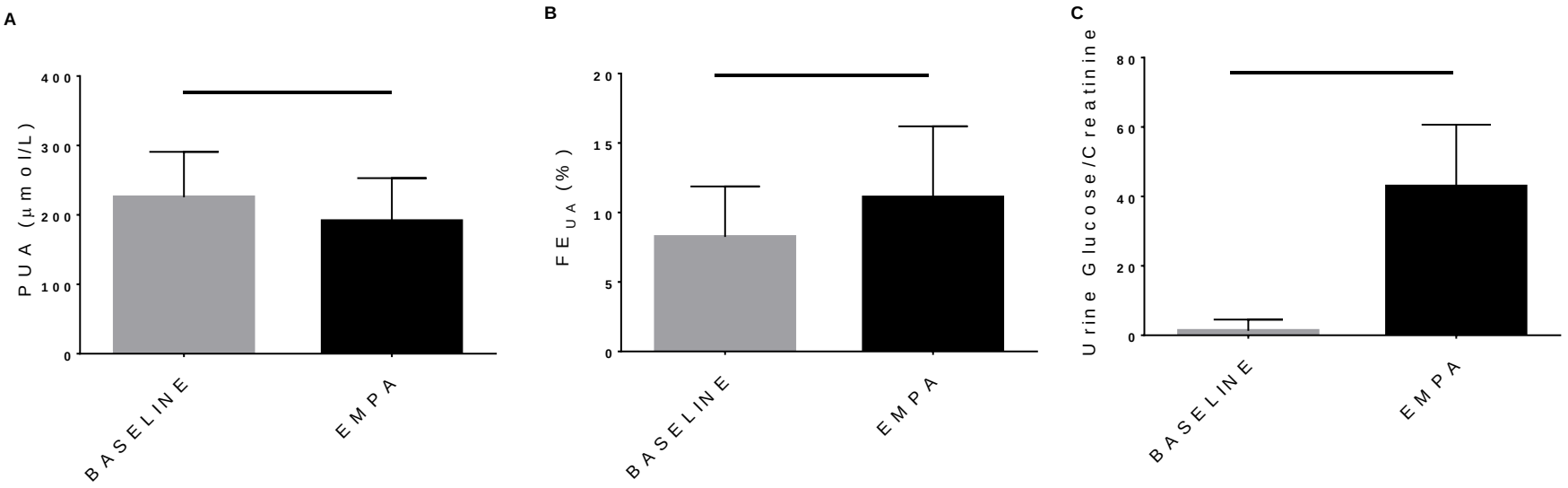
During clamped euglycemic conditions, after empagliflozin treatment, the anticipated increase in $U_{glucose/creatinine}$ (1.3±3.2 to 42.9±17.8, $p<0.0001$) was accompanied by a decline in PUA (225±65 to 191±62 mmol/L, $p<0.0001$) and increases in $U_{UA/creatinine}$ (290±110 to 327±103 mmol/mmol, $p=0.0075$) and FE_{UA} (8.2±3.6 to 11.1±5.1, $p<0.0001$) (Figure 3.3).

3.5 Discussion

Observational associations between higher UA levels and metabolic abnormalities, cardiovascular disease and kidney dysfunction show consistency among independent healthy and disease state cohorts, in both animals and humans (114). The potential renal protective effects of UA lowering in T1D patients are being studied as part of the NIH funded Protecting Early Renal Function Loss or "PERL" study (NCT02017171) (66), highlighting the promising future role for UA-based therapies in T1D. However UA levels during euglycemia compared to hyperglycemia have not been clearly defined in otherwise healthy T1D patients. Our first goal was to compare PUA levels in HC with levels in patients with T1D. Our second goal was to determine if acute clamped hyperglycemia, which promotes deleterious hemodynamic effects such as increased blood pressure, influences PUA levels.

Due to the strong association between PUA levels and cardiovascular and renal abnormalities, especially in the context of diabetes, it was initially hypothesized that T1D patients would have higher PUA levels compared to HC. Our first major observation, however, was that T1D patients had lower PUA levels under euglycemic conditions compared to HC, in conjunction with increased urinary glucose that did not correlate with the degree of UA excretion. Hyperglycemia in T1D patients was associated with a significant increase in urinary sodium, glucose and UA excretion and thus a further PUA decrease, highlighting an important physiological link between

Figure 3.3. PUA (A), FE_{UA} (B) and urine glucose/creatinine (C) levels in T1D (n=40) during euglycemic conditions at baseline and after treatment with the SGLT2 inhibitor empagliflozin (EMPA).



The bars above cohorts in each figure represent significance levels of $p<0.05$ hyperglycemia, which promotes deleterious hemodynamic effects such as increased blood pressure, influences PUA levels.

renal handling of UA, glucose and sodium (111). Furthermore, the negative correlation between PUA and FE_{UA} during euglycemia and hyperglycemia suggests that PUA decreased as a result of increased renal excretion. The lack of elevated UA excretion in T1D compared to HC under euglycemic conditions may suggest that T1D patients produce less UA in plasma or consume less UA-containing products, although the similar protein intake based on urine urea excretion in these groups suggests that differences in intake of UA-containing foods were not relevant to our findings.

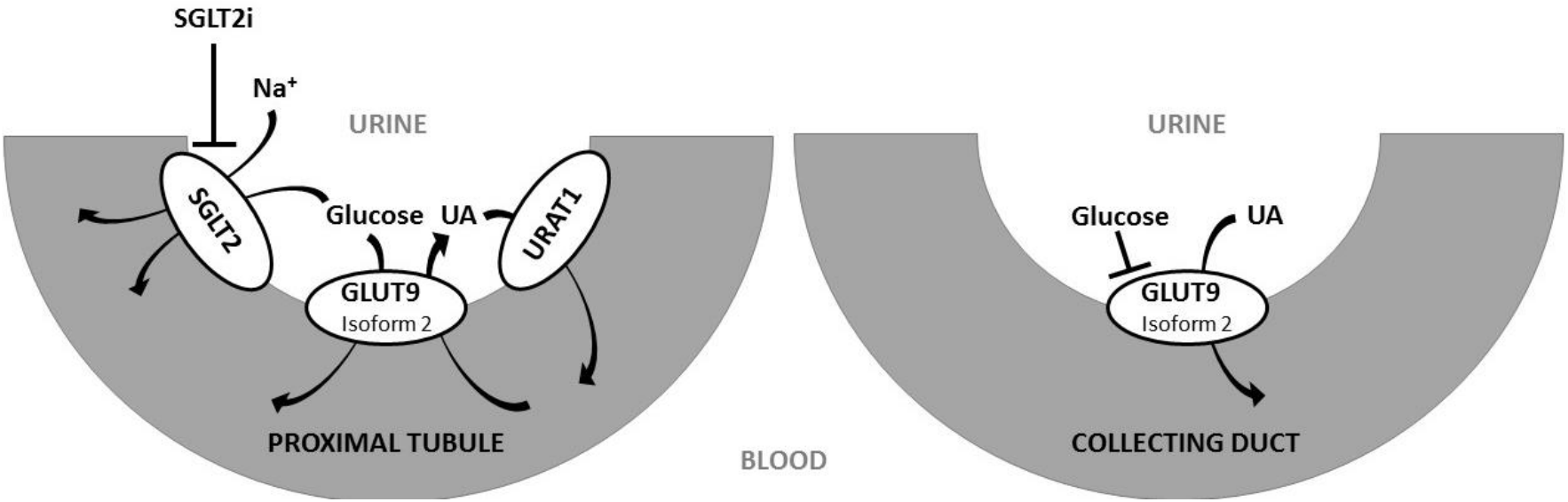
Our observations support several studies showing an increase in UA excretion in response to intravenous D-glucose infusion (115, 116). More recently, an association was found between lower PUA and poor glycemic control (117-119). Previous studies have shown that insulin levels are positively correlated with PUA and insulin administration decreases UA excretion (120). However, it is not known whether this is a direct effect of insulin or the result of insulin-mediated normalization of glycemia, leading to reduced glycosuria. Worsening glycemic control resulting in hyperglycemia and glycosuria has been correlated with a decrease in PUA (118, 121). Thus, it is perhaps not surprising that epidemiological studies have shown a decreased risk of UA-related conditions, such as gout, in diabetic compared to non-diabetic individuals – especially in the context of T1D (122). The mechanisms behind the glucose-mediated PUA lowering effects have been explained by osmotic diuresis caused by increased plasma glucose levels (115), proximal tubule alterations (119) or the effect of glucose on renal UA handling (115, 121).

Our second aim was to determine whether PUA lowering with hyperglycemia in T1D was due to systemic hyperglycemia causing decreased UA production or renal glycosuria causing increased UA excretion. SGLT2 inhibition with empagliflozin under clamped euglycemic conditions was used to increase urinary glucose excretion to determine if glycosuria during euglycemia results in a persistent decrease in PUA through increased renal UA excretion. Previous trials with SGLT2 inhibitors in patients with T2D have reported consistent and clinically relevant decreases in PUA levels, however urine UA excretion was not measured and the mechanisms responsible have not, to our knowledge, been clearly elucidated (112, 113). *O*ur *post-hoc* analysis demonstrated a decline in PUA during euglycemia with glycosuria induced with SGLT2 inhibition, an effect that was accompanied by an increase in UA excretion. Consistent with our observations, in a recent study using healthy controls, SGLT2 inhibition with luseogliflozin resulted in a positive correlation between urine UA and urine glucose excretion (99). The results of the present study

provide the first evidence in the T1D population suggesting that hyperglycemia-mediated uricosuria is likely due to renal glycosuria rather than a direct effect of systemic hyperglycemia. PUA lowering effects reported with SGLT2 inhibition may be of clinical relevance, since this may in part explain the potential protective renal and cardiovascular physiological profile that has been linked with this emerging drug class (111).

The molecular mechanisms responsible for the uricosuric effect of glucose are not clear. PUA levels depend on the exogenous pool which varies with dietary intake, while the endogenous pool is mainly regulated by hepatic production, intestinal secretion and renal excretion (114). Approximately 70% of UA is excreted into urine, but is easily filtered into the renal tubule and about 90% of filtered UA is reabsorbed by the S1 segment of the proximal convoluted tubule (114). Approximately 10% of filtered UA is excreted (114). Accordingly, our HC showed a FE_{UA} of 6.1±4.1% and T1D during euglycemia 7.3±3.8%. UA reabsorption occurs by intracellular anion transporters on the basolateral membrane – mainly by URAT1 and a more recently discovered GLUT9 isoform 2 (123, 124), and on the apical membrane OAT4 and OAT10 (125, 126). Recently, transport experiments in *Xenopus* oocytes showed that none of the transporters involved in UA reabsorption were influenced by luseogliflozin (99). GLUT9 isoform 2 is a facilitative glucose transporter mostly expressed in the kidney and the liver, located on the apical membrane (124). GLUT9 isoform 2 secretes UA in exchange for glucose at 10mM (123). Additionally, GLUT9 isoform 2 is expressed in the collecting ducts where it plays a role in the reabsorption of UA (127). Plasma glucose is mostly filtered in the glomerulus and is concentrated in the proximal tubule. It is possible that during euglycemia the concentration needed for GLUT9 stimulation is not reached in the proximal tubule and the lower PUA in T1D during euglycemia vs. HC could occur by mechanisms other than glycosuria- mediated uricosuria. Based on these findings, the results of our study could be explained as follows: glycosuria during SGLT2 inhibition stimulates excretion of UA by GLUT9 isoform 2 on the apical membrane of the proximal tubule and possibly inhibits reabsorption of UA in the collecting ducts (Figure 3.4). Our conclusion may reflect recent *in vitro* data showing that stimulation of *Xenopus* oocytes expressing GLUT9 isoform 2 with 10mM D-glucose resulted in UA efflux and stimulation of the oocytes with 100mM D-glucose - thought to be the concentration in the collecting ducts - inhibited the uptake of UA (99). Finally, increased glycosuria and uricosuria could, in the appropriate context, suggest the presence of more generalized, “Fanconi-like” proximal tubular dysfunction. Since SGLT2 inhibition causes minor but statistically significant *increases*, rather than *decreases*, in serum potassium, phosphate and

Figure 3.4. Proposed hypothesis for glycosuria mediated hyperuricemia in T1D patients, supported by findings in this study and by Chino *et al.*(99).



SGLT2i: Sodium glucose transporter 2 inhibitor; GLUT9: Glucose transporter 9; URAT1: Urate Transporter 1; UA: Uric Acid.

bicarbonate, a proximal tubulopathy with this class of agents is very unlikely and has not been reported (128).

To examine the functional role of PUA in this otherwise healthy cohort of T1D patients, we correlated PUA with blood pressure and renal hemodynamic function. We found a significant positive correlation between PUA and SBP, negative correlations between PUA and ERPF and PUA and GFR in T1D. In contrast, PUA did not correlate with any of these measures in HC. These observations in T1D patients are consistent with the vasoconstrictive phenotype, as suggested by observational studies. For example, the independent association between PUA and blood pressure has been reported in various cohorts, including a subset of the Framingham Heart Study (30, 73). The deleterious effect of PUA on cardiovascular function may be worsened by the hypertensive effect of hyperglycemia in T1D patients (26, 27, 72). Hyperglycemia induces systemic vascular abnormalities such as endothelial dysfunction in humans (26, 27). As a result of the effects of hyperglycemia and neurohormonal activation of the renin angiotensin aldosterone system, UA levels are independently associated with endothelial dysfunction, thereby promoting hypertension, even when UA levels are within the normal range (29, 30). Therefore, lower PUA levels in T1D patients, especially under hyperglycemic conditions, do not necessarily indicate that T1D patients are protected from the deleterious effects of UA. The effects of PUA may be exacerbated by hyperglycemia in T1D patients, leading to exaggerated deleterious hemodynamic consequences despite lower absolute PUA levels. From a clinical perspective, small trials have already started to show that lowering UA exerts anti-proteinuric and antihypertensive effects and could prevent renal functional loss and vascular injury (61, 62, 64, 73, 78, 114). Thus, despite the lower UA levels in T1D versus HC, which are further lowered during hyperglycemia, studying UA lowering agents in T1D patients could be a critical step towards preventing progression of diabetes-related complications.

Our study has limitations. First, the study cohort consisted of a carefully selected group of patients with uncomplicated disease, limiting the generalizability of the data to populations outside of T1D, or to patients with existing complications. Additionally, although the similar urine urea excretion and thus protein intake suggest that differences in dietary intake of high UA-containing foods were unlikely, consumption of UA was not recorded. Fructose is another exogenous source of UA, which was not recorded in this study, and should be considered in future analyses. Finally, while we propose a possible explanation for glycosuria-mediated uricosuria, we could not

determine the mechanistic basis at the molecular level. Future studies are needed in order to confirm our hypothesis.

In conclusion, glycosuria, rather than the direct effect of hyperglycemia, is responsible for increased uricosuria in T1D patients, and may be mediated by glucose-mediated activation of GLUT9 isoform 2 on the apical membrane of the proximal tubule. Since PUA lowering may lead to renal and vascular protective effects, our data suggests that PUA lowering by SGLT2 inhibition via increased uricosuria may be clinically important. Finally, future studies examining the effect of UA lowering therapies should account for the impact of ambient glucose levels, which cause a clinically relevant uricosuric and consequent PUA lowering effect.

Chapter 4: Plasma Uric Acid Effects on Glomerular Hemodynamic Profile of Patients with Uncomplicated Type 1 Diabetes Mellitus

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A version of this chapter is published in: Diabetic Medicine. (2015) Epub ahead of print.

4.1 Abstract

Aims: Increased plasma uric acid (PUA) levels are associated with impaired renal function in patients with type 1 diabetes (T1D), but the mechanisms are not well understood. Our aim was to evaluate whether higher PUA levels are associated with increased afferent arteriolar resistance in T1D vs. healthy controls (HC), thereby influencing renal function.

Methods: PUA, glomerular filtration rate (GFR - inulin) and effective renal plasma flow (ERPF - paraaminohippurate) were measured in 70 otherwise healthy T1D patients and 60 HC. Gomez's equations were used to estimate afferent (R_A) and efferent (R_E) arteriolar resistances, glomerular hydrostatic pressure (P_{GLO}) and filtration pressure (ΔP_F). The relationships between PUA and glomerular hemodynamic parameters were evaluated by univariable linear regression correlation coefficients.

Results: In T1D, higher PUA correlated with lower P_{GLO} (p=0.002) and ΔP_F (p=0.0007), with higher R_A (p=0.001), but not with R_E (p=0.55). These associations were accompanied by correlations between higher PUA with lower GFR (p=0.0007), ERPF (p=0.008), RBF (p=0.047) and higher RVR (p=0.021). There were no significant correlations between PUA and renal hemodynamic parameters in the HC.

Conclusions: The association between higher PUA with lower GFR and lower ERPF in T1D patients is driven by alterations in the estimated R_A. PUA-mediated R_A may be caused by increased tone or thickening of the afferent renal arteriole, which could potentiate renal injury by causing ischemia to the renal microcirculation.

4.2 Introduction

Uric acid (UA) is an inflammatory factor that mediates production of reactive oxygen species and stimulates the renin angiotensin aldosterone system (RAAS). Evidence from animal models suggests a positive correlation between UA levels and hyperfiltration with a subsequent increase in proteinuria and risk of renal failure (80), while lowering of UA reduces proteinuria, preserves GFR and suppresses expression of renal inflammatory interleukins (80). In patients with diabetes mellitus (DM), hyperfiltration is associated with an early decline in GFR and with macrovascular abnormalities (47). Subsequently, UA was found to be associated with impaired renal function in type 1 DM (T1D) and in healthy controls (HC) even when UA levels are in the normal range (80). For example, the 355 T1D participants in the second Joslin Study on the Natural History of Microalbuminuria showed a significant association between high UA levels within the normal range and early GFR loss over a 6-year follow up period (55). Additionally, a *post hoc* analysis of 116 hyperuricemic gout patients treated with febuxostat for 5 years in the Febuxostat Open-Label Clinical Trial of Urate-Lowering Efficacy and Safety (FOCUS) Study showed an inverse correlation between UA reduction and the rate of eGFR decline (62). The promising renal protective effect of UA lowering on 3-year change in GFR in the T1D population is currently being investigated in the Protecting Early Renal Function Loss (PERL) Study (NCT02017171) (66).

The mechanisms responsible for the association between UA and renal dysfunction are not well understood. Animal models suggest that hyperuricemia could cause preglomerular (afferent) arteriolar injury, characterized by hyalinosis and wall thickening (41, 129). In a histological analysis of human renal biopsy specimens, similar observed hyalinotic changes to the afferent arteriole may disrupt glomerular hemodynamic autoregulation, leading to ischemia and renal injury (130). Furthermore, micropuncture studies in hyperuricemic animals have shown that medial thickening of the afferent arteriole correlates with serum UA and glomerular capillary pressure (131). These animal models concluded that UA-mediated afferent arteriolar injury leads to disruption of renal haemodynamic function, resulting in ischemia. Despite what is known in preclinical studies, such an association has not yet been identified in humans.

Due to the inability to differentiate between afferent and efferent arteriolar resistances in humans, observational associations between UA and renal haemodynamic function have relied primarily on assessments of glomerular filtration rate (GFR). In order to better understand the

pathophysiological mechanisms responsible for the association between UA and renal disease in humans, a more detailed description of *in vivo* afferent and efferent arteriolar function is required. In 1951, Gomez et al derived equations for indirect measurements of afferent (R_A) and efferent (R_E) arteriolar resistances, filtration pressure (P_F) and glomerular hydrostatic pressure (P_{GLO}) (132). Recently, these equations were used successfully in various conditions (133-135) as well as by our group to characterize the renal hemodynamic profile in patients with T1D (92) and in response to SGLT2 inhibition (136).

The goal of this analysis was to assess the correlation of plasma UA levels (PUA) with glomerular haemodynamic parameters estimated using Gomez's equations (R_A, R_E, P_F and P_{GLO}) in patients with uncomplicated T1D and a similar group of normal healthy controls. Based on existing data in animal models of diabetes, we hypothesized that PUA would be positively associated with R_A, but not R_E in T1D patients.

4.3 Research Design and Methods

4.3.1 Subject Inclusion Criteria and Study Preparation

All the studies were approved by the University Health Network Research Ethics Board and all subjects gave written informed consent. Sixty HC and 70 T1D patients were included in this physiological analysis using archived blood samples obtained as part of previous physiological studies based on the following criteria (6, 45, 93, 137): 18-40 years of age, blood pressure <140/90 mmHg, normoalbuminuria based on a 24-hour urine collection, GFR>60mL/min/1.73^{2}m diabetes duration >1 year, no history of renal or cardiovascular complications and no intake of concomitant medications that would alter blood pressure or cardiovascular outcomes. All participants followed a 7-day diet prior to the study visit: sodium ≥150 mmol/day and protein ≤1.5 g/kg/day as previously described (92).

4.3.2 Experimental Procedures

HC were studied during normoglycemia and patients with T1D were studied during euglycemia (4-6 mmol/L) maintained using a modified glucose clamp technique (107) at the Renal Physiology Laboratory at the Toronto General Hospital. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were estimated using inulin and paraaminohippurate (PAH) steady state

infusion clearance techniques (107), respectively, using previously described methods. The results of the 2 clearance periods were averaged. Brachial artery blood pressure measurements were obtained at 30-minute intervals throughout the study days (Critikon, Tampa, Florida, USA). After clamped euglycemia was achieved for at least 2 hours, blood was collected for measurements of inulin, PAH, PUA and aldosterone and was processed as previously described (85). The following parameters were calculated:

Filtration fraction (FF) = $\frac{GFR}{ERPF}$

Renal blood flow (RBF) = $\frac{ERPF}{1-Hematocrit}$

Renal vascular resistance (RVR) = $\frac{MAP}{RBF}$

4.3.3 Gomez's Equations for Intraglomerular Hemodynamic Analysis

Indirect intraglomerular hemodynamic parameters were estimated using equations estimated by Gomez based on data from animal studies (132). These equations were successfully used in a similar manner to evaluate patients with conditions such as hypertension, endocrine disorders and T1D (133-136). Assumptions imposed by Gomez's equations were the following: i) intrarenal vascular resistances are divided into afferent, post-glomerular and efferent; ii) hydrostatic pressures within the renal tubules, venules, Bowman's space and interstitium (P_{Bow}) are in equilibrium of 10 mmHg; iii) glomerulus is in filtration disequilibrium; iv) the gross filtration coefficient (K_{FG}) is 0.0867 ml/s per mmHg given a normal kidney physiology (GFR = 130 ml/min, oncotic pressure π_G is 25 mmHg and P_{GLO} = 60 mmHg, given Winton's indirect estimates in dogs that glomerular pressure is roughly two-thirds of the MAP (132)). Previous micropuncture studies in Munich-Wistar rats suggest different P_{GLO} values in diabetic and control conditions. Thus, to replicate potential differences in HC vs. T1D patients, Gomez's equations were also used to calculate a second set of intraglomerular hemodynamic parameters assuming P_{GLO} values of 47.5 mmHg (K_{FG} = 0.1733 ml/s per mmHg) for HC and 56.4 mmHg (K_{FG} = 0.1012 ml/s per mmHg) for T1D participants(138).

MAP (mmHg), ERPF (ml/s), GFR (mL/s) and total protein (g/dL) were used to calculate R_E (dyne•sec•cm^{-5}) and R_A (dyne•sec•cm^{-5}), P_{GLO} (mmHg), ΔP_F (mmHg) and π_G (mmHg):

The filtration pressure across glomerular capillaries (ΔP_F):

$$\Delta P_F = GFR / K_{FG}$$

The glomerular oncotic pressure (π_G) from the plasma protein mean concentration (C_M) within the capillaries:

$$C_M = TP/FF \times \ln(1/1-FF)$$

$$\pi_G = 5 \text{ x } (C_M - 2)$$

Glomerular hydrostatic pressure (P_{GLO}):

$$P_{GLO} = \Delta P_F + P_{Bow} + \pi_G$$

R_A and R_E were estimated using principles of Ohm's law, where 1328 is the conversion factor to dyne•sec•cm^{-5} (132):

$$R_A = [(MAP-P_{GLO})/RBF] \times 1328$$

$$R_E = [GFR/(K_{FG} \times (RBF-GFR)] \times 1328$$

4.3.4 Statistical analysis

Data are presented as mean ± standard deviation (SD). To assess for *between-group* differences, analysis of variance with *post-hoc* Tukey's test was used. Linear regression analysis was used to determine correlations between renal haemodynamic responses and PUA levels. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using SAS v9.1.3 and GraphPad Prism software (version 5.0).

4.4 Results

4.4.1 Baseline Characteristics

Participants were young, normotensive and normoalbuminuric. Baseline parameters, such as age, BMI and gender distribution were similar between HC and T1D patients (Table 4.1). While there were no significant differences in DBP and SBP, there was a significantly higher heart rate in T1D patients although still within the normal range. Out of the 70 T1D patients, 44 exhibited

Table 4.1. Baseline subject characteristics and intraglomerular hemodynamic parameters in HC and patients with uncomplicated T1D during euglycemia (mean ± standard deviation).

Parameter	HC (n=60)	T1D (n=70)
Baseline parameters		
Males	28 (47%)	35 (50%)
Age (years)	26.1±5.5	25.2±5.6
Diabetes duration (years)	-	16.7±6.6
Weight (kg)	70.7±12.6	75.3±12.4
Height (m)	1.73±0.10	1.74±0.09
Body mass index (kg/m^2)	23.5±3.1	24.9±3.3
Hemoglobin A1c - mmol/mol (%)	31.2±10.8 (5.2±0.3)	65.5±17.6 (8.1±1.6)*
24 hour urine sodium (mmol/day)	158±90	142±87
24 hour protein intake (g/kg/day)	1.0±0.3	1.0±0.3
Systemic hemodynamic function		
Heart Rate (HR, beats per minute)	61±9	70±12*
Systolic Blood Pressure (SBP, mmHg)	109±9	114±10*
Diastolic Blood Pressure (DBP, mmHg)	65±6	66±6
Renal hemodynamic function		
Effective Renal Plasma Flow (ERPF, $mL/min/1.73m^2$)	662±118	756±264*
Glomerular Filtration Rate (GFR, $mL/min/1.73m^2$)	117±14	130±29*
Filtration fraction	0.18±0.03	0.18±0.04
Renal Blood Flow (RBF, $mL/min/1.73m^2$)	1053±197	1207±418*
Renal Vascular Resistance (RVR, mmHg/L/min)	0.078±0.017	0.074±0.023
Plasma Analysis		
Aldosterone (ng/dL)	274±258	38±38*
Plasma uric acid (µmol/L)	312±72	232±61*
Intraglomerular hemodynamic parameters		
Filtration pressure (ΔP_F, mmHg)	22.4±2.6	25.1±5.5*
Glomerular hydrostatic pressure (P_{GLO}, mmHg)	59.7±3.3	58.9±6.1
Oncotic pressure (π_G, mmHg)	27.2±2.2	23.8±2.6*
Afferent arteriolar resistance (R_A, $dyne \bullet sec \bullet cm^{-5}$)	1577±695	1723±877
Efferent arteriolar resistance (R_E, $dyne \bullet sec \bullet cm^{-5}$)	2067±588	1971±469

24 hour protein intake: estimated by the formula ([urine urea X 0.18] + 14) / weight in kg. Values are mean ± standard deviation. *$p<0.05$ for HC vs. T1D. HC: healthy controls; T1D: type 1 diabetic patients.

normofiltration (63%) and 26 hyperfiltration (37%), where hyperfiltration was defined as GFR ≥135 mL/min/1.73m^2.

Consistent with previous observations, T1D participants had significantly lower levels of circulating aldosterone ($p<0.0001$), compared to HC (85). As expected, T1D subjects had higher GFR and RBF compared to HC ($p=0.0008$ and $p=0.026$ respectively). PUA levels were significantly lower in the T1D group vs. HC (232±61 vs. 312±72 μmol/L, $p<0.0001$), which is consistent with our previous findings (85).

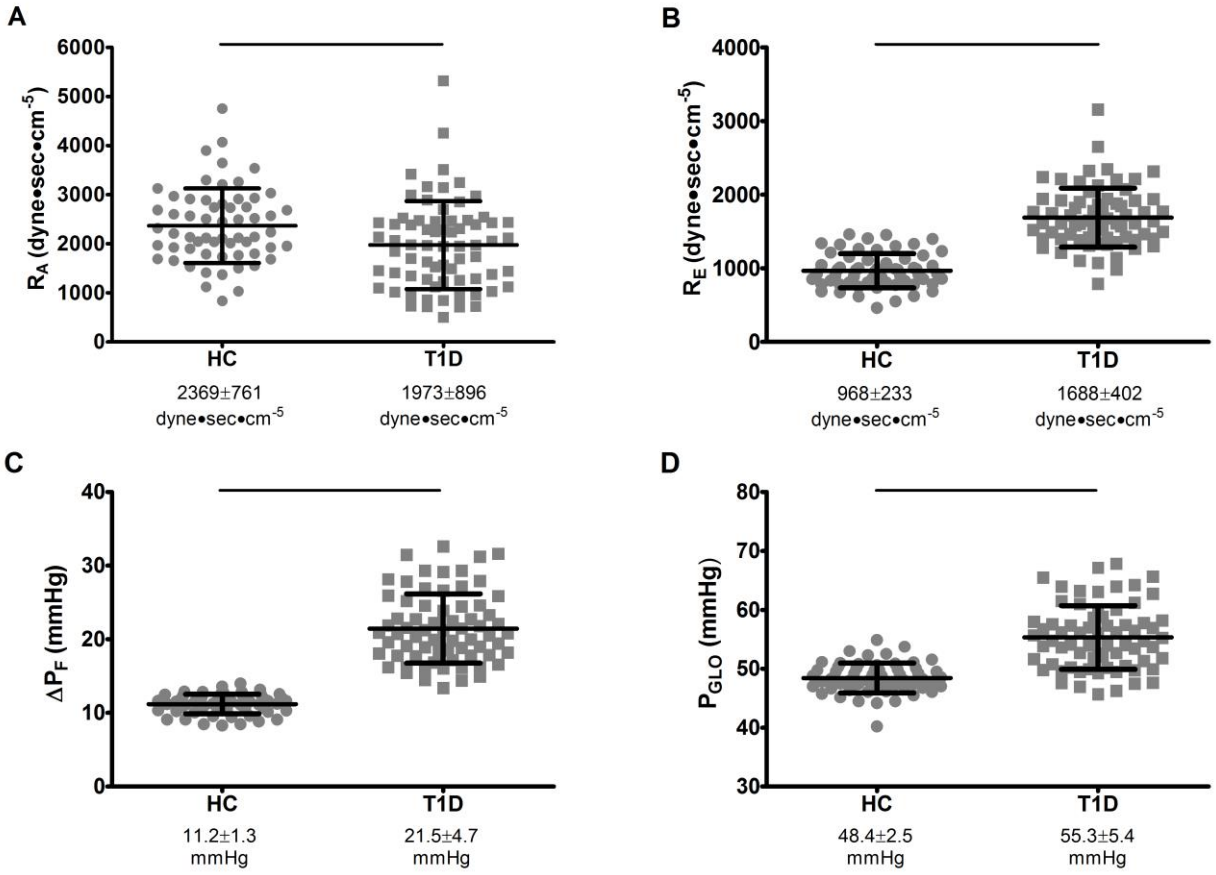
4.4.2 Intraglomerular Hemodynamic Parameters

There was a significantly higher filtration pressure observed in T1D vs. HC population ($p=0.0008$), the oncotic pressure was significantly lower in T1D patients ($p<0.0001$), and no significant difference was observed in afferent and efferent resistances between HC and T1D patients ($p=0.14$ and $p=0.67$ respectively). When the intraglomerular hemodynamic parameters were re-calculated using P_{GLO} values consistent with the micropuncture values obtained in animal models of 47.5 mmHg for HC and 56.4 mmHg for T1D participants, R_A was lower in T1D compared to HC ($p=0.008$) and R_E, ΔP_F and P_{GLO} were higher ($p<0.0001$ for each comparison, Figure 4.1).

4.4.3 PUA Correlations with Renal Hemodynamic Parameters

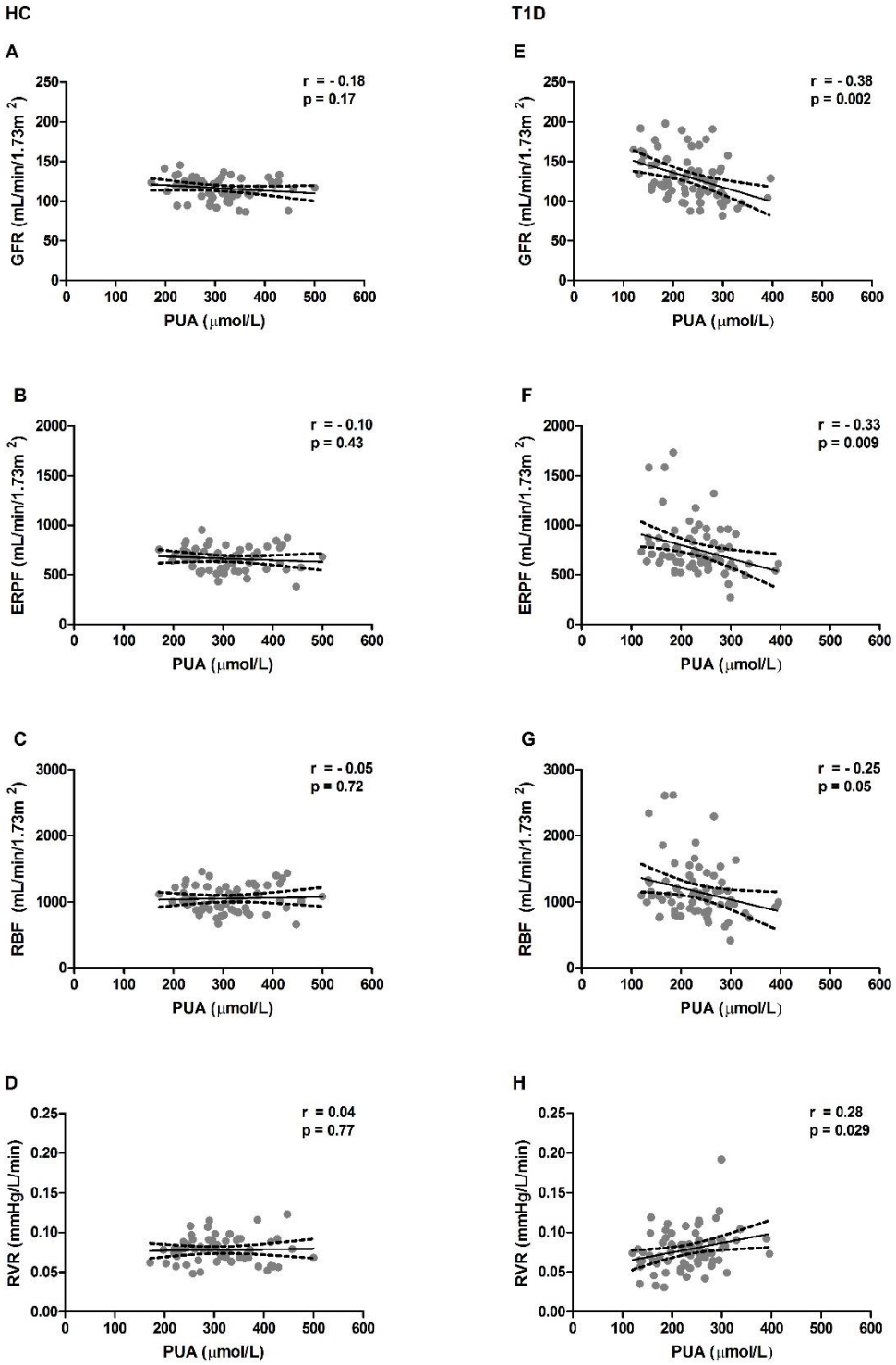
As was previously reported by our group, PUA levels were negatively correlated with GFR ($r=-0.38$, $p=0.002$), ERPF ($r=-0.33$, $p=0.009$), RBF ($r=-0.25$, $p=0.05$) and positively with RVR ($r=0.28$, $p=0.029$) in T1D patients (Figure 4.2) (85). PUA was negatively correlated with filtration pressure ($r=-0.40$, $p=0.0007$) and glomerular hydrostatic pressure ($r=-0.36$, $p=0.002$), positively correlated with afferent resistance ($r=0.38$, $p=0.001$), but not with efferent resistance ($r=-0.07$, $p=0.55$) in the T1D population (Figure 4.3). There were no significant correlations observed between PUA and any of the measured renal hemodynamic parameters in the HC population. The direction and statistical significance of all observed correlations were similar when R_A, R_E, ΔP_F and P_{GLO} were estimated using the P_{GLO} assumption of 47.5 mmHg in HC and 56.4 mmHg in T1D (data not shown).

Figure 4.1. R_A (A), R_E (B), ΔP_F (C) and P_{GLO} (D) in HC and T1D calculated by Gomez's equations (assumption: P_{GLO} of 47.5 mmHg in HC and 56.4 mmHg in T1D).



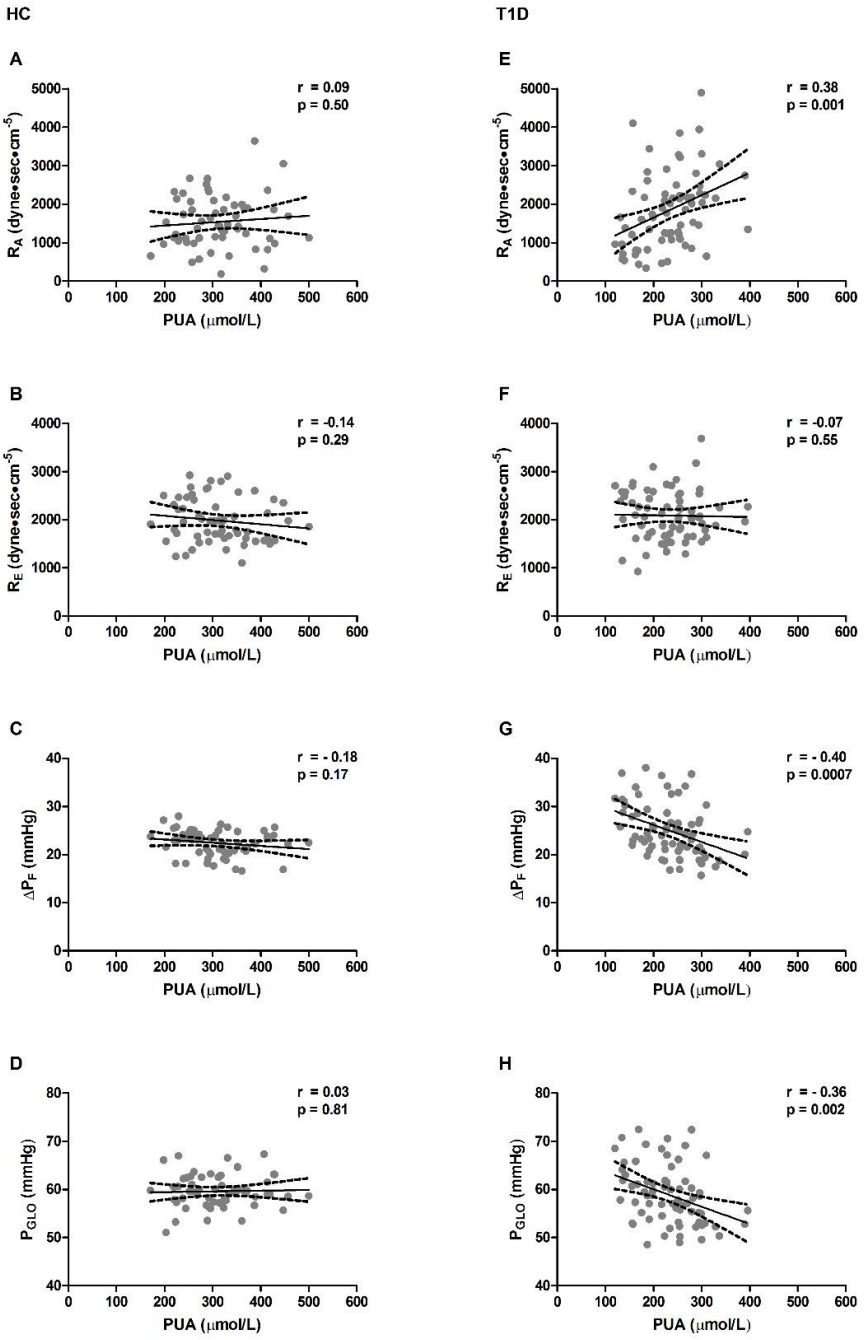
HC n=60 and T1D n=70; R_A: afferent arteriolar resistance; R_E: efferent arteriolar resistance; ΔP_F: filtration pressure; P_{GLO}: glomerular hydrostatic pressure. Values are mean ± standard deviation. The bars in each figure represent significance levels of $p<0.05$.

Figure 4.2. Linear regression analysis of PUA with GFR (A), ERPF (B), RBF (C) and RVR (D) in HC and with GFR (E), ERPF (F), RBF (G) and RVR (H) inT1D participants.



HC n=60 and T1D n=70; GFR: glomerular filtration rate; ERPF: effective renal plasma flow; RBF: renal blood flow; RVR: renal vascular resistance.

Figure 4.3. Linear regression analysis of PUA with R_A (A), R_E (B), ΔP_F (C) and P_{GLO} (D) in HC and with R_A (E), R_E (F), ΔP_F (G) and P_{GLO} (H) inT1D participants.



HC n=60 and T1D n=70; R_A: afferent arteriolar resistance; R_E: efferent arteriolar resistance; ΔP_F: filtration pressure; P_{GLO}: glomerular hydrostatic pressure. Direction and significance of all correlations were also observed when R_A, R_E, ΔP_F and P_{GLO} were calculated using Gomez's equations assuming P_{GLO} of 47.5 mmHg in HC and 56.4 mmHg in T1D.

4.5 Discussion

Patients with microalbuminuria are at high risk of progressing to renal function decline leading to end stage renal disease (57). The mechanisms that initiate and sustain this early renal function decline are unknown, but are potentially important for the identification of therapies that will preserve renal function. Given that higher PUA levels are consistently associated with renal dysfunction in human and animal models, it is important to further study the mechanisms linking PUA with early renal function decline to identify predictors and targets for intervention. In preclinical studies, hyperuricemic rats exhibited increased afferent resistance and a reduction in single-nephron GFR, which was prevented with allopurinol treatment (131). To date, human observational studies that have associated PUA and renal function have relied primarily on isolated direct and indirect measures of GFR, without further characterization of other renal measures such as renal blood flow or vascular resistance. Detailed mechanistic insights into PUA-mediated changes in segmental renal arteriolar resistances have therefore remained unknown. Therefore, our goal was to clarify whether PUA influences mathematically calculated pre-glomerular or post-glomerular arteriolar resistances in patients with uncomplicated T1D, thereby gaining a better understanding of the relationship between PUA and early manifestations of diabetic nephropathy including decline in GFR. We assessed the correlation between PUA and glomerular hemodynamic parameters (filtration pressure, glomerular hydrostatic pressure, afferent and efferent arteriolar resistances) in an otherwise healthy T1D population compared to a similar group of HC participants.

Consistent with our previous observations (85), we found a significant negative correlation between PUA and ERPF, GFR, RBF and a positive correlation with RVR in this otherwise healthy T1D cohort. These correlations were absent in the HC group. Although these observations suggest early hemodynamic changes prior to onset of early renal complications in the T1D patients, it was only with the use of the Gomez's equations that we were able to gain additional insight into differences in afferent vs. efferent arteriolar resistance. In support of our hypothesis based on preclinical data was the novel observation that PUA-associated hemodynamic alterations may occur due to increased afferent arteriolar resistance. In addition to interactions with R_A, PUA was negatively correlated with filtration pressure and glomerular hydrostatic pressure, but did not correlate with the efferent resistance in the T1D population. These correlations were absent in the HC group. Our finding suggests that PUA may influence afferent arteriolar resistance, which

results in decreased filtration pressure and glomerular hydrostatic pressure – an effect that is potentiated in the presence of diabetes. To our knowledge, this observation has not been previously made in humans. We recognize that although we have hypothesized that UA is responsible for mediating renal vasoconstriction, neurohormonal activation of angiotensin II and norepinephrine can lead to both vasoconstriction and decreased UA excretion in healthy humans (139). Therefore, a "cause and effect" interaction between UA and R_A has not yet been established. To better define this physiological relationship, future mechanistic studies should consider repeating these measurements in subjects with hyperuricemia before and after lowering serum UA.

The mechanisms responsible for UA-mediated renal injury are complex and range from excessive UA crystallization and intrarenal obstruction, to effects on activating neurohormonal and pro-inflammatory pathways (80). However, as observed in multiple recent studies as well as our current observations, even lower PUA levels within the normal range are associated with changes in renal function (85, 100). For example, Krolewski et al. recently reported that mildly elevated PUA is an independent predictor of early GFR loss, even in normoalbuminuric patients with T1D, such that each 0.5 mg/dl (29.7 μmol/L) was associated with an odds-ratio of 1.86 for early GFR decline (100).

Although we are unable to determine the molecular mechanisms and signaling pathways responsible for UA mediated afferent renal arteriole vasoconstriction in patients with T1D from this clinical study, some insights may be obtained through previous observations in human and animal studies. For example, oxonic acid-induced hyperuricemia in rats leads to the development of thickening of the afferent arteriole with proliferation of smooth muscle cells and macrophage infiltration, causing impaired renal function (41, 129). A positive correlation between PUA and arteriolar wall thickening and hyalinosis was also observed in renal biopsy samples of 167 chronic kidney disease patients (140). The presence of renal arteriolar hyalinosis was reported as a potential marker for impaired renal hemodynamic autoregulation promoting an increased risk of focal segmental glomerulosclerosis or glomerular ischemia (130). The authors suggested that alterations in afferent arteriolar morphology and function may have been on the basis of altered neurohormonal bioactivity. For example, in the hyperuricemic rat model, the hypertrophic vascular remodelling coincided with increased expression of juxtaglomerular renin and cyclooxygenase-2 in the afferent arterioles as well as a decrease in nitric oxide synthase-1 in the

macula densa (16). PUA levels and the degree of smooth muscle cell proliferation correlated with cyclooxygenase-2 levels (16) and upon further *in vitro* investigation, incubation of cultured smooth muscle cells with UA resulted in cyclooxygenase-2 production and smooth muscle cell proliferation (16). UA also inhibits nitric oxide release from endothelial cells resulting in vasoconstriction (80) while preservation of endothelial NO levels was shown to partially protect the kidney from ischemic renal injury (141).

Changes in cyclooxygenase-2 and nitric oxide bioactivity may also be compounded by UA-mediated RAAS activation (42) and suppressed nitric oxide bioavailability causing increased sodium reabsorption at the proximal tubule, thereby altering the tubuloglomerular feedback mechanism leading to hyperfiltration and potentially more structural damage to the afferent arteriole (142). Consequently, micropuncture studies in hyperuricemic rat models have demonstrated that the renal arteriolopathy induced by hyperuricemia correlates with increased glomerular capillary pressure, which may further promote renal injury (131). We did not observe a similar positive correlation between UA and P_{GLO} in our data, possibly due to the uncomplicated nature of the study cohort or due to UA levels being within the normal range. The afferent arteriole remodelling can result in an obliterative arteriopathy, thereby potentiating renal injury by causing ischemia to the postglomerular circulation (143), including the S3 proximal tubule and the medullary thick ascending limb (144). Ischemic tubules recruit inflammatory cells, which may stimulate oxidant release and further augment renal injury (145). The observed PUA-mediated increase in afferent arteriolar resistance in T1D participants may be caused by either increased tone or thickening of the afferent glomerular arteries. Thus, UA could potentiate renal injury by causing ischemia, thereby contributing to early function decline, even in the absence of albuminuria.

As a final comment, we recognize that the Gomez's equations do not make *a priori* distinctions for the physiological assumptions used to calculate renal hemodynamic parameters in HC vs. T1D. In contrast, classic micropuncture studies in murine models have reported different values for factors used to calculate segmental resistances by the Gomez's equations. We therefore performed a second set of analyses using P_{GLO} values from these previous micropuncture studies in Munich-Wistar rats in an effort to mirror differences in non-diabetic and diabetic conditions. With this approach, differences in the intraglomerular hemodynamic profile of T1D patients vs. HC more closely resembled the profiles observed in diabetic vs. control rats (138). Varying this

P_{GLO} assumption in the Gomez's equations may therefore more accurately reflect early renal hemodynamic abnormalities in patients with T1D vs. HC. Future work is required to determine whether Gomez's P_{GLO} assumptions should be altered to more accurately reflect the intraglomerular hemodynamic profile in humans in various disease states, including differences that may exist at various levels of GFR in the impaired, normal and hyperfiltration ranges.

Our study has limitations. The study cohort consisted of a carefully selected group of HC and patients with uncomplicated T1D, limiting the generalizability of the data to other populations. For example, since patients with hypertension and reduced GFR were not included in this analysis, we were not able to study the association between PUA and glomerular hypertension, which could be a consequence of long term renal arteriolopathy and chronic systemic hypertension. Additionally, while we have hypothesized that presence of T1D potentiates the interaction between PUA and renal hemodynamic function, we recognize that the absence of the association between PUA and renal hemodynamic function in HC could be due to the smaller GFR range and higher PUA values when compared to the T1D cohort. Although our observations were made using some data from several previously conducted studies, measurement bias was decreased by including all participants that were studied under identical procedures, laboratory environment, and study staff. Though UA consumption in the form of purines and fructose was not recorded, we observed a similar urinary urea excretion and thus protein intake. This finding suggests that differences in dietary intake of high UA-containing foods were unlikely to explain the findings. Next, we recognize that the Gomez's equations that were used to calculate intraglomerular haemodynamic parameters are indirect estimates. Although these equations are based on a few physiological assumptions, they have been carefully studied and were successfully used in various healthy and disease populations over the last sixty years, and do appear to reflect dynamic changes in renal hemodynamic function (92). Finally, while we propose that PUA mediates early renal hemodynamic changes via decreased resistance at the afferent arteriole, we could not determine the mechanistic basis at the molecular level. None of the studies examining the association between PUA and renal hemodynamic function can exclude the possibility that the association between higher PUA and lower GFR and ERPF is based on increased renal clearance leading to lower PUA levels. The cause and effect relationship could be established in future work from the currently ongoing Protecting Early Renal Function Loss (PERL) Study (NCT02017171) examining the potential renal and vascular protective effects of PUA lowering in a T1D

population with microalbuminuria (66). Future studies are needed to elucidate the mechanisms of our findings.

In conclusion, the inverse relationship between PUA with GFR and ERPF in otherwise healthy T1D patients may be on the basis of increased afferent renal arteriole resistance, which could contribute to early renal function decline. The PUA-mediated increase in afferent arteriolar resistance may be caused either by increased tone, or by altered arterial structure characterized by thickening of the afferent arteriole, leading to renal ischemia. UA lowering therapies in patients with diabetes may lead to renal protection by modifying the relationship between PUA and the renal microcirculation, thereby avoiding renal ischemia and the associated decline in renal function – an effect that is being studied in ongoing renal protection trials.

Chapter 5: Renal and Vascular Effects of Uric Acid Lowering in Patients with Uncomplicated Type 1 Diabetes Mellitus

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Manuscript in preparation.

5.1 Abstract

Objective: Even within the normal range, higher plasma uric acid (PUA) levels are associated with lower GFR and higher blood pressure (BP) in young adults with type 1 diabetes (T1D). Our aim was to determine the impact of PUA lowering on renal and vascular function in patients with uncomplicated T1D.

Methods: T1D patients (n=49) and healthy controls (HC, n=24) were recruited into this mechanistic study. T1D patients were studied under euglycemic and hyperglycemic conditions at baseline and after treatment with the xanthine oxidase inhibitor febuxostat (FBX) for 8 weeks. HCs were studied under euglycemic conditions. PUA, GFR (inulin), effective renal plasma flow (ERPF, paraaminohippurate) and BP were measured pre- and post-FBX and in response to a graded angiotensin II infusion to assess the intrarenal renin angiotensin aldosterone system (RAAS). Arterial stiffness was measured pre- and post-FBX. Gomez's equations were used to estimate afferent (R_A) and efferent (R_E) arteriolar resistances and glomerular hydrostatic pressure (P_{GLO}).

Results: FBX decreased PUA in HC (303±71µmol/L to 131±55µmol/L, $p<0.0001$) and T1D (240±62µmol/L to 124±53µmol/L, $p<0.0001$). FBX decreased systolic BP in T1D patients (112±10 to 109±9, $p=0.049$), but not in HC. FBX treatment augmented the filtration fraction response to hyperglycemia in T1D patients, which was accompanied by larger increases in R_E and P_{GLO}. Other renal parameters, arterial stiffness measurements, plasma RAAS hormones and hemodynamic responses to angiotensin II were not affected by FBX in T1D or HC.

Conclusions: In summary, FBX treatment for 8 weeks had a modest BP lowering effect in patients with uncomplicated T1D. PUA lowering enhanced the filtration fraction response to clamped hyperglycemia through an increase in R_E, but without impacting the RAAS, suggesting that PUA may augment the vasoconstrictor or vasodilatory mechanisms which mediate the renal response to hyperglycemia at the efferent arteriole. Longitudinal outcome trials are required to determine whether our physiologic findings can be applied to chronic PUA lowering effects on renal and cardiovascular outcomes in T1D patients.

5.2 Introduction

Accumulating evidence suggests that PUA is associated with multiple key pathways involved in the pathogenesis of diabetic complications, such as metabolic abnormalities (hyperglycemia and insulin resistance), cardiovascular disease (hypertension, arterial stiffness, endothelial dysfunction) and kidney injury (80). Interestingly, extracellular PUA levels are lower in young adults and adolescence with type 1 diabetes (T1D) compared to well matched healthy controls (HC) (85, 86, 98), likely due to a stimulatory effect of increased urinary glucose on the GLUT 9 isoform 2 transporter on the apical membrane of the proximal tubule, which increases PUA excretion (99). Thus, PUA-mediated target organ injury in T1D could instead be related to intracellular uric acid effects, increased urinary PUA excretion and tubular cell exposure (146), increased sequestration of PUA along the vascular endothelium, or possibly PUA-mediated inflammation and activation of the renin angiotensin aldosterone system (RAAS) (80). Consequently, within the normal range PUA is associated with impaired renal function (1, 54), early GFR loss (55) and increased risk of developing proteinuria in T1D patients, (58). Even in young T1D adults and adolescents without complications, higher PUA levels within the normal range are associated with lower GFR (85, 147, 148). This association may be driven by PUA-mediated increases in resistance at the afferent renal arteriole, which could potentiate renal injury by causing ischemia to the renal microcirculation (147). Additionally, accumulating evidence suggests that PUA levels are independently associated with intimal medial thickness, endothelial dysfunction and vascular stiffness (35), promoting the development of hypertension, cardiovascular disease and chronic kidney disease (CKD) (1, 7, 29-31). Although not observed in adolescents with T1D (148), the association of higher within the normal range PUA levels with higher blood pressure emerges in young adults with uncomplicated T1D (85). Such consistently established relationships between PUA and early renal and cardiovascular risk factors in young T1D patients suggest that lowering PUA may be an important strategy to reduce renal and vascular disease progression and prevent diabetes-related complications.

From the limited data available from PUA lowering studies in T1D patients with CKD, gout and type 2 diabetes, PUA lowering may slow GFR decline, reduce proteinuria and suppress expression of renal inflammatory interleukins (60-64, 80). Conversely, withdrawal of PUA lowering therapy leads to a significant acceleration in the rate of kidney function loss and an increase in urinary excretion of inflammatory markers (20). PUA lowering was also shown to improve endothelial

function (74-77) and lower blood pressure in adult and pediatric cohorts with normal renal function (73, 149-152). Currently, the promising effects of PUA lowering on renal protection in T1D patients with albuminuria and above normal PUA is being examined as part of the NIH funded Protecting Early Renal Function Loss or "PERL" study (NCT02017171) (66). Given that traditional RAAS blockade provides only partial protection from the development of T1D complications and the compelling association between PUA and renal and vascular dysfunction, it is of the utmost importance to evaluate the potentially protective renal and vascular effects of pharmacologic PUA lowering in young T1D adults without any complications.

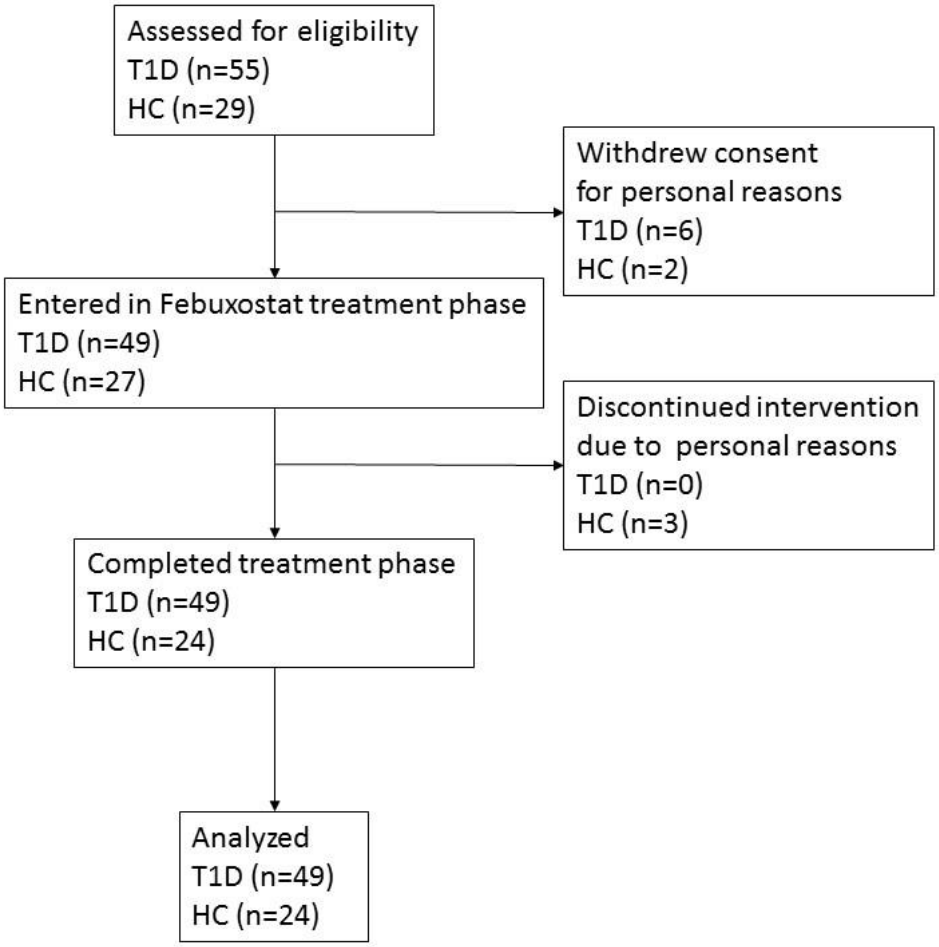
Accordingly, the goals of this hypothesis generating, physiological study were to determine if PUA lowering modifies (1) the effect of hyperglycemia and infused angiotensin II on renal hemodynamic function, (2) systemic blood pressure and (3) arterial stiffness during clamped euglycemia and hyperglycemia in young uncomplicated T1D adults. It was hypothesized that PUA lowering will ameliorate early hemodynamic abnormalities characteristic of T1D, including renal hyperfiltration, systemic hypertensive responses to hyperglycemia and arterial stiffness.

5.3 Research Design and Methods

5.3.1 Subject Inclusion Criteria and Study Preparation

The flow chart of participants is shown in figure 5.1. Twenty-four HC and 49 T1D patients completed this open-label, proof of principle, 8-week febuxostat treatment study (ClinicalTrials.gov identifier NCT02344602). T1D study participants included 42 patients with normofiltration (T1D-N, GFR <135 mL/min/1.73m^2) and 7 patients with hyperfiltration (T1D-H, GFR ≥135 mL/min/1.73m^2). Detailed inclusions criteria were as follows: (1) male and female participants 18-40 years old; (2) normoalbuminuria on a 24 hour urine collection; (3) normal values for body mass index (18-35 kg/m^2); (4) normal renal and liver function; (5) normal electrocardiogram; (6) clinic blood pressure <140/90 mmHg; (7) T1D duration >5 years; (8) able to take medications every day and (9) normal PUA levels. Exclusion criteria were as follows: (1) history of cardiac or lung disease (except for treated asthma) or hypothyroidism; (2) history of stroke or peripheral vascular disease; (3) history of proliferative retinopathy; (4) regular medications aside from thyroid or asthma medications if needed; (5) pregnancy or breastfeeding (women would have to be using two forms of reliable contraception to be eligible for the study); (6) alcohol or tobacco within 72 hours prior to the study; (7) PUA ≥ 450 μmol/L; (8) hypertension,

Figure 5.1. Flow diagram for study participants.



or on BP-lowering medicine; (9) coronary heart disease; (10) eGFR<60 mL/min; (11) active cancer (except non-melanoma skin cancer); (12) taking medication for hyperuricemia; (13) gout, anemia, cirrhosis, active/chronic hepatitis; (14) known allergy to either allopurinol or probenecid and (15) current use of agents that influence renal function or that interfere with purine metabolism such as didanosine, azothioprine, methotrexate, ketoprofen, ketorolac, mycophenolate. The study was approved by the University Health Network Research Ethics Board (Toronto, Canada) and all subjects gave written informed consent.

5.3.2 Experimental Design

The experimental design is shown in Figure 5.2. T1D patients were studied at baseline (1 euglycemic and 1 hyperglycemic day) and after 8 weeks of febuxostat (1 euglycemic and 1 hyperglycemic day). During clamped euglycemic study days, blood glucose was maintained between 4-6 mmol/L, and during clamped hyperglycemic study days, blood glucose was maintained between 9-11 mmol/L (Figure 5.3). Studies were performed after 7 days on a controlled diet consisting of ≥150 mmol/day sodium and ≤1.5 g/kg/day protein. The sodium-replete diet was used to avoid circulating volume contraction, RAAS activation, *between-subject* heterogeneity and in an attempt to keep study conditions similar to typical North American dietary patterns. Pre-study protein intake was modest to avoid the hyperfiltration effect of high protein diets (105). Compliance was ascertained by measurement of 24-hour urine sodium and urea excretion on the seventh day prior to the studies. HC subjects were studied during normal euglycemic conditions and were compared with the T1D group under clamped euglycemic conditions, before and after PUA lowering. All study participants were instructed to avoid caffeine- containing products and to have the same light breakfast on the morning of each study visit.

Subjects were then treated with 80mg daily febuxostat (FBX) for 8 weeks and the same tests were performed during clamped euglycemia and hyperglycemia. Blood was drawn at the study midpoint to monitor renal function, electrolytes, PUA levels and blood pressure was measured. HC subjects were similarly studied during normoglycemic conditions only.

Figure 5.2. Study outline.

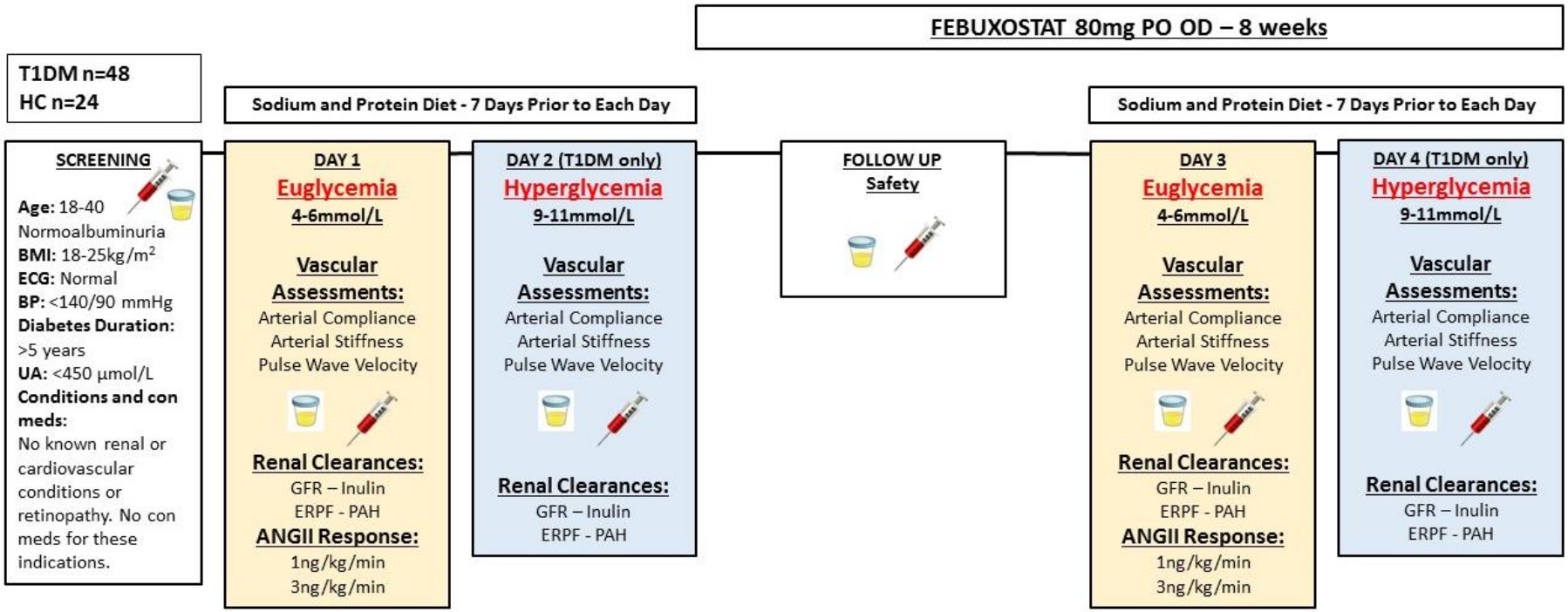
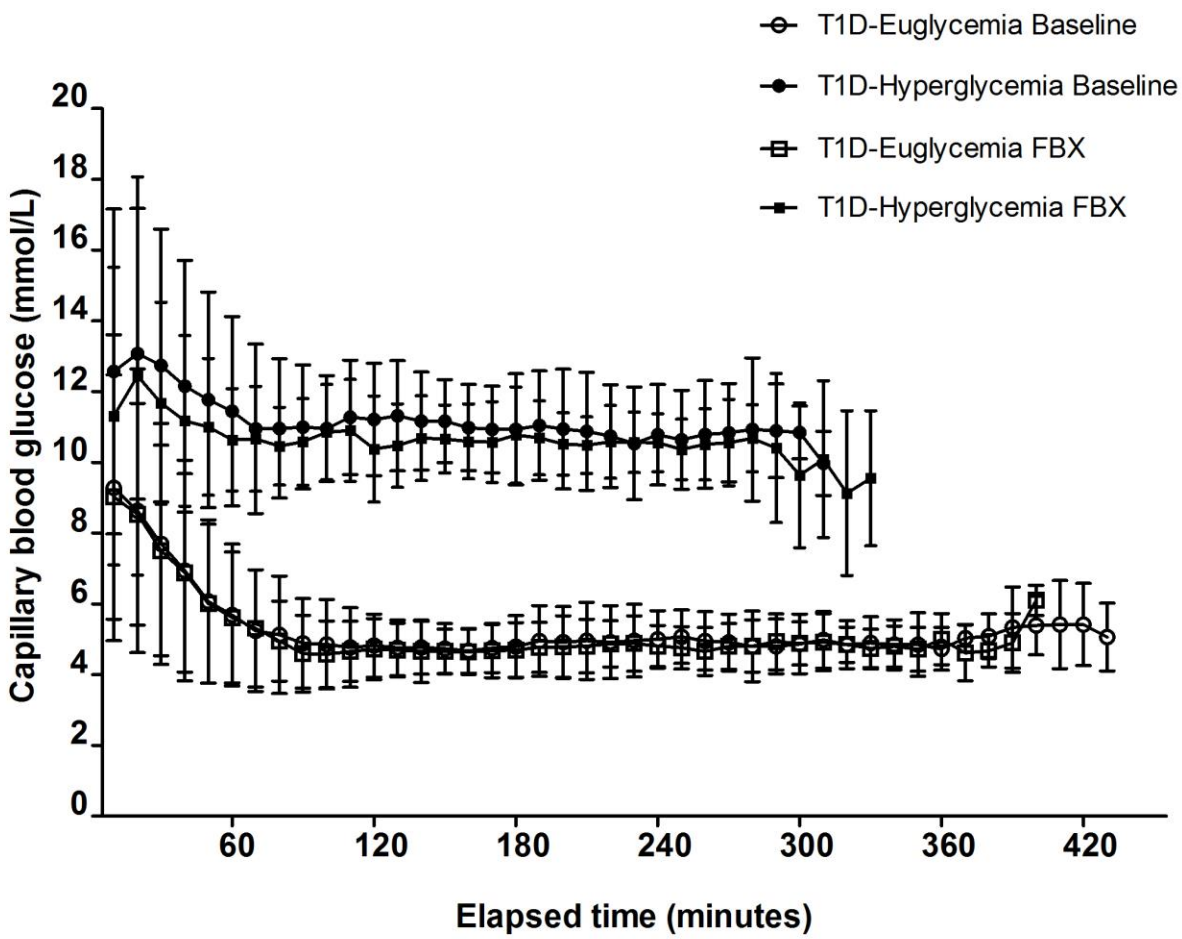


Figure 5.3. Capillary blood glucose levels during the euglycemic and hyperglycemic clamp studies at baseline and after FBX treatment in T1D patients (mean ± SD). Glucose levels were recorded every 10 minutes during the clamp.



5.3.3 Assessment of Renal Hemodynamic Function

Subjects presented to the Renal Physiology Laboratory on Day 1 for the baseline euglycemic and consequently hyperglycemic studies on Day 2. After clamped euglycemia was achieved for 5 hours, blood samples were collected for inulin and PAH blank and for baseline circulating RAAS mediators (plasma renin concentration and aldosterone) (137). Oscillometric brachial artery blood pressure measurements were obtained in a reclining position at 30-minute intervals throughout the study (Critikon, Tampa, Florida, USA). Blood pressure measurements were obtained in duplicated, and an average was taken for each time point. Subjects remained supine at all times. Baseline renal hemodynamic function (GFR and ERPF) was measured using inulin and PAH clearance according to the plasma disappearance technique (37-39, 46-48, 52, 72, 107, 153-159). The mean of the final 2 clearance periods represented baseline GFR and ERPF, expressed per 1.73 m^2.

The following parameters were calculated:

Filtration fraction (FF) = $\frac{GFR}{ERPF}$

Renal blood flow (RBF) = $\frac{ERPF}{1-Hematocrit}$

Renal vascular resistance (RVR) = $\frac{MAP}{RBF}$

Indirect intraglomerular hemodynamic parameters were estimated using formulae estimated by Gomez based on data from animal studies (132). These equations were successfully used in a similar manner to evaluate patients with conditions such as hypertension, endocrine disorders and T1D (92, 133-136, 147). Assumptions imposed by Gomez's equations were the following: i) intrarenal vascular resistances are divided into afferent, post-glomerular and efferent; ii) hydrostatic pressures within the renal tubules, venules, Bowman's space and interstitium (P_{Bow}) are in equilibrium of 10 mmHg; iii) glomerulus is in filtration disequilibrium; iv) the gross filtration coefficient (K_{FG}) is 0.1733 ml/s per mmHg (P_{GLO} = 47.5 mmH) for HC and K_{FG} = 0.1012 ml/s per mmHg (P_{GLO} = 56.4 mmHg) for T1D to reflect the different P_{GLO} values in diabetic and control conditions observed in previous micropuncture studies in Munich-Wistar rats (138). MAP (mmHg), ERPF (ml/s), GFR (mL/s) and total protein (g/dL) were used to calculate R_E (dyne•sec•cm^{-5}) and R_A (dyne•sec•cm^{-5}), P_{GLO} (mmHg), ΔP_F (mmHg) and π_G (mmHg).

The filtration pressure across glomerular capillaries (ΔP_F):

$$\Delta P_F = GFR / K_{FG}$$

The glomerular oncotic pressure (π_G) from the plasma protein mean concentration (C_M) within the capillaries:

$$C_M = TP/FF \times \ln(1/1-FF)$$

$$\pi_G = 5 \text{ x } (C_M - 2)$$

Glomerular hydrostatic pressure (P_{GLO}):

$$P_{GLO} = \Delta P_F + P_{Bow} + \pi_G$$

R_A and R_E were estimated using principles of Ohm's law, where 1328 is the conversion factor to dyne•sec•cm^{-5} (132):

$$R_A = [(MAP-P_{GLO})/RBF] \times 1328$$

$$R_E = [GFR/(K_{FG} \times (RBF-GFR)] \times 1328$$

5.3.4 Assessment of Angiotensin II Infusion Response

After baseline clearance periods were complete, Ang II (Clinalpha, Laüfelfingen, Switzerland) was administered at incremental doses of 1 ng/kg/min and 3 ng/kg/min, each over 30 minutes, followed by a 30-minute recovery phase (38, 72). Blood was collected during each Ang II infusion period for HCT, inulin and PAH. Oscillometric brachial artery blood pressure measurements were obtained in a reclining position every 5 minutes during each Ang II infusion (Critikon, Tampa, Florida, USA). Blood pressure measurements were obtained in duplicated, and an average was taken for each time point. A further collection of blood was obtained at the end of the Ang II infusion and after a 30-minute recovery period. Renal hemodynamic parameters were assessed at the end of each Ang II infusion period and at recovery. On Day 2 subjects were similarly studied under hyperglycemic conditions, but did not undergo the Ang II infusion.

5.3.5 Vascular Assessments

Vascular assessments were performed on each of the 4 study days after ambient glycemia has been stabilized and before renal hemodynamic function testing. In brief, arterial compliance was measured non-invasively using a Sphygmocor device (SphygmoCor, AtCor Medical Systems Inc., Sydney, Australia). Right carotid artery waveforms were recorded with a high-fidelity micromanometer (SPC-301, Millar Instruments) and using the validated transfer function, corresponding central aortic pressure waveform data was generated. MAP and heart rate were determined using the integral software. Augmentation index, an estimate of arterial stiffness was calculated as the difference between the second systolic peak and inflection point, expressed as a percentage of the central pulse pressure corrected to a heart rate of 75 beats per minute. The aortic pulse wave velocity was measured using the same device.

5.3.6 Statistical Analyses

The primary endpoint of this study was change from baseline in GFR after an 8 week FBX treatment under stable euglycemic and hyperglycemic conditions. Our previous data have shown that the standard deviation of the ΔGFR in response to RAAS modulation is approximately 19 ml/min/1.73 m^2 (5, 72, 160). To detect a 10 ml/min/1.73m^2 between-group difference in the GFR response to PUA lowering, for a two-sided test with p=0.01 (to correct for multiple comparisons) and with Z_α = 2.58 the sample size equals 24 in each group (T1D-H and T1D-N). We therefore studied 48 T1D patients, so that the analysis could be performed on the basis of filtration status during clamped euglycemia (24 hyperfiltering, 24 normofiltering subjects) (5). We could not distinguish T1D-H from T1D-N patients until the inulin clearances were reported and although our previous work has shown that approximately 50% of young patients with T1D exhibit inulin-based hyperfiltration, we only observed 7 T1D-H patients and 42 T1D-N in our recruited cohort.

The difference between renal hemodynamic parameters at euglycemic clamp and hyperglycemic clamp was used to analyze the hyperglycemic response pre- and post- FBX treatment. The difference between renal hemodynamic parameters at baseline euglycemic clamp and 30 minutes after the 1 and 3ng/kg/min Ang II infusions were used to analyze the Ang II response pre- and post- FBX treatment.

One T1D-H patient was excluded from the analysis examining the effect of FBX on renal hemodynamic function during the euglycemic clamp only due to issues with the blood sample

collected for the inulin and PAH measurements. For similar reasons, 1 T1D-N patient was excluded from the analysis of Ang II infusion responses pre- and post- FBX. One T1D-N patient was excluded from the analysis of radial AIX during hyperglycemia due to an inability to obtain measurements pre- and post- FBX treatment. PUA levels did not decrease significantly in response to FBX treatment in 3 T1D patients, however these patients were included in the analysis.

Data are presented as mean ± standard deviation (SD). Within-group differences and responses to FBX treatment were analyzed using paired t-tests. To assess for *between-group* differences, analysis of variance with *post-hoc* Tukey's test was used. Linear regression analysis was used to determine correlations between renal haemodynamic responses and PUA levels. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using SAS v9.1.3 and GraphPad Prism software (version 5.0).

5.4 Results

5.4.1 Baseline Characteristics

The study population comprised of 49 T1D (42 T1D-N, 7 T1D-H) and 28 HC participants (Table 5.1). All T1D patients had T1D duration of >5 years with a mean of 14.3±7.2 years. Overall, baseline characteristics were similar between T1D and HC groups, whereas 24 hour protein intake tended to be lower and hemoglobin A1c was higher in the T1D group. Sex distribution, age, BMI, HDL cholesterol, LDL cholesterol, triglycerides, 24 hour urine sodium and estradiol and progesterone levels (females only) did not differ between HC and T1D groups.

5.4.2 Effect of Febuxostat on PUA Levels

As expected, T1D patients at baseline under euglycemic conditions had lower PUA levels compared to HC (240±62µmol/L vs 303±71µmol/L respectively, $p=0.0002$), and were further lowered in T1D patients during hyperglycemic conditions (240±62µmol/L vs 221±61 µmol/L, $p<0.0001$). An 8 week treatment with FBX significantly decreased PUA levels by approximately a half in each group: HC (303±71µmol/L to 131±55µmol/L, $p<0.0001$), T1D during euglycemic (240±62µmol/L to 124±53µmol/L, $p<0.0001$) and hyperglycemic (221±61µmol/L to 108±42µmol/L, $p<0.0001$) conditions (Table 5.2).

Table 5.1. Baseline Demographic Characteristics of Healthy Controls (HC) and Patients with Type 1 Diabetes (T1D).

Parameter	HC (n=24)	T1D (n=49)
Males	12 (50%)	25 (51%)
Age (years)	25.5±4.5	26.3±5.4
Diabetes duration (years)	-	14.3±7.2
Body mass index (kg/m^2)	23.6±3.4	25.1±3.4
Cholesterol	4.2±0.8	4.6±0.8
HDL Cholesterol	1.5±0.5	1.5±0.4
LDL Cholesterol	2.4±0.7	2.6±0.7
Triglyceride	1.0±0.7	1.1±0.7
Hemoglobin A1c – mmol/mol (%)	31.7± 2.4 (5.0±0.2%)	62.3±14.8 (7.8±1.3%)*
24 hour urine sodium (mmol/day)	155±65	150±76
24 hour protein intake (g/kg/day)	1.1±0.3	1.0±0.3*
Estradiol (females only)	226±169	217±250
Progesterone (females only)	3.3±4.2	3.1±7.5

24 hour protein intake: estimated by the formula ([urine urea X 0.18] + 14) / weight in kg. n, number of participants. *$p<0.05$ vs. HC; HC: healthy controls; T1D: type 1 diabetic patients.

Table 5.2. Diet Parameters and Plasma Marker Response to FBX Treatment in Healthy Controls (HC) and Patients with Type 1 Diabetes (T1D) Studied Under Euglycemic and Hyperglycemic Clamp Conditions.

	HC (n=24)			T1D (n=49)					
				Euglycemia			Hyperglycemia		
Parameter	**Baseline**	**Febuxostat**	**p-value**	**Baseline**	**Febuxostat**	**p-value**	**Baseline**	**Febuxostat**	**p-value**
Diet parameters									
Hemoglobin A1c - mmol/mol (%)	5.05±0.22	4.97±0.23	0.0167	62.3±14.8 (7.8±1.3%)	62.4±13.9 (7.9±1.3%)	0.8831	-	-	-
24 hour urine sodium (mmol/day)	155±65	152±74	0.7968	150±76	132±83	0.1126	-	-	-
24 hour protein intake (g/kg/day)	1.1±0.3	1.1±0.3	0.2726	1.0±0.3	1.0±0.4	0.8264	-	-	-
Plasma Analysis									
Aldosterone (ng/dL)	291±164	283±260	0.8852	76±56	67±39	0.1265	60±54	60±47	0.9976
Renin (ng/L)	14.4±10.4	12.1±8.5	0.3343	10.3±22.9	10.1±16.5	0.9376	6.8±15.6	5.1±5.8	0.4028
PUA (µmol/L)	303±71	131±55	<0.0001	240±62	124±53	<0.0001	221±61	108±42	<0.0001
Estradiol (females only)	226±169	285±220	0.1997	217±249	245±260	0.6742	-	-	-
Progesterone (females only)	3.3±4.2	4.3±5.9	0.5248	3.1±7.5	2.1±3.8	0.5487	-	-	-

24 hour protein intake: estimated by the formula ([urine urea X 0.18] + 14) / weight in kg. Values are mean ± standard deviation. n, number of participants. *p<0.05 vs. HC; HC: healthy controls; T1D: patients with type 1 diabetes; PUA: plasma uric acid.

5.4.3 Effect of Febuxostat on Renal Function, Blood Pressure and Vascular Parameters

5.4.3.1 Normoglycemic Conditions in HC

After 8 weeks of treatment, FBX did not significantly alter the renal hemodynamic function (ERPF, GFR, FF, RBF and RVR, Table 5.3), intraglomerular hemodynamics (P_{GLO}, R_A, R_E and R_A/R_E), blood pressure (SBP, DBP and HR, Table 5.4) or vascular parameters (aortic AIX, carotid AIX, carotid femoral and carotid radial PWVs, Table 5.2).

5.4.3.2 Euglycemic Clamp Conditions in T1D

While there were no differences observed in renal hemodynamic function (ERPF, GFR, FF, RBF and RVR) under euglycemic conditions in response to FBX treatment, P_{GLO} decreased (54.4±4.1 to 53.4±3.2, p=0.0497) without significant changes in R_A, R_E and R_A/R_E in T1D patients (Table 5.2). In the overall T1D group, FBX treatment lead to a modest decrease in SBP (112±9 to 109±9, p=0.0491, Figure 5.4), but not in DBP (67±6 to 66±7, p=0.2823) or HR (67±11 to 66±10, p=0.8605). FBX did not alter vascular stiffness parameters (aortic AIX, carotid AIX, carotid femoral and carotid radial PWVs) (Table 5.4).

Further analysis revealed that GFR in each of the 6 patients with T1D-H decreased (150±13 mL/min/1.73m^2 to 129±10 mL/min/1.73m^2, p=0.0113, Figure 5.5), whereas there was no change in GFR in patients with T1D-N (111±14mL/min/1.73m^2 to 111±16 mL/min/1.73m^2, p=0.9227).

5.4.3.3 Hyperglycemic Clamp Conditions in T1D

After 8 weeks of treatment, FBX did not significantly alter renal hemodynamic function (ERPF, GFR, FF, RBF and RVR), intraglomerular hemodynamics (P_{GLO}, R_A, R_E and R_A/R_E), blood pressure (SBP, DBP and HR) or vascular parameters (aortic AIX, carotid AIX, carotid femoral and carotid radial PWVs, Table 5.4) in T1D patients under hyperglycemic conditions (Table 5.4).

5.4.4 PUA Correlations with Renal Hemodynamic Parameters

As observed in our previous studies (85, 147, 148), higher PUA was correlated with lower GFR (r=-0.37, p=0.009 during euglycemia, r=-0.46, p=0.0009 during hyperglycemia) and lower ERPF (r=-0.29, p=0.047 during euglycemia, r=-0.39, p=0.006 during hyperglycemia). After FBX

Table 5.3. Renal Hemodynamic Function and Intraglomerular Hemodynamic Response to FBX Treatment in Healthy Controls (HC) and Patients with Type 1 Diabetes (T1D) Studied Under Euglycemic and Hyperglycemic Clamp Conditions.

	HC (n=24)			T1D (n=49)					
				Euglycemia			Hyperglycemia		
Parameter	**Baseline**	**Febuxostat**	**p-value**	**Baseline**	**Febuxostat**	**p-value**	**Baseline**	**Febuxostat**	**p-value**
Renal hemodynamic function									
ERPF (mL/min/1.73m^2)	654±111	639±91	0.3818	647±131	657±113	0.5329	676±133	665±124	0.4235
GFR (mL/min/1.73m^2)	117±17	119±15	0.2836	115±19	113±16	0.1893	130±21	133±19	0.2142
Filtration fraction	0.18±0.04	0.19±0.03	0.3124	0.18±0.04	0.17±0.03	0.1019	0.20±0.04	0.21±0.05	0.1064
RBF (mL/min/1.73m^2)	1058±202	1035±178	0.4044	1051±219	1063±185	0.6300	1071±209	1052±203	0.3956
RVR (mmHg/L/min)	0.077±0.015	0.077±0.015	0.6896	0.081±0.022	0.077±0.014	0.1327	0.080±0.016	0.082±0.020	0.4171
Intraglomerular hemodynamic parameters									
P_{GLO} (mmHg)	48.9±2.7	49.4±2.5	0.2275	54.4±4.1	53.4±3.2	0.0497	54.9±4.0	56.1±4.1	0.0664
R_A (dyne•sec•cm^{-5})	2246±640	2208±704	0.6845	2167±885	2010±662	0.1905	2170±721	2132±815	0.7373
R_E (dyne•sec•cm^{-5})	994±263	1028±197	0.3931	1690±424	1604±331	0.1299	1871±423	1997±573	0.0829
R_A/R_E	2.39±0.86	2.20±0.76	0.1504	1.31±0.52	1.29±0.47	0.6641	1.21±0.44	1.11±0.43	0.1667

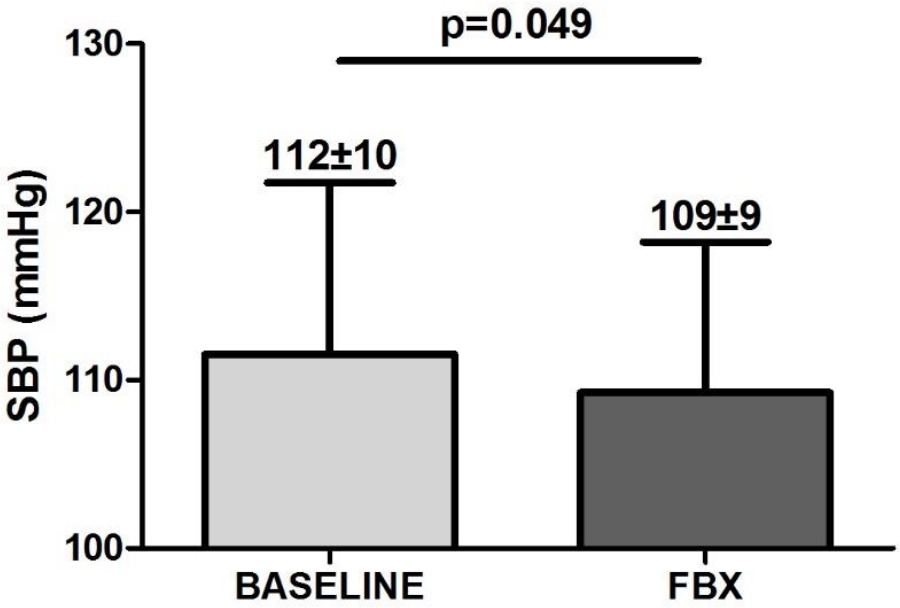
Values are mean ± standard deviation. n, number of participants. *p<0.05 vs. HC; HC: healthy controls; T1D: patients with type 1 diabetes; ERPF: effective renal plasma flow; GFR: glomerular filtration rate; RBF: renal blood flow; RVR: renal vascular resistance; P_{GLO}: Glomerular hydrostatic pressure; R_A: Afferent arteriolar resistance; R_E: Efferent arteriolar resistance.

Table 5.4. Systemic Hemodynamic Function and Vascular Parameter Response to FBX Treatment in Healthy Controls (HC) and Patients with Type 1 Diabetes (T1D) Studied Under Euglycemic and Hyperglycemic Clamp Conditions.

	HC (n=24)			T1D (n=49)					
				Euglycemia			Hyperglycemia		
Parameter	**Baseline**	**Febuxostat**	**p-value**	**Baseline**	**Febuxostat**	**p-value**	**Baseline**	**Febuxostat**	**p-value**
Systemic hemodynamic function									
HR (bpm)	62±8	61±9	0.4042	67±11	66±10	0.8605	64±11	64±12	0.7484
SBP (mmHg)	107±9	106±8	0.3267	112±10	109±9	0.0491	113±9	112±9	0.4405
DBP (mmHg)	64±6	64±7	0.6106	67±6	66±7	0.2823	69±7	69±7	0.6546
Vascular Parameters									
Aortic AIX (%)	-7.7±9.7	-9.6±8.1	0.4353	-3.0±11.5	-4.1±11.8	0.4247	-1.8±14.3	-1.5±12.5	0.8605
Carotid AIX (%)	-3.6±13.6	-4.3±14.1	0.7529	0.5±15.0	1.6±14.7	0.4740	4.4±15.7	2.5±14.9	0.1723
Carotid radial PWV (m/s)	7.1±1.0	6.8±1.1	0.2340	7.3±1.1	7.2±1.1	0.7043	7.6±1.0	7.3±1.3	0.0728
Carotid femoral PWV (m/s)	5.5±1.1	5.3±1.0	0.5332	5.8±1.0	5.6±1.2	0.2979	5.8±0.9	5.6±1.1	0.0972

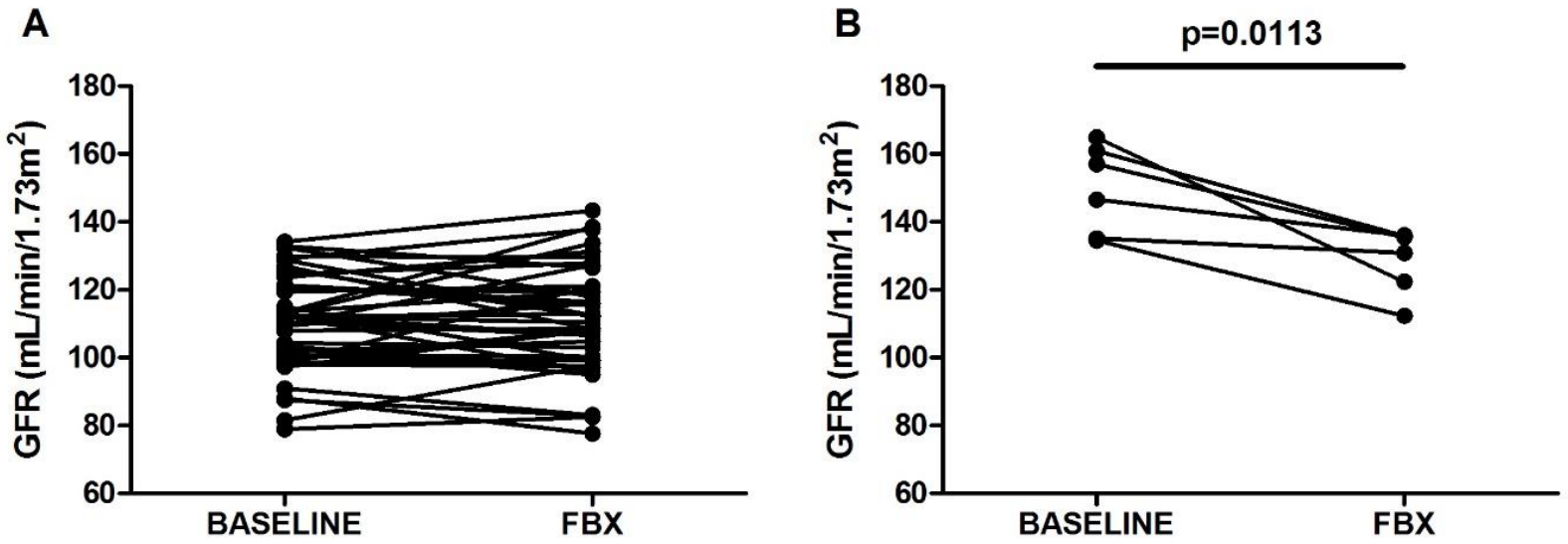
Values are mean ± standard deviation. n, number of participants. *p<0.05 vs. HC; HC: healthy controls; T1D: patients with type 1 diabetes; HR: heart rate; bpm: beats per minute; SBP: systolic blood pressure; DBP: diastolic blood pressure; AIX: augmentation index; PWV: Pulse Wave Velocity.

Figure 5.4. Systolic blood pressure (SBP) response during a euglycemic clamp day at baseline and after an 8 week treatment with febuxostat in patients with T1D.



Values are mean ± standard deviation.

Figure 5.5. Glomerular filtration rate (GFR) response during a euglycemic clamp day at baseline and after an 8 week treatment with febuxostat in individual patients with T1D and (A) normofiltration (T1D-N, GFR <135 mL/min/1.73m^2, n=42) and (B) hyperfiltration (T1D-H, GFR ≥135 mL/min/1.73m^2, n=6).



treatment, high PUA was still correlated with lower GFR during euglycemia (r=-0.30, p=0.038) but not during hyperglycemia (r=-0.18, p=0.22) or with ERPF (r=-0.087, p=0.56 during euglycemia, r=-0.15, p=0.29 during hyperglycemia). No correlations were observed between PUA and GFR or ERPF in HC pre- or post-FBX treatment.

5.4.5 Effect of Febuxostat on Renal Hyperglycemic Responses

FBX treatment lead to a significantly higher increase in FF in response to hyperglycemia (+0.01±0.04 to +0.03±0.04, 0.0296), which was accompanied by a larger increase in R_E (+195±384 dyne•sec•cm^{-5} to +400±525dyne•sec•cm^{-5}, p=0.0271) and P_{GLO} (+0.5±3.6mmHg to 2.7±3.7mmHg, p=0.0053), but not R_A, GFR or ERPF (Figure 5.6). No significant differences were observed in blood pressure (SBP, DBP and HR) or vascular parameters (aortic AIX, carotid AIX, carotid femoral and carotid radial PWVs) responses to hyperglycemia.

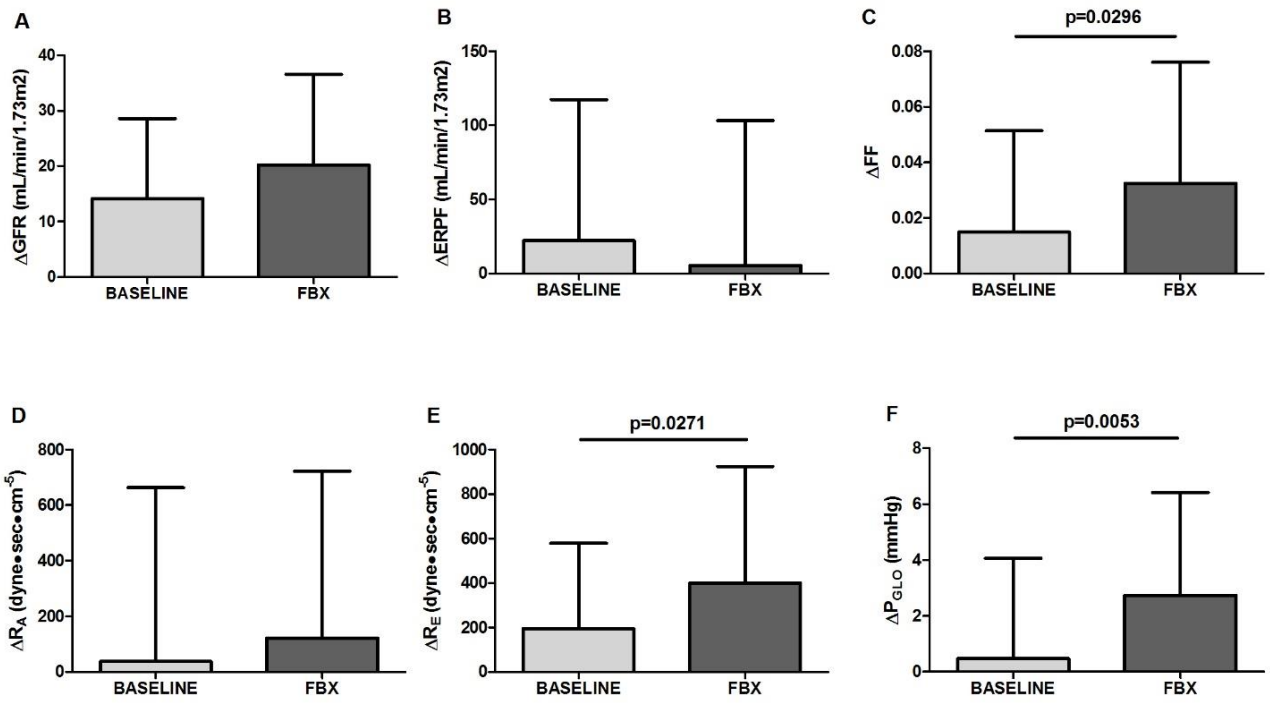
5.4.6 Effect of Febuxostat on Ang II Infusion Responses and Plasma RAAS Markers

FBX treatment did not alter plasma aldosterone or renin levels in HC or T1D under euglycemic and hyperglycemic conditions (Table 5.2) and did not alter the renal hemodynamic response to 1 ng/kg/min or 3 ng/kg/min Ang II infusions (Figure 5.7), nor were there changes in intraglomerular hemodynamic parameters (P_{GLO}, R_A, R_E and R_A/R_E) or blood pressure (SBP, DBP and HR) in either group.

5.4.7 Effect of Febuxostat on Glucose Control, Laboratory Parameters and Adverse Events

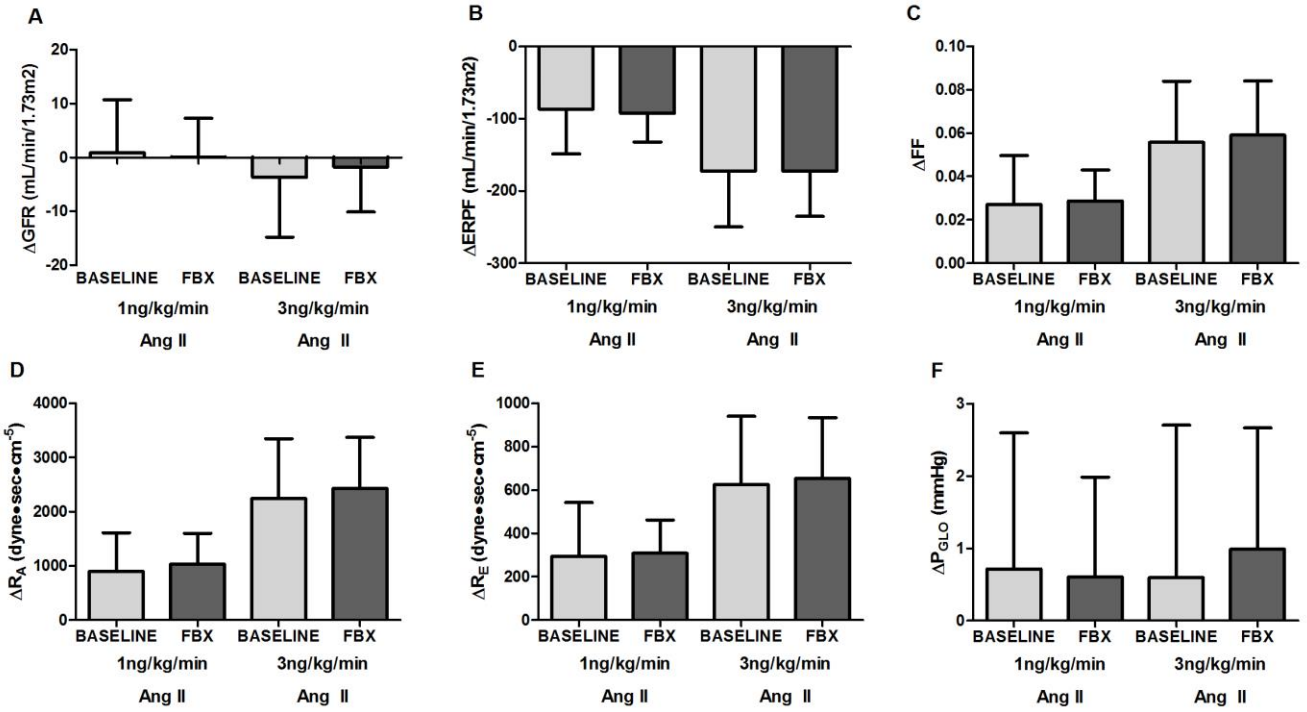
FBX treatment decreased hemoglobin A1c levels (5.05±0.22% to 4.97±0.23%, p=0.0167) in HC participants, but not in T1D during euglycemic or hyperglycemic conditions. FBX did not alter 24 hour protein intake, 24 hour urine sodium excretion, BMI, estradiol or progesterone levels (females only), or any other clinically relevant biochemical and hematological parameters assessed (including serum sodium, potassium, calcium, magnesium, chloride, phosphate and liver enzymes) in T1D or HC participants. No adverse events were reported, aside from some mild nausea after FBX intake in several patients which resolved after taking the agent with food.

Figure 5.6. GFR (A), ERPF (B), FF (C), R_A (D), R_E (E) and P_{GLO} (F) response to clamped hyperglycemia in T1D at baseline and after an 8 week treatment with febuxostat.



T1D n=48; GFR: Glomerular filtration rate; ERPF: Effective renal plasma flow; FF: Filtration fraction; P_{GLO}: Glomerular hydrostatic pressure; R_A: Afferent arteriolar resistance; R_E: Efferent arteriolar resistance. Δ in each outcome represents the difference between the outcome measured at hyperglycemic clamp day and euglycemic clamp day. R_A, R_E and P_{GLO} in T1D calculated by Gomez's equations (assumption: P_{GLO} of 56.4 mmHg in T1D). Values are mean ± standard deviation).

Figure 5.7. GFR (A), ERPF (B), FF (C), R_A (D), R_E (E) and P_{GLO} (F) response to Ang II infusion (1ng/kg/min and 3ng/kg/min) during a euglycemic clamp day in T1D at baseline and after an 8 week treatment with febuxostat.



T1D n=48; GFR: Glomerular filtration rate; ERPF: Effective renal plasma flow; FF: Filtration fraction; P_{GLO}: Glomerular hydrostatic pressure; R_A: Afferent arteriolar resistance; R_E: Efferent arteriolar resistance. Δ in each outcome represents the difference between the outcome measured post- and pre- the 3ng/kg/min Ang II infusion during a euglycemic clamp day. R_A, R_E and P_{GLO} in T1D calculated by Gomez's equations (assumption: P_{GLO} of 56.4 mmHg in T1D). Values are mean ± standard deviation.

5.5 Discussion

In patients with T1D, PUA levels are linked with the early loss of renal function (54). While the association between PUA levels and renal risk is compelling, only a few studies have measured the effects of PUA lowering therapies on renal outcomes. Goicoechea et al. examined the effect of allopurinol in CKD patients (eGFR <60 ml/min) and found a slower rate of progression in the allopurinol (+1.3 ml/min/1.73m^2) versus the control group (-3.3 ml/min/1.73m^2) after controlling for important clinical variables (61). Allopurinol also reduces the risk of the composite endpoint of deterioration in renal function or dialysis (60). Finally, in patients with stage 3 - 4 CKD, withdrawal from allopurinol is associated with an increase in PUA levels, deteriorating renal function, hypertension and an increase in the urinary excretion of the pro-fibrotic transforming factor-beta (TGF-β) (20). PUA lowering agents such as allopurinol also exert antihypertensive effects (73, 149-152) and improve endothelial function in older normotensive and hypertensive patients with type 2 DM and in patients with CKD (74, 161). Instead of focusing on older, hypertensive type 2 DM patients with established CKD, our study examined the effect PUA lowering on renal hemodynamic and vascular function in young, normotensive T1D patients with normal renal function and normal baseline PUA levels. Moreover, our use of FBX as a physiological probe is novel due to the greater potency and better safety profile of this agent compared with older agents. Furthermore, due to its mechanism of action, FBX does not stimulate the production of reactive oxygen species, which may limit vascular protective effects of allopurinol (162-165). FBX is therefore appealing in terms of its potential for long term clinical use and inclusion in future clinical trials.

Our first major observation was that the 8 week FBX treatment did not change baseline renal function parameters, such as GFR and ERPF in HC or T1D during euglycemic and hyperglycemic conditions. Although our observation may suggest that PUA lowering does not affect renal function in uncomplicated T1D patients, we observed a GFR decrease of about 20 mL/min/1.73m^2 in each of the 6 patients that presented with hyperfiltration at baseline and no renal hemodynamic change in patients with normofiltration or HCs. Given the role of hyperfiltration in predicting the subsequent development of microalbuminuria and nephropathy in T1D patients (166), PUA lowering may promote renal protection by normalizing hyperfiltration without any renal effects in individuals with normal renal function in our study cohort. Moreover, lowering PUA during hyperfiltration may reduce the exposure of renal tubular cells to increased uric acid excretion,

which can form crystals in the setting of the urinary acidification observed in T1D adolescents, thereby promoting kidney injury through stimulation of inflammasomes, Toll-like receptors and chemotactic factors (146). It is also unclear whether GFR normalized in the 6 T1D-H patients due to the regression-dilution bias phenomenon, where a variable that shows an extreme value during the first measurement is less likely to be so extreme at the subsequent measurement (167). We were unable to evaluate the regression-dilution bias due to the lack of placebo group data that would quantify a natural GFR change over an 8 week time period. Unfortunately, the small number of T1D-H patients that were recruited into the study does not allow us to make a definitive conclusion regarding PUA lowering in T1D-H patients.

We previously reported that in a similar T1D cohort, higher PUA was associated with lower GFR and ERPF via increased resistance at the afferent renal arteriole (147) suggesting that chronic exposure to within normal higher PUA levels leads to renal vasoconstrictive effects at the afferent arteriole leading to ischemia and potential renal injury. As observed in our previous studies (85, 147, 148), in our current T1D cohort within the normal range higher PUA at baseline was correlated with lower GFR and ERPF during euglycemia and hyperglycemia. After FBX, such a correlation remained only with ERPF during euglycemic conditions, which is likely a result of significantly decreased PUA range in response to FBX treatment limiting our power to detect significant correlations. Existing data therefore suggest that chronic PUA exposure does not cause hyperfiltration and that acute lowering of PUA may normalize hyperfiltration through as-yet undefined mechanisms. Our data examining the role of PUA lowering in renal hemodynamic function does not clearly indicate whether PUA is a cause or a consequence of altered renal hemodynamic function in T1D. Further studies are required to understand the causality of the association between PUA, GFR and ERPF in this population.

Consistent with previous studies reporting allopurinol mediated antihypertensive effects (73, 149-152), our second major observation was the modest decrease in SBP observed in T1D patients under euglycemic conditions, but not hyperglycemic conditions in response to FBX treatment. Studies with larger sample size are required to determine whether FBX treatment indeed significantly lowers blood pressure in uncomplicated T1D adults. To further understand PUA-mediated effects on vascular function in our study cohort we examined the effects of PUA lowering on arterial stiffness, which has not yet been tested in patients with uncomplicated T1D. Our data suggests that at early stages in the natural history of T1D, FBX does not improve arterial

stiffness under euglycemic or hyperglycemic conditions. Other mechanisms that have been implicated in the relationship between PUA and high blood pressure include RAAS activation, increased reactive oxygen species (ROS) production and inhibition of the nitric oxide synthase (NOS) (9, 25, 40). In our study, FBX treatment did not change the levels of plasma RAAS markers or the hemodynamic responses to an exogenous infusion of Ang II, suggesting that blood pressure lowering effect of FBX are not attributed to changes in the RAAS activation. Thus, further studies are required to determine whether FBX mediates changes in nitric oxide (NO) bioavailability or ROS production leading to alterations in endothelial function or blood pressure.

Whether PUA influences the vascular response to clamped hyperglycemia is unknown in patients with uncomplicated T1D. Our third major observation, contrary to what we expected, was that PUA lowering enhanced the renal filtration fraction response to clamped hyperglycemia through an increase in R_E. Constriction at the efferent renal arteriole is a known mechanism of hyperfiltration mediated by intrarenal RAAS activation in T1D (35). Moreover, PUA is positively associated with plasma renin activity in humans, even when PUA levels are within the normal range (<450μmol/L) (9, 168-170). The association between PUA and RAAS activation has been further strengthened by previous findings demonstrating a negative correlation between PUA and the renal hemodynamic response to an angiotensin II infusion in a cohort of individuals with a wide range of PUA values (100 - 595 μmol/L) (42). Specifically, Perlstein et al reported a negative correlation ($r=-0.37$, $p<0.001$) between PUA and renal plasma flow responses to infused Ang II, suggesting a state of baseline intrarenal RAAS activation with higher PUA levels, resulting in blunted responsiveness to an exogenous Ang II infusion (42). Contrary to these previous observations, Ang II infusion responses and plasma renin and aldosterone levels did not change after an FBX treatment for 8 weeks in our young uncomplicated T1D cohort. The observation that PUA lowering enhanced the renal filtration fraction response to clamped hyperglycemia through an increase in R_E, but without impacting the RAAS, suggests that PUA may augment the vasoconstrictor or vasodilatory mechanisms which mediate the renal response to hyperglycemia at the efferent arteriole. Such mechanisms may include NO, endothelin-1, COX-2, vasopressin, prostaglandins and bradykinins, which require further investigation. Our observations suggest that there may be U-shaped relationship between PUA lowering and potential beneficial effects, where PUA lowering is associated with a greater response to hyperglycemia and higher PUA levels are associated with lower renal function and higher blood pressure. This observation is consistent with a previously observed J-shaped relation between PUA and the rate of cardiovascular events

in patients with essential hypertension (171). Future studies should examine a range of FBX doses to find an optimal level of PUA that maximize cardiorenal protection.

One of the limitations of our study was the relatively small sample size, and especially the unexpected small number of T1D-H patients who were recruited. The lack of a placebo group did not allow us to compare natural GFR variability over 8 weeks to determine the PUA lowering effect on GFR after 8 weeks of FBX treatment. As a result, placebo-controlled studies with larger T1D-H cohorts are needed to determine whether PUA lowering can normalize hyperfiltration and promote renal protection. In an attempt to minimize the effects of a small sample size, we recruited homogenous study groups with careful pre-study preparation, with an emphasis on a special sodium and protein diet to reduce the potential influence of neurohormonal factors on renal hemodynamic responses. As a result of the carefully selected group of patients and controls, our data is limited in generalizability to other populations, such as T1D patients with longer diabetes duration, degree of proteinuria, nephropathy and others. Although endogenous production or exogenous consumption of uric acid in the form of purines and fructose were not recorded, we observed similar levels of urinary urea excretion (as a surrogate marker of protein intake) pre- and post- FBX treatment. Each study participant acted as his/her own control to decrease possible intra-individual variability. Next, we recognize that the Gomez formulae that were used to estimate intraglomerular hemodynamic parameters are indirect estimates and are based on a few physiological assumptions. Nevertheless, Gomez formulae have been carefully studied and were successfully used in various healthy and disease populations over the last sixty years, including in patients with uncomplicated T1D, and do appear to reflect dynamic changes in renal hemodynamic function (92, 147).

In conclusion, FBX treatment for 8 weeks had a modest BP lowering effect in patients with uncomplicated T1D. Although PUA lowering did not demonstrate significant changes to renal hemodynamics in the overall T1D cohort, our data suggests that it may normalize hyperfiltration. Our study is also the first to demonstrate that PUA lowering enhanced the renal filtration fraction response to clamped hyperglycemia through an increase in R_E, but without impacting the RAAS, suggesting that PUA may augment the vasoconstrictor or vasodilatory mechanisms which mediate the renal response to hyperglycemia at the efferent arteriole. Longitudinal outcome trials are required to determine whether our physiologic findings can be applied to chronic PUA lowering effects on renal and cardiovascular outcomes in T1D patients. Placebo-controlled studies with

larger T1D-H cohorts are needed to determine whether PUA lowering can normalize hyperfiltration and thus, promote renal protection.

Chapter 6: Conclusions and Future Directions

It is of paramount importance to identify new risk biomarkers and safe, new agents that protect patients with T1D from initiation and progression of DN. Recognizing the limitations of extending observations in patients with type 2 diabetes, metabolic abnormalities and chronic kidney disease to a cohort of patients without complications, a major objective of the studies described in this thesis was to examine the role of baseline PUA in renal and cardiovascular function and to determine the effects of pharmacologic lowering of PUA in young patients with uncomplicated T1D. Although we originally hypothesized that PUA levels will be higher in T1D compared to HC, our first unexpected observation in Chapter 2 were the lower PUA levels in the early subclinical adolescent cohort with T1D compared to well matched HC (148). Despite being lower, such "suppressed" within normal range PUA levels negatively correlate with eGFR in adolescents with T1D. The absence of the association between PUA and blood pressure, FMD, PWV and ACR in adolescents with T1D suggests that PUA may not yet be associated with cardiovascular pathophysiology at an early stage of T1D.

In Chapter 3, our findings in the adolescent cohort with T1D were extended to a cohort of young adults with uncomplicated T1D. We originally hypothesized that even within the normal range PUA levels would be higher in young adults with T1D compared to HC and that the acute hyperglycemic stimulus, which promotes negative hemodynamic effects, would further increase baseline PUA levels. As in the adolescent cohort, adults with T1D had lower PUA levels, which were unexpectedly further decreased during hyperglycemia. By inducing glycosuria during euglycemic clamp conditions via SGLT2i for 8 weeks, we showed that it is the glycosuria, which stimulates increased PUA excretion, likely through the GLUT9 isoform 2 transporter on the apical membrane of the proximal tubule (85). We also observed that in contrast to adolescents with T1D, young adults with T1D had a positive correlation between PUA and systolic blood pressure suggesting the advent of the association between PUA and cardiovascular pathophysiology with longer diabetes duration.

In Chapter 4 of my thesis, similar to findings in adolescents with T1D, we showed that in young adults with T1D higher PUA levels were associated with lower GFR and lower ERPF, which appeared to be driven by increased resistance at the afferent renal arteriole (147). Our observation suggests that PUA may mediate increased tone or thickening of the afferent renal arteriole, which

could eventually potentiate renal injury by causing ischemia to the renal microcirculation. Alternatively, this association could be driven by increased renal clearance leading to lower PUA levels.

Our retrospective analyses in chapters 2 to 4 did not allow us to determine the causality of the association between PUA and renal and vascular parameters. Thus, for the final experimental chapter of this thesis we conducted a hypothesis generating, physiological study intended to determine the impact of PUA lowering on renal and vascular function in young uncomplicated adults with T1D. The main aims of this study were to investigate if PUA lowering with FBX treatment for 8 weeks modifies (1) the effect of hyperglycemia and infused angiotensin II on renal hemodynamic function, (2) systemic blood pressure and (3) arterial stiffness during clamped euglycemia and hyperglycemia in young uncomplicated adults with T1D compared to HC. We observed that PUA lowering with FBX for 8 weeks had a modest blood pressure lowering effect in young adult patients with uncomplicated T1D, which requires further investigation in larger cohort studies with longer PUA lowering treatment. Although in the overall cohort with T1D PUA lowering did not demonstrate significant changes to the renal hemodynamic function, our data suggests that it may normalize hyperfiltration, a mechanism thought to be involved in the early pathogenesis of renal function decline. Unfortunately, the small number of patients with T1D-H that were recruited into the study did not allow us to make a definitive conclusion regarding PUA lowering in patients with T1D-H and thus future studies with a larger T1D-H cohort and longer PUA lowering treatment duration are required to investigate our observation and determine how it can be applied to the clinical setting. PUA lowering enhanced the renal hyperfiltration response to clamped hyperglycemia through an increase in resistance at the efferent renal arteriole, but without impacting the RAAS, suggesting that PUA may augment the vasoconstrictor or vasodilatory mechanisms which mediate the renal response to hyperglycemia at the efferent arteriole. Future studies should examine whether PUA modulates other important mediators such as NO, endothelin-1, COX-2, vasopressin, prostaglandins or bradykinins, in response to hyperglycemia, thereby influencing renal hemodynamic responses to hyperglycemia after PUA lowering. Augmenting such important mediators that maintain intrarenal microcirculation, especially during hyperglycemia could potentially lead to increased hyperfiltration-mediated kidney injury due to PUA lowering. Thus, it is important to further study the mechanisms of such an observation to determine whether PUA lowering agents should be used with caution in patients

with suboptimal glycemic control or as concomitant therapy with medication that is intended to act on these mediators of intrarenal microcirculation.

Overall, we showed an association between PUA, blood pressure, GFR and ERPF in an adolescent and an adult cohort with T1D. Such observations are currently being extended to those with over 50 years of T1D duration in the ongoing Longevity study, which will allow us to compare observations in a range of patients with and without complications. Since our cross-sectional analyses included only uncomplicated patients with T1D, it does not allow us to extend our observations to the subset of patients that are most likely to develop diabetic nephropathy. To observe the progression of T1D in our adolescent cohort over 3 years, longitudinal observations will be made at the completion of the AdDIT trial. It is therefore not yet clear whether PUA is a biomarker of cardiovascular and renal function complications in patients with T1D, where the NIH definition of a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." (172) Finally, we were not able to determine whether PUA is a cause or a consequence of altered renal and cardiovascular hemodynamic function in uncomplicated T1D. Further studies are required to understand the causality of the association between PUA, GFR, ERPF and blood pressure in this population as well as to determine whether these associations persist in cohorts of even longer T1D duration, and in patients with microvascular or macrovascular complications.

Although acute PUA lowering was not associated with a decrease in resistance at the afferent renal arteriole or with blunted responses to hyperglycemia as was predicted from our retrospective analyses in Chapters 2 to 4, we showed some potentially beneficial effects of acute PUA lowering on blood pressure and hyperfiltration. Longitudinal outcome trials are required to determine whether our retrospective physiologic findings and those with acute PUA lowering can be applied to chronic PUA lowering effects on renal and cardiovascular outcomes in patients with T1D, especially in patient with hyperfiltration and other early adverse renal phenotype markers, such as albuminuria or decreased GFR. The promising effect of PUA lowering on renal protection in T1D with albuminuria and above normal PUA is already being examined as part of the NIH funded Protecting Early Renal Function Loss or "PERL" study (NCT02017171) (66). Trials examining the protective effects of PUA lowering in a cohort with an even earlier phenotype of renal function decline, such as those with microalbuminuria and preserved GFR or

normoalbuminuria and early GFR decline, would provide additional information beyond the data that will emerge from the PERL study. The outcomes of such trials have the potential to reduce the health risks associated with DM and to decrease the morbidity, mortality and social costs associated with T1D.

References

1. Lewis G, Maxwell AP. Risk factor control is key in diabetic nephropathy. The Practitioner. 2014;258(1768):13-7, 2.
2. de Boer IH. Kidney disease and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. Diabetes care. 2014;37(1):24-30.
3. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care. 2005;28(1):164-76.
4. Ali MK, Bullard KM, Saaddine JB, Cowie CC, Imperatore G, Gregg EW. Achievement of goals in U.S. diabetes care, 1999-2010. The New England journal of medicine. 2013;368(17):1613-24.
5. Sochett EB, Cherney DZ, Curtis JR, Dekker MG, Scholey JW, Miller JA. Impact of renin angiotensin system modulation on the hyperfiltration state in type 1 diabetes. Journal of the American Society of Nephrology : JASN. 2006;17(6):1703-9.
6. Cherney DZ, Scholey JW, Jiang S, Har R, Lai V, Sochett EB, et al. The effect of direct renin inhibition alone and in combination with ACE inhibition on endothelial function, arterial stiffness, and renal function in type 1 diabetes. Diabetes care. 2012;35(11):2324-30.
7. Stanton RC. Clinical challenges in diagnosis and management of diabetic kidney disease. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2014;63(2 Suppl 2):S3-21.
8. Filiopoulos V, Hadjiyannakos D, Vlassopoulos D. New Insights into Uric Acid Effects on the Progression and Prognosis of Chronic Kidney Disease. Ren Fail. 2012;34(4):510-20.
9. Yu MA, Sanchez-Lozada LG, Johnson RJ, Kang DH. Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction. J Hypertens. 2010;28(6):1234-42.
10. Wang DD, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Cozma AI, et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. The Journal of nutrition. 2012;142(5):916-23.
11. Bobulescu IA, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. Advances in chronic kidney disease. 2012;19(6):358-71.
12. Watanabe S, Kang DH, Feng L, Nakagawa T, Kanellis J, Lan H, et al. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. Hypertension. 2002;40(3):355-60.
13. Jalal DI, Chonchol M, Chen W, Targher G. Uric acid as a target of therapy in CKD. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2013;61(1):134-46.
14. Har R, Scholey JW, Daneman D, Mahmud FH, Dekker R, Lai V, et al. The effect of renal hyperfiltration on urinary inflammatory cytokines/chemokines in patients with uncomplicated type 1 diabetes mellitus. Diabetologia. 2013;56(5):1166-73.
15. Cherney DZ, Scholey JW, Sochett E, Bradley TJ, Reich HN. The acute effect of clamped hyperglycemia on the urinary excretion of inflammatory cytokines/chemokines in uncomplicated type 1 diabetes: a pilot study. Diabetes care. 2011;34(1):177-80.
16. Chaudhary K, Malhotra K, Sowers J, Aroor A. Uric Acid - Key Ingredient in the Recipe for Cardiorenal Metabolic Syndrome. Cardiorenal medicine. 2013;3(3):208-20.
17. Jalal DI, Maahs DM, Hovind P, Nakagawa T. Uric acid as a mediator of diabetic nephropathy. Seminars in nephrology. 2011;31(5):459-65.

18. Kirilmaz B, Asgun F, Alioglu E, Ercan E, Tengiz I, Turk U, et al. High inflammatory activity related to the number of metabolic syndrome components. Journal of clinical hypertension (Greenwich, Conn). 2010;12(2):136-44.
19. Griffith JW, Sun T, McIntosh MT, Bucala R. Pure Hemozoin is inflammatory in vivo and activates the NALP3 inflammasome via release of uric acid. Journal of immunology (Baltimore, Md : 1950). 2009;183(8):5208-20.
20. Talaat KM, el-Sheikh AR. The effect of mild hyperuricemia on urinary transforming growth factor beta and the progression of chronic kidney disease. American journal of nephrology. 2007;27(5):435-40.
21. Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y, et al. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. The Journal of clinical investigation. 1997;99(1):144-50.
22. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? Free radical biology & medicine. 2002;33(6):774-97.
23. Cantu-Medellin N, Kelley EE. Xanthine oxidoreductase-catalyzed reactive species generation: A process in critical need of reevaluation. Redox biology. 2013;1(1):353-8.
24. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am J Physiol Regul Integr Comp Physiol. 2003;284(1):R1-12.
25. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, et al. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes. 2013;62(10):3307-15.
26. Miller JA. Impact of hyperglycemia on the renin angiotensin system in early human type 1 diabetes mellitus. Journal of the American Society of Nephrology : JASN. 1999;10(8):1778-85.
27. Gordin D, Ronnback M, Forsblom C, Makinen V, Saraheimo M, Groop PH. Glucose variability, blood pressure and arterial stiffness in type 1 diabetes. Diabetes Res Clin Pract. 2008;80(3):e4-7.
28. Lee IK, Kim HS, Bae JH. Endothelial dysfunction: its relationship with acute hyperglycaemia and hyperlipidemia. Int J Clin Pract Suppl. 2002(129):59-64.
29. Kanbay M, Yilmaz MI, Sonmez A, Turgut F, Saglam M, Cakir E, et al. Serum uric acid level and endothelial dysfunction in patients with nondiabetic chronic kidney disease. American journal of nephrology. 2011;33(4):298-304.
30. Erdogan D, Gullu H, Caliskan M, Yildirim E, Bilgi M, Ulus T, et al. Relationship of serum uric acid to measures of endothelial function and atherosclerosis in healthy adults. Int J Clin Pract. 2005;59(11):1276-82.
31. Tsai WC, Huang YY, Lin CC, Li WT, Lee CH, Chen JY, et al. Uric acid is an independent predictor of arterial stiffness in hypertensive patients. Heart Vessels. 2009;24(5):371-5.
32. Kanbay M, Segal M, Afsar B, Kang DH, Rodriguez-Iturbe B, Johnson RJ. The role of uric acid in the pathogenesis of human cardiovascular disease. Heart (British Cardiac Society). 2013;99(11):759-66.
33. Syme H. Hypertension in small animal kidney disease. The Veterinary clinics of North America Small animal practice. 2011;41(1):63-89.
34. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiological reviews. 2007;87(1):245-313.
35. Zatz R, Meyer TW, Rennke HG, Brenner BM. Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. Proceedings of the National Academy of Sciences of the United States of America. 1985;82(17):5963-7.
36. Cherney DZ, Scholey JW, Miller JA. Insights into the regulation of renal hemodynamic function in diabetic mellitus. Curr Diabetes Rev. 2008;4(4):280-90.

37. Cherney DZ, Lai V, Scholey JW, Miller JA, Zinman B, Reich HN. Effect of direct renin inhibition on renal hemodynamic function, arterial stiffness, and endothelial function in humans with uncomplicated type 1 diabetes: a pilot study. Diabetes care. 2010;33(2):361-5.
38. Cherney DZ, Reich HN, Scholey JW, Lai V, Slorach C, Zinman B, et al. Systemic hemodynamic function in humans with type 1 diabetes treated with protein kinase Cbeta inhibition and renin-angiotensin system blockade: a pilot study. Canadian journal of physiology and pharmacology. 2012;90(1):113-21.
39. Cherney DZI, Scholey JW, Shannon J, Har H, Lai V, Sochett EB, et al. The Effect of Direct Renin Inhibition Alone and in Combination with ACE Inhibition on Endothelial Function, Arterial Stiffness and Renal Function in Type 1 Diabetes Mellitus. Diabetes Care. 2012;in press.
40. Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension. 2001;38(5):1101-6.
41. Mazzali M, Kanellis J, Han L, Feng L, Xia YY, Chen Q, et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. American journal of physiology Renal physiology. 2002;282(6):F991-7.
42. Perlstein TS, Gumieniak O, Hopkins PN, Murphey LJ, Brown NJ, Williams GH, et al. Uric acid and the state of the intrarenal renin-angiotensin system in humans. Kidney international. 2004;66(4):1465-70.
43. Messerli FH, Frohlich ED, Dreslinski GR, Suarez DH, Aristimuno GG. Serum uric acid in essential hypertension: an indicator of renal vascular involvement. Annals of internal medicine. 1980;93(6):817-21.
44. Portugal-Cohen M, Kohen R. Exposure of human keratinocytes to ischemia, hyperglycemia and their combination induces oxidative stress via the enzymes inducible nitric oxide synthase and xanthine oxidase. J Dermatol Sci. 2009;55(2):82-90.
45. Cherney DZI, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, et al. The Renal Hemodynamic Effect of SGLT2 Inhibition in Patients with Type 1 Diabetes. Circulation. 2014;129:587-97.
46. Cherney DZ, Miller JA, Scholey JW, Nasrallah R, Hebert RL, Dekker MG, et al. Renal hyperfiltration is a determinant of endothelial function responses to cyclooxygenase 2 inhibition in type 1 diabetes. Diabetes care. 2010;33(6):1344-6.
47. Cherney DZ, Sochett EB. Evolution of renal hyperfiltration and arterial stiffness from adolescence into early adulthood in type 1 diabetes. Diabetes care. 2011;34(8):1821-6.
48. Cherney DZ, Sochett EB, Lai V, Dekker MG, Slorach C, Scholey JW, et al. Renal hyperfiltration and arterial stiffness in humans with uncomplicated type 1 diabetes. Diabetes care. 2010;33(9):2068-70.
49. Sanchez-Lozada LG, Tapia E, Soto V, Avila-Casado C, Franco M, Zhao L, et al. Treatment with the xanthine oxidase inhibitor febuxostat lowers uric acid and alleviates systemic and glomerular hypertension in experimental hyperuricaemia. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2008;23(4):1179-85.
50. Wang C, Pan Y, Zhang QY, Wang FM, Kong LD. Quercetin and allopurinol ameliorate kidney injury in STZ-treated rats with regulation of renal NLRP3 inflammasome activation and lipid accumulation. PloS one. 2012;7(6):e38285.
51. Kim SM, Choi YW, Seok HY, Jeong KH, Lee SH, Lee TW, et al. Reducing Serum Uric Acid Attenuates TGF-beta(1)-Induced Profibrogenic Progression in Type 2 Diabetic Nephropathy. Nephron Experimental nephrology. 2013;121(3-4):e108-e20.

52. Cherney DZ, Sochett EB, Dekker MG, Perkins BA. Ability of cystatin C to detect acute changes in glomerular filtration rate provoked by hyperglycaemia in uncomplicated Type 1 diabetes. Diabetic medicine : a journal of the British Diabetic Association. 2010;27(12):1358-65.
53. Hovind P, Rossing P, Tarnow L, Johnson RJ, Parving HH. Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes: an inception cohort study. Diabetes. 2009;58(7):1668-71.
54. Rosolowsky ET, Ficociello LH, Maselli NJ, Niewczas MA, Binns AL, Roshan B, et al. High-normal serum uric acid is associated with impaired glomerular filtration rate in nonproteinuric patients with type 1 diabetes. Clinical journal of the American Society of Nephrology : CJASN. 2008;3(3):706-13.
55. Ficociello LH, Rosolowsky ET, Niewczas MA, Maselli NJ, Weinberg JM, Aschengrau A, et al. High-normal serum uric acid increases risk of early progressive renal function loss in type 1 diabetes: results of a 6-year follow-up. Diabetes care. 2010;33(6):1337-43.
56. Perkins BA, Ficociello LH, Ostrander BE, Silva KH, Weinberg J, Warram JH, et al. Microalbuminuria and the risk for early progressive renal function decline in type 1 diabetes. Journal of the American Society of Nephrology : JASN. 2007;18(4):1353-61.
57. Perkins BA, Krolewski AS. Early nephropathy in type 1 diabetes: the importance of early renal function decline. Current opinion in nephrology and hypertension. 2009;18(3):233-40.
58. Jalal DI, Rivard CJ, Johnson RJ, Maahs DM, McFann K, Rewers M, et al. Serum uric acid levels predict the development of albuminuria over 6 years in patients with type 1 diabetes: findings from the Coronary Artery Calcification in Type 1 Diabetes study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(6):1865-9.
59. Zoppini G, Targher G, Chonchol M, Ortalda V, Abaterusso C, Pichiri I, et al. Serum uric acid levels and incident chronic kidney disease in patients with type 2 diabetes and preserved kidney function. Diabetes care. 2012;35(1):99-104.
60. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2006;47(1):51-9.
61. Goicoechea M, de Vinuesa SG, Verdalles U, Ruiz-Caro C, Ampuero J, Rincon A, et al. Effect of allopurinol in chronic kidney disease progression and cardiovascular risk. Clinical journal of the American Society of Nephrology : CJASN. 2010;5(8):1388-93.
62. Whelton A, Macdonald PA, Zhao L, Hunt B, Gunawardhana L. Renal function in gout: long-term treatment effects of febuxostat. Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases. 2011;17(1):7-13.
63. Miao Y, Ottenbros SA, Laverman GD, Brenner BM, Cooper ME, Parving HH, et al. Effect of a reduction in uric acid on renal outcomes during losartan treatment: a post hoc analysis of the reduction of endpoints in non-insulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan Trial. Hypertension. 2011;58(1):2-7.
64. Momeni A, Shahidi S, Seirafian S, Taheri S, Kheiri S. Effect of allopurinol in decreasing proteinuria in type 2 diabetic patients. Iran J Kidney Dis. 2010;4(2):128-32.
65. Bose B, Badve SV, Hiremath SS, Boudville N, Brown FG, Cass A, et al. Effects of uric acid-lowering therapy on renal outcomes: a systematic review and meta-analysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2014;29(2):406-13.
66. Maahs DM, Caramori L, Cherney DZ, Galecki AT, Gao C, Jalal D, et al. Uric Acid Lowering to Prevent Kidney Function Loss in Diabetes: The Preventing Early Renal Function Loss (PERL) Allopurinol Study. Current diabetes reports. 2013;13(4):550-9.

67. Feig DI, Madero M, Jalal DI, Sanchez-Lozada LG, Johnson RJ. Uric acid and the origins of hypertension. The Journal of pediatrics. 2013;162(5):896-902.
68. Cannon PJ, Stason WB, Demartini FE, Sommers SC, Laragh JH. Hyperuricemia in primary and renal hypertension. The New England journal of medicine. 1966;275(9):457-64.
69. Lu Z, Dong B, Wu H, Chen T, Zhang Y, Wu J, et al. Serum uric acid level in primary hypertension among Chinese nonagenarians/centenarians. Journal of human hypertension. 2009;23(2):113-21.
70. Bjornstad P, Paul Wadwa R, Sirota JC, Snell-Bergeon JK, McFann K, Rewers M, et al. Serum uric acid and hypertension in adults: a paradoxical relationship in type 1 diabetes. Journal of clinical hypertension (Greenwich, Conn). 2014;16(4):283-8.
71. Ciarla S, Struglia M, Giorgini P, Striuli R, Necozione S, Properzi G, et al. Serum uric acid levels and metabolic syndrome. Arch Physiol Biochem. 2014;120(3):119-22.
72. Cherney DZ, Reich HN, Miller JA, Lai V, Zinman B, Dekker MG, et al. Age is a determinant of acute hemodynamic responses to hyperglycemia and angiotensin II in humans with uncomplicated type 1 diabetes mellitus. Am J Physiol Regul Integr Comp Physiol. 2010;299(1):R206-14.
73. Feig DI, Rodriguez-Iturbe B, Nakagawa T, Johnson RJ. Nephron number, uric acid, and renal microvascular disease in the pathogenesis of essential hypertension. Hypertension. 2006;48(1):25-6.
74. Kao MP, Ang DS, Gandy SJ, Nadir MA, Houston JG, Lang CC, et al. Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease. Journal of the American Society of Nephrology : JASN. 2011;22(7):1382-9.
75. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. Circulation. 2006;114(23):2508-16.
76. Higgins P, Dawson J, Lees KR, McArthur K, Quinn TJ, Walters MR. Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. Cardiovascular therapeutics. 2012;30(4):217-26.
77. Waring WS, McKnight JA, Webb DJ, Maxwell SR. Lowering serum urate does not improve endothelial function in patients with type 2 diabetes. Diabetologia. 2007;50(12):2572-9.
78. Kanbay M, Huddam B, Azak A, Solak Y, Kadioglu GK, Kirbas I, et al. A randomized study of allopurinol on endothelial function and estimated glomular filtration rate in asymptomatic hyperuricemic subjects with normal renal function. Clinical journal of the American Society of Nephrology : CJASN. 2011;6(8):1887-94.
79. Hoieggen A, Alderman MH, Kjeldsen SE, Julius S, Devereux RB, De Faire U, et al. The impact of serum uric acid on cardiovascular outcomes in the LIFE study. Kidney international. 2004;65(3):1041-9.
80. Lytvyn Y, Perkins BA, Cherney DZ. Uric acid as a biomarker and a therapeutic target in diabetes. Can J Diabetes. 2015;39(3):239-46.
81. Ford ES, Li C, Cook S, Choi HK. Serum concentrations of uric acid and the metabolic syndrome among US children and adolescents. Circulation. 2007;115(19):2526-32.
82. Civantos Modino S, Guijarro de Armas MG, Monereo Mejias S, Montano Martinez JM, Iglesias Bolanos P, Merino Viveros M, et al. Hyperuricemia and metabolic syndrome in children with overweight and obesity. Endocrinologia y nutricion : organo de la Sociedad Espanola de Endocrinologia y Nutricion. 2012;59(9):533-8.
83. Pacifico L, Cantisani V, Anania C, Bonaiuto E, Martino F, Pascone R, et al. Serum uric acid and its association with metabolic syndrome and carotid atherosclerosis in obese children. European journal of endocrinology / European Federation of Endocrine Societies. 2009;160(1):45-52.

84. Valle M, Martos R, Canete MD, Valle R, van Donkelaar EL, Bermudo F, et al. Association of serum uric acid levels to inflammation biomarkers and endothelial dysfunction in obese prepubertal children. Pediatric diabetes. 2014.
85. Lytvyn Y, Skrtic M, Yang GK, Yip PM, Perkins BA, Cherney DZ. Glycosuria-mediated urinary uric acid excretion in patients with uncomplicated type 1 diabetes mellitus. American journal of physiology Renal physiology. 2015;308(2):F77-83.
86. Lytvyn Y, Škrtić M, Yang GK, Lai V, Scholey JW, Yip PM, et al. Plasma Uric Acid Effect on Glomerular Hemodynamic Profile of Patients with Uncomplicated Type 1 Diabetes Mellitus. Diabetic Medicine. 2015;In Press.
87. Adolescent type 1 Diabetes Cardio-renal Intervention Trial (AdDIT). BMC Pediatr. 2009;9:79.
88. Dunger DB, Schwarze CP, Cooper JD, Widmer B, Neil HA, Shield J, et al. Can we identify adolescents at high risk for nephropathy before the development of microalbuminuria? Diabetic medicine : a journal of the British Diabetic Association. 2007;24(2):131-6.
89. Schultz CJ, Konopelska-Bahu T, Dalton RN, Carroll TA, Stratton I, Gale EA, et al. Microalbuminuria prevalence varies with age, sex, and puberty in children with type 1 diabetes followed from diagnosis in a longitudinal study. Oxford Regional Prospective Study Group. Diabetes care. 1999;22(3):495-502.
90. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. Scandinavian journal of clinical and laboratory investigation. 2004;64(1):25-30.
91. Har RL, Reich HN, Scholey JW, Daneman D, Dunger DB, Moineddin R, et al. The urinary cytokine/chemokine signature of renal hyperfiltration in adolescents with type 1 diabetes. PloS one. 2014;9(11):e111131.
92. Skrtic M, Lytvyn Y, Yang GK, Yip P, Lai V, Silverman M, et al. Glomerular haemodynamic profile of patients with Type 1 diabetes compared with healthy control subjects. Diabetic medicine : a journal of the British Diabetic Association. 2015.
93. Cherney DZ, Reich HN, Jiang S, Har R, Nasrallah R, Hebert RL, et al. Hyperfiltration and the effect of nitric oxide inhibition on renal and endothelial function in humans with uncomplicated type 1 diabetes mellitus. Am J Physiol Regul Integr Comp Physiol. 2012;303(7):R710-8.
94. Kostraba JN, Dorman JS, Orchard TJ, Becker DJ, Ohki Y, Ellis D, et al. Contribution of diabetes duration before puberty to development of microvascular complications in IDDM subjects. Diabetes care. 1989;12(10):686-93.
95. Lawson ML, Sochett EB, Chait PG, Balfe JW, Daneman D. Effect of puberty on markers of glomerular hypertrophy and hypertension in IDDM. Diabetes. 1996;45(1):51-5.
96. Bjornstad P, Maahs DM, Rivard CJ, Pyle L, Rewers M, Johnson RJ, et al. Serum uric acid predicts vascular complications in adults with type 1 diabetes: the coronary artery calcification in type 1 diabetes study. Acta Diabetol. 2014;51(5):783-91.
97. Palmer TM, Nordestgaard BG, Benn M, Tybjaerg-Hansen A, Davey Smith G, Lawlor DA, et al. Association of plasma uric acid with ischaemic heart disease and blood pressure: mendelian randomisation analysis of two large cohorts. BMJ (Clinical research ed). 2013;347:f4262.
98. Bjornstad P, Snell-Bergeon JK, McFann K, Wadwa RP, Rewers M, Rivard CJ, et al. Serum uric acid and insulin sensitivity in adolescents and adults with and without type 1 diabetes. Journal of diabetes and its complications. 2014;28(3):298-304.
99. Chino Y, Samukawa Y, Sakai S, Nakai Y, Yamaguchi JI, Nakanishi T, et al. SGLT2 inhibitor lowers serum uric acid through alteration of uric acid transport activity in renal tubule by increased glycosuria. Biopharmaceutics & drug disposition. 2014.

100. Krolewski AS, Niewczas MA, Skupien J, Gohda T, Smiles A, Eckfeldt JH, et al. Early progressive renal decline precedes the onset of microalbuminuria and its progression to macroalbuminuria. Diabetes care. 2014;37(1):226-34.
101. Quattrin T, Waz WR, Duffy LC, Sheldon MW, Campos SP, Albini CH, et al. Microalbuminuria in an adolescent cohort with insulin-dependent diabetes mellitus. Clinical pediatrics. 1995;34(1):12-7.
102. Loeffler LF, Navas-Acien A, Brady TM, Miller ER, 3rd, Fadrowski JJ. Uric acid level and elevated blood pressure in US adolescents: National Health and Nutrition Examination Survey, 1999-2006. Hypertension. 2012;59(4):811-7.
103. Odutayo A, Cherney D. Cystatin C and acute changes in glomerular filtration rate. Clinical nephrology. 2012;78(1):64-75.
104. Perkins BA, Sochett EB, Cherney DZ. Ability of Cystatin C to detect changes in glomerular filtration rate after ACE inhibition in patients with uncomplicated type 1 diabetes. Clinical and experimental hypertension (New York, NY : 1993). 2012;34(8):606-11.
105. Jones SL, Kontessis P, Wiseman M, Dodds R, Bognetti E, Pinto J, et al. Protein intake and blood glucose as modulators of GFR in hyperfiltering diabetic patients. Kidney international. 1992;41(6):1620-8.
106. Cherney DZI, Miller JA, Scholey JW, Bradley TJ, Slorach C, Curtis JR, et al. The effect of cyclooxygenase-2 inhibition on renal hemodynamic function in humans with type 1 diabetes. Diabetes. 2008;57(3):688-95.
107. Cherney DZ, Miller JA, Scholey JW, Bradley TJ, Slorach C, Curtis JR, et al. The effect of cyclooxygenase-2 inhibition on renal hemodynamic function in humans with type 1 diabetes. Diabetes. 2008;57(3):688-95.
108. Cherney DZI, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation. 2014;129(5):587-97.
109. Florijn KW, Barendregt JN, Lentjes EG, van Dam W, Prodjosudjadi W, van Saase JL, et al. Glomerular filtration rate measurement by "single-shot" injection of inulin. Kidney international. 1994;46(1):252-9.
110. Yang GK, Maahs DM, Perkins B, Cherney DZI. Renal Hyperfiltration and Systemic Blood Pressure in Patients with Uncomplicated Type 1 Diabetes Mellitus. PloS one. 2013;8(7):e68908.
111. Cherney DZ, Perkins BA. Sodium-Glucose Cotransporter 2 Inhibition in Type 1 Diabetes: Simultaneous Glucose Lowering and Renal Protection? Can J Diabetes. 2014.
112. Wilding JP, Ferrannini E, Fonseca VA, Wilpshaar W, Dhanjal P, Houzer A. Efficacy and safety of ipragliflozin in patients with type 2 diabetes inadequately controlled on metformin: a dose-finding study. Diabetes, obesity & metabolism. 2013;15(5):403-9.
113. Ferrannini E, Seman L, Seewaldt-Becker E, Hantel S, Pinnetti S, Woerle HJ. A Phase IIb, randomized, placebo-controlled study of the SGLT2 inhibitor empagliflozin in patients with type 2 diabetes. Diabetes, obesity & metabolism. 2013;15(8):721-8.
114. Lytvyn Y, Perkins BA, Cherney DZI. Uric Acid as a Biomarker and a Therapeutic Target in Diabetes. Canadian Journal of Diabetes. 2014;In Press.
115. Skeith MD, Healey LA, Cutler RE. Effect of phloridzin on uric acid excretion in man. The American journal of physiology. 1970;219(4):1080-2.
116. Bonsnes RW, Dana ES. On the increased uric acid clearance following the intravenous infusion of hypertonic glucose solutions. The Journal of clinical investigation. 1946;25:386-8.
117. Choi HK, Ford ES. Haemoglobin A1c, fasting glucose, serum C-peptide and insulin resistance in relation to serum uric acid levels--the Third National Health and Nutrition Examination Survey. Rheumatology (Oxford, England). 2008;47(5):713-7.

118. Herman JB, Goldbourt U. Uric acid and diabetes: observations in a population study. Lancet. 1982;2(8292):240-3.
119. Gonzalez-Sicilia L, Garcia-Estan J, Martinez-Blazquez A, Fernandez-Pardo J, Quiles JL, Hernandez J. Renal metabolism of uric acid in type I insulin-dependent diabetic patients: relation to metabolic compensation. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 1997;29(10):520-3.
120. Quinones Galvan A, Natali A, Baldi S, Frascerra S, Sanna G, Ciociaro D, et al. Effect of insulin on uric acid excretion in humans. The American journal of physiology. 1995;268(1 Pt 1):E1-5.
121. Gotfredsen A, McNair P, Christiansen C, Transbol I. Renal hypouricaemia in insulin treated diabetes mellitus. Clinica chimica acta; international journal of clinical chemistry. 1982;120(3):355-61.
122. Rodriguez G, Soriano LC, Choi HK. Impact of diabetes against the future risk of developing gout. Annals of the rheumatic diseases. 2010;69(12):2090-4.
123. Anzai N, Ichida K, Jutabha P, Kimura T, Babu E, Jin CJ, et al. Plasma urate level is directly regulated by a voltage-driven urate efflux transporter URATv1 (SLC2A9) in humans. The Journal of biological chemistry. 2008;283(40):26834-8.
124. Augustin R, Carayannopoulos MO, Dowd LO, Phay JE, Moley JF, Moley KH. Identification and characterization of human glucose transporter-like protein-9 (GLUT9): alternative splicing alters trafficking. The Journal of biological chemistry. 2004;279(16):16229-36.
125. Hagos Y, Stein D, Ugele B, Burckhardt G, Bahn A. Human renal organic anion transporter 4 operates as an asymmetric urate transporter. Journal of the American Society of Nephrology : JASN. 2007;18(2):430-9.
126. Bahn A, Hagos Y, Reuter S, Balen D, Brzica H, Krick W, et al. Identification of a new urate and high affinity nicotinate transporter, hOAT10 (SLC22A13). The Journal of biological chemistry. 2008;283(24):16332-41.
127. Kimura T, Takahashi M, Yan K, Sakurai H. Expression of SLC2A9 isoforms in the kidney and their localization in polarized epithelial cells. PloS one. 2014;9(1):e84996.
128. Weir MR, Kline I, Xie J, Edwards R, Usiskin K. Effect of canagliflozin on serum electrolytes in patients with type 2 diabetes in relation to estimated glomerular filtration rate (eGFR). Current medical research and opinion. 2014;30(9):1759-68.
129. Kang DH, Nakagawa T, Feng L, Watanabe S, Han L, Mazzali M, et al. A role for uric acid in the progression of renal disease. Journal of the American Society of Nephrology : JASN. 2002;13(12):2888-97.
130. Hill GS, Heudes D, Bariety J. Morphometric study of arterioles and glomeruli in the aging kidney suggests focal loss of autoregulation. Kidney international. 2003;63(3):1027-36.
131. Sanchez-Lozada LG, Tapia E, Avila-Casado C, Soto V, Franco M, Santamaria J, et al. Mild hyperuricemia induces glomerular hypertension in normal rats. American journal of physiology Renal physiology. 2002;283(5):F1105-10.
132. Gomez DM. Evaluation of renal resistances, with special reference to changes in essential hypertension. The Journal of clinical investigation. 1951;30(10):1143-55.
133. Tsuda A, Ishimura E, Ohno Y, Ichii M, Nakatani S, Mori K, et al. Significant association of poor glycemic control with increased resistance in efferent arterioles--study of inulin and para-aminohippuric acid clearance in humans. Diabetes Res Clin Pract. 2014;104(2):234-40.
134. Ott C, Schneider MP, Raff U, Ritt M, Striepe K, Alberici M, et al. Effects of manidipine vs. amlodipine on intrarenal haemodynamics in patients with arterial hypertension. Br J Clin Pharmacol. 2013;75(1):129-35.

135. Tsuda A, Inaba M, Ichii M, Ochi A, Ohno Y, Nakatani S, et al. Relationship between serum TSH levels and intrarenal hemodynamic parameters in euthyroid subjects. European journal of endocrinology / European Federation of Endocrine Societies. 2013;169(1):45-50.
136. Skrtic M, Yang GK, Perkins BA, Soleymanlou N, Lytvyn Y, von Eynatten M, et al. Characterisation of glomerular haemodynamic responses to SGLT2 inhibition in patients with type 1 diabetes and renal hyperfiltration. Diabetologia. 2014;57(12):2599-602.
137. Miller JA, Cherney DZ, Duncan JA, Lai V, Burns KD, Kennedy CR, et al. Gender differences in the renal response to Renin-Angiotensin system blockade. Journal of the American Society of Nephrology : JASN. 2006;17(9):2554-60.
138. Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. Kidney international. 1981;19(3):410-5.
139. Ferris TF, Gorden P. Effect of angiotensin and norepinephrine upon urate clearance in man. The American journal of medicine. 1968;44(3):359-65.
140. Kohagura K, Kochi M, Miyagi T, Kinjyo T, Maehara Y, Nagahama K, et al. An association between uric acid levels and renal arteriolopathy in chronic kidney disease: a biopsy-based study. Hypertension research : official journal of the Japanese Society of Hypertension. 2013;36(1):43-9.
141. Brodsky SV, Yamamoto T, Tada T, Kim B, Chen J, Kajiya F, et al. Endothelial dysfunction in ischemic acute renal failure: rescue by transplanted endothelial cells. American journal of physiology Renal physiology. 2002;282(6):F1140-9.
142. Welch WJ, Wilcox CS, Thomson SC. Nitric oxide and tubuloglomerular feedback. Seminars in nephrology. 1999;19(3):251-62.
143. Fine LG, Orphanides C, Norman JT. Progressive renal disease: the chronic hypoxia hypothesis. Kidney international Supplement. 1998;65:S74-8.
144. Epstein FH, Brezis M, Silva P, Rosen S. Physiological and clinical implications of medullary hypoxia. Artificial organs. 1987;11(6):463-7.
145. Ejaz AA, Mu W, Kang DH, Roncal C, Sautin YY, Henderson G, et al. Could uric acid have a role in acute renal failure? Clinical journal of the American Society of Nephrology : CJASN. 2007;2(1):16-21.
146. Bjornstad P, Roncal C, Milagres T, Pyle L, Lanaspa MA, Bishop FK, et al. Hyperfiltration and uricosuria in adolescents with type 1 diabetes. Pediatric nephrology (Berlin, Germany). 2016;31(5):787-93.
147. Lytvyn Y, Skrtic M, Yang GK, Lai V, Scholey JW, Yip PM, et al. Plasma uric acid effects on glomerular haemodynamic profile of patients with uncomplicated Type 1 diabetes mellitus. Diabetic medicine : a journal of the British Diabetic Association. 2015.
148. Lytvyn Y, Mahmud FH, Daneman D, Deda L, Dunger DB, Deanfield J, et al. Association Between Plasma Uric Acid Levels and Cardiorenal Function in Adolescents With Type 1 Diabetes. Diabetes care. 2016.
149. Feig DI, Kang DH, Nakagawa T, Mazzali M, Johnson RJ. Uric acid and hypertension. Curr Hypertens Rep. 2006;8(2):111-5.
150. Feig DI, Mazzali M, Kang DH, Nakagawa T, Price K, Kannelis J, et al. Serum uric acid: a risk factor and a target for treatment? Journal of the American Society of Nephrology : JASN. 2006;17(4 Suppl 2):S69-73.
151. Alper AB, Jr., Chen W, Yau L, Srinivasan SR, Berenson GS, Hamm LL. Childhood uric acid predicts adult blood pressure: the Bogalusa Heart Study. Hypertension. 2005;45(1):34-8.
152. Sundstrom J, Sullivan L, D'Agostino RB, Levy D, Kannel WB, Vasan RS. Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. Hypertension. 2005;45(1):28-33.

153. Cherney D, Reich H, Lai V, Hebert RL, Nasrallah R, Jiang S, et al. Hyperfiltration and the effect of nitric oxide inhibition on renal hemodynamic and endothelial function in humans with uncomplicated type 1 diabetes mellitus. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2012;August 1.
154. Cherney DZ, Lai V, Miller JA, Scholey JW, Reich HN. The angiotensin II receptor type 2 polymorphism influences haemodynamic function and circulating RAS mediators in normotensive humans. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(12):4093-6.
155. Cherney DZ, Scholey JW, Cattran DC, Kang AK, Zimpelmann J, Kennedy C, et al. The effect of oral contraceptives on the nitric oxide system and renal function. American journal of physiology Renal physiology. 2007;293(5):F1539-44.
156. Cherney DZ, Scholey JW, Nasrallah R, Dekker MG, Slorach C, Bradley TJ, et al. Renal hemodynamic effect of cyclooxygenase 2 inhibition in young men and women with uncomplicated type 1 diabetes mellitus. American journal of physiology Renal physiology. 2008;294(6):F1336-41.
157. Cherney DZ, Scholey JW, Zhou J, Zimpelmann J, Kennedy C, Burns KD, et al. Endothelial nitric oxide synthase gene polymorphisms and the renal hemodynamic response to L-arginine. Kidney international. 2009;75(3):327-32.
158. Cherney DZ, Sochett EB, Miller JA. Gender differences in renal responses to hyperglycemia and angiotensin-converting enzyme inhibition in diabetes. Kidney international. 2005;68(4):1722-8.
159. Cherney DZ, Konvalinka A, Zinman B, Diamandis EP, Soosaipillai A, Reich H, et al. Effect of protein kinase Cbeta inhibition on renal hemodynamic function and urinary biomarkers in humans with type 1 diabetes: a pilot study. Diabetes care. 2009;32(1):91-3.
160. Miller JA, Curtis JR, Sochett EB. Relationship between diurnal blood pressure, renal hemodynamic function, and the renin-angiotensin system in type 1 diabetes. Diabetes. 2003;52(7):1806-11.
161. Butler R, Morris AD, Belch JJ, Hill A, Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. Hypertension. 2000;35(3):746-51.
162. Malik UZ, Hundley NJ, Romero G, Radi R, Freeman BA, Tarpey MM, et al. Febuxostat inhibition of endothelial-bound XO: implications for targeting vascular ROS production. Free radical biology & medicine. 2011;51(1):179-84.
163. Nishino T, Okamoto K, Kawaguchi Y, Hori H, Matsumura T, Eger BT, et al. Mechanism of the conversion of xanthine dehydrogenase to xanthine oxidase: identification of the two cysteine disulfide bonds and crystal structure of a non-convertible rat liver xanthine dehydrogenase mutant. The Journal of biological chemistry. 2005;280(26):24888-94.
164. Okamoto K, Eger BT, Nishino T, Kondo S, Pai EF, Nishino T. An extremely potent inhibitor of xanthine oxidoreductase. Crystal structure of the enzyme-inhibitor complex and mechanism of inhibition. The Journal of biological chemistry. 2003;278(3):1848-55.
165. Okamoto K, Eger BT, Nishino T, Pai EF, Nishino T. Mechanism of inhibition of xanthine oxidoreductase by allopurinol: crystal structure of reduced bovine milk xanthine oxidoreductase bound with oxipurinol. Nucleosides, nucleotides & nucleic acids. 2008;27(6):888-93.
166. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. Diabetologia. 2009;52(4):691-7.
167. Tripepi G, Jager KJ, Dekker FW, Wanner C, Zoccali C. Bias in clinical research. Kidney international. 2008;73(2):148-53.

168. Saito I, Saruta T, Kondo K, Nakamura R, Oguro T, Yamagami K, et al. Serum uric acid and the renin-angiotensin system in hypertension. Journal of the American Geriatrics Society. 1978;26(6):241-7.
169. Milicevic Z, Jelakovic B, Marinkovic M. [Hyperuricemia and plasma renin activity in essential arterial hypertension]. Lijec Vjesn. 1994;116(1-2):14-7.
170. Zhou X, Matavelli L, Frohlich ED. Uric acid: its relationship to renal hemodynamics and the renal renin-angiotensin system. Curr Hypertens Rep. 2006;8(2):120-4.
171. Verdecchia P, Schillaci G, Reboldi G, Santeusanio F, Porcellati C, Brunetti P. Relation between serum uric acid and risk of cardiovascular disease in essential hypertension. The PIUMA study. Hypertension. 2000;36(6):1072-8.
172. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical pharmacology and therapeutics. 2001;69(3):89-95.