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Elevated plasma cyclic guanosine monophosphate may explain greater efferent arteriolar tone in adults with longstanding type 1 diabetes: A brief report

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ABSTRACT

Cyclic guanosine monophosphate (cGMP) influences intrarenal hemodynamics in animal models, but the relationship between cGMP and renal function in adults with type 1 diabetes (T1D) remains unclear. In this study, plasma cGMP correlated with efferent arteriolar resistance, effective renal plasma flow, and renal vascular resistance in adults with T1D.

1. Introduction

Longstanding type 1 diabetes is associated with increased afferent (R_A) and efferent arteriolar (R_E) tone with decreased glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), which relate to exaggerated renin-angiotensin-aldosterone system (RAAS) activation.[1] Cyclic guanosine monophosphate (cGMP) is also thought to play a role in regulating intrarenal hemodynamic function.[2] In response to natriuretic peptide (NP) and nitric oxide (NO) activation, cGMP is synthesized by particulate (pGC) and soluble (sGC) guanylyl cyclases, respectively.[3] While both sGC and pGC activation increase intracellular concentrations of cGMP, pGC activation accounts for the majority of the circulating cGMP.[3,4] In the NP/pGC/cGMP pathway, NP receptor A (NPR-A) and B (NRP—B) are activated by all three NPs with resultant increased circulating cGMP. Animal data suggest that activation of NPR-A results in increased cGMP, triggering R_A vasodilation and R_E

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Table 1
Clinical and biochemical characteristics of the study participants.

	Controls $n = 73$	DKD resistors $n = 44$	DKD $n = 22$	P for trend	P for controls vs DKD resistors	P for DKD resistors vs DKD
Clinical characteristics						
Sex M/F	32/41	22/22	8/14	0.57	0.52	0.29
Age (years)	65 ± 8	65 ± 7	68 ± 8	0.18	0.73	0.08
Duration type 1 diabetes (yr)	–	55 ± 6	55 ± 5	–	–	0.92
Weight (kg)	75.7 ± 16.2	73.3 ± 12.5	73.1 ± 12.1	0.60	0.39	0.95
BMI (kg/m^2)	27.2 ± 5.5	26.4 ± 3.5	27.0 ± 4.8	0.65	0.36	0.61
RAAS inhibition	10 (14%)	34 (78%)	20 (91%)	<0.001	<0.001	0.18
SBP (mmHg)	129 ± 19	133 ± 16	134 ± 14	0.31	0.24	0.73
DBP (mmHg)	79 ± 10	71 ± 10	69 ± 9	<0.001	<0.001	0.37
Neurohormonal markers						
cGMP (pmol/L)	4.2 (3.1–5.8)	4.8 (3.4–6.3)	6.6 (5.4–10.1)	<0.0001	<0.0001	<0.0001
NO	66.4 ± 15.5	68.7 ± 20.7	77.6 ± 22.4	0.10	0.56	0.11
Tubular injury markers						
NGAL (ng/mL)	174.4 (158.1–192.3)	139.9 (123–158.5)	225.7 (189.1–269.3)	<0.0001	0.007	<0.0001
β2M (ng/mL)	1488.5 (1357.2–1632.5)	1158.1 (1029.1–1303.2)	1771.0 (1498.7–2092.9)	<0.0001	0.001	<0.0001
Measured parameters of intrarenal hemodynamic function						
GFR_{INULIN} (mL/min/1.73 m^2)	105 ± 19	108 ± 16	93 ± 15	0.005	0.51	0.002
$ERPF_{PAH}$ (mL/min/1.73 m^2)	497 ± 131	478 ± 101	385 ± 70	<0.001	0.39	0.002
RVR (mmHg/L/min·1000)	115 ± 38	125 ± 32	157 ± 30	<0.001	0.15	<0.001
Derived parameters of intrarenal hemodynamic function						
P_{GLO} (mmHg)	44.6 ± 2.8	49.3 ± 4.1	49.1 ± 3.7	<0.001	<0.001	0.74
R_A (dyne·s·cm^{-5})	4448 ± 2055	4400 ± 1614	5652 ± 1622	<0.001	0.89	0.01
R_E (dyne·s·cm^{-5})	1215 ± 267	2310 ± 451	2592 ± 656	<0.001	<0.001	0.01
Biochemical characteristics						
HbA1c (%)	5.7 ± 0.4	7.3 ± 0.8	7.6 ± 1.0	<0.001	<0.001	0.03
HbA1c (mmol/mol)	39 ± 4	56 ± 9	60 ± 11	<0.001	<0.001	0.03
Glucose (mmol/L)	5.4 ± 1.6	8.1 ± 3.4	9.4 ± 4.1	<0.001	<0.001	0.09
$eGFR_{MDRD}$ (mL/min/1.73 m^2)	84 ± 14	81 ± 12	57 ± 14	<0.001	0.19	<0.001
Urine ACR (mg/mmol)	1.0 [0.7, 2.2]	1.0 [0.7, 1.6]	9.5 [5.1, 16.3]	<0.001	0.69	<0.001

Data expressed as mean ± SD, median [interquartile range], or *n* (%). DN: diabetic nephropathy; type 1 diabetes: type 1 diabetes: RAAS, renin-aldosterone-angiotensin system; ACR, albumin to creatinine ratio.

vasoconstriction.[5] It is, however, unclear whether cGMP contributes to the intrarenal hemodynamic dysfunction of longstanding type 1 diabetes in humans. Accordingly, our aim was to define the relationship between plasma cGMP, intrarenal hemodynamic function and plasma markers of tubular injury in longstanding type 1 diabetes.

2. Methods

This study represents a secondary analysis of the *Canadian Study of Longevity in Type 1 Diabetes*. The demographics and composition of this cross-sectional study have been previously described.[1] In the subset undergoing in-hospital phenotyping procedures, adults with type 1 diabetes of duration ≥50 years ($n = 66$) and age- and sex-matched comparators without diabetes ($n = 73$) had GFR by plasma inulin clearance, ERPF by plasma *p*-aminohippurate (PAH) clearance, plasma NO, cGMP, NGAL and β2M measured by methods as previously described.[1,6] Per study design, participants with type 1 diabetes were categorized as diabetic kidney disease (DKD) resistors if they had $eGFR_{MDRD}$ ≥60 ml/min/1.73 m^2 and 24-hour urine albumin excretion <30 mg/day, otherwise they were assigned to the DKD group.

Statistical analyses were performed using SAS version 9.4 for Windows (SAS Institute, Cary, NC). Continuous variables were assessed for normality (Shapiro-Wilk test and inspection of histograms). Comparisons of clinical characteristics between controls, DKD resistors, and DKD subgroups were made using ANOVA, the Kruskal-Wallis test, or the χ^2-test, depending on variable distribution. Comparisons between adults with and without type 1 diabetes were made with student *t*-test and Mann–Whitney *U* test as appropriate. The relationships were examined by Pearson correlation and multivariable linear regression models, adjusted for age, sex, SBP and HbA1c. Positively skewed variables were natural log transformed for inclusion in the linear regression models. We also evaluated whether type 1 diabetes and DKD resistor status were effect modifiers on the relationships between plasma NO, cGMP and parameters of intrarenal hemodynamic function. Analyses were considered exploratory and hypothesis generating and adjustments for multiple comparisons were not employed. An α-level of 0.05 (two-sided) was used to test for statistical significance.

3. Results

Adults with type 1 diabetes had greater plasma cGMP than their normoglycemic peers (geometric means [95% CI]: 5.4 [3.8, 6.6] vs. 4.2 [3.1–5.8] pmol/mL, $p = 0.004$), whereas plasma NO was not significantly different ($p = 0.15$). Plasma cGMP was also higher in adults with diabetic kidney disease (DKD) compared to those without DKD (Table 1). No difference in plasma NO was observed between participants with and without DKD (Table 1). Plasma cGMP strongly correlated with R_E (Table 2) in adults with type 1 diabetes (Table 2). There was a significant interaction between cGMP and R_E by type 1 diabetes status ($p < 0.0001$). cGMP also positively correlated with NGAL and

Table 2
Pearson correlation and multivariable linear regression models.

Multivariable linear regression models		Adults with longstanding type 1 diabetes							
		GFR	ERPF	RVR	P_{GLO}	R_A	R_E	NGAL[a]	β2M[a]
NO	r (R^2)	−0.08 (<1%), $p = 0.60$	−0.04 (<1%), $p = 0.77$	0.10 (1%), $p = 0.47$	−0.02 (<1%), $p = 0.91$	0.11 (1%), $p = 0.45$	0.03 (<1%), $p = 0.83$	0.07 (<1%), $p = 0.64$	0.001 (<1%), $p = 0.99$
	β ± SE[b]	−0.1 ± 0.1, $p = 0.51$	−0.3 ± 0.6, $p = 0.64$	0.2 ± 0.2, $p = 0.31$	−0.02 ± 0.03, $p = 0.54$	11.5 ± 9.4, $p = 0.23$	0.9 ± 3.4, $p = 0.79$	0.00 ± 0.00, $p = 0.60$	−0.00 ± 0.00, $p = 0.60$
cGMP[a]	r (R^2)	−0.01 (<0.1%), $p = 0.92$	*−0.38 (14%), $p = 0.004$*	*0.38 (14%), $p = 0.005$*	0.01 (<0.1%), $p = 0.94$	0.22 (5%), $p = 0.11$	*0.52 (27%), $p < 0.0001$*	*0.49 (24%), $p = 0.0002$*	*0.49 (24%), $p = 0.0002$*
	β ± SE[b]	4.2 ± 5.2, $p = 0.42$	−41.3 ± 24.8, $p = 0.10$	13.7 ± 8.4, $p = 0.11$	0.7 ± 1.2, $p = 0.54$	181.4 ± 413.1, $p = 0.66$	*461.2 ± 144.5, $p = 0.003$*	*0.6 ± 0.1, $p < 0.0001$*	*0.6 ± 0.1, $p < 0.0001$*

GFR = glomerular filtration rate, ERPF = effective renal plasma flow, RVR = renal vascular resistance, P_{GLO} = glomerular pressure, R_A = afferent arteriolar tone, R_E = efferent arteriolar tone, NGAL = neutrophil gelatinase-associated lipocalin (NGAL) and B2M = β2-microglobulin.
[a] Natural log-transformed.
[b] Adjusted for age, sex, SBP and HbA1c. β-estimates represent the change in the dependent variable per a 1-unit change in the independent variable.

β2M in adults with type 1 diabetes (Table 2). In contrast, these relationships were not evident in adults without type 1 diabetes (Table 2).

4. Discussion

Based on our analysis, elevated R_E observed in longstanding type 1 diabetes is related to greater plasma cGMP concentrations compared to normoglycemic peers. We speculate that the greater plasma cGMP concentration observed in adults with type 1 diabetes may relate to ANP activation in response to renal hypoxia. In rat models, hypoxia increases urinary cGMP without changing GFR, possibly due to an attempt to sustain filtration via R_A vasodilation and R_E vasoconstriction.[7] Atrial natriuretic peptide (ANP) is recognized to be regulated in response to renal hypoxia, and also exert cytoprotective effects.[8] While we did not observe a relationship between plasma cGMP and R_A in our study, this may relate to the RAAS-mediated predominant R_A vasoconstriction in longstanding type 1 diabetes.[1] A substantial amount of urinary cGMP is derived from plasma via tubular secretion. Accordingly, elevated plasma cGMP in longevity study participants may be in part due to tubular injury and impaired secretion, thus explaining the relationship between plasma cGMP and NGAL and β2M. It is also important to note that there are data suggesting decreased urinary and plasma cGMP in diabetes models, and in particular impairment of the NO/cGMP pathway.[9] While the reasons for these inconsistencies remain unclear, it may at least be partially explained by the type of biological fluid used to measure cGMP, i.e. urine vs. blood since the NP/pGC/cGMP pathway is a stronger contributor of circulating cGMP.

This is to our knowledge the first study examining the relationships between NO, cGMP, GFR, ERPF and calculated parameters of intrarenal hemodynamic function in participants with longstanding type 1 diabetes. The gold standard techniques to quantify GFR and ERPF by inulin and PAH clearance methods are significant strengths of our study. This study is subject to survivorship bias, since inclusion required participants to have lived with type 1 diabetes for 50 years or more. Therefore, potential participants with progressive or advanced DKD may not have been captured in this longevity study because of related mortality, which limits the overall generalizability of these findings. Further research is needed to define the role of the NP/pGC/cGMP pathway in the pathogenesis of DKD in type 1 diabetes, and whether better understanding of this pathway can be leveraged to develop novel therapies to combat DKD.

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