Title: Cardiac autonomic dysfunction is associated with high risk albumin: creatinine ratio in young adolescents with type 1 diabetes in AdDIT (Adolescent Type 1 Diabetes Cardio-Renal Interventional Trial)

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Running title (47 characters): Autonomic and renal dysfunction in adolescence

Objective: To examine the association between cardiac autonomic dysfunction and high albumin:creatinine ratio (ACR) in adolescents with type 1 diabetes.

Research Design and Methods: Adolescents recruited as part of a multicentre screening study (n=445, 49% female, aged 10-17 years, mean duration 6.9 years, mean HbA1c 8.4%, 68mmol/mol) underwent a 10-minute continuous ECG-recording for heart rate variability analysis. Time domain heart rate variability measures included: baseline heart rate, SDNN (standard deviation of the R-R interval), RMSSD (root mean squared difference of successive RR intervals); and spectral analysis included: sympathetic (low frequency) and parasympathetic (high frequency) components. Standardised ACR were calculated from 6 early morning urine collections using an established algorithm, reflecting age, gender and duration, and stratified into ACR tertiles where the upper tertile reflects higher nephropathy risk.

Results: The upper tertile ACR group had faster heart rate (76 vs 73bpm; p<0.01) and less heart rate variability (SDNN 68 vs 76ms, p=0.02; RMSSD 63 vs 71ms, p=0.04). HbA1c was 8.5% (69 mmol/mmol) in the upper tertile vs 8.3% (67mmol/mol) in the lower tertiles (p=0.07). In multivariable analysis, upper tertile ACR was associated with faster heart rate (β=2.5, CI 0.2 to 4.8; p=0.03) and lower RMSSD (β=-9.5, CI -18.2 to -0.8; p=0.03), independent of age and HbA1c.

Conclusions: Adolescents at potentially higher risk for nephropathy show an adverse cardiac autonomic profile, indicating sympathetic overdrive, compared to the lower risk group. Longitudinal follow up of this cohort will further characterize the relationship between autonomic and renal dysfunction, and the impact of interventions in this population.

Cardiac autonomic dysfunction is an early subclinical complication of type 1 diabetes (1; 2). It is associated with microalbuminuria both in adults and adolescents (3; 4), and the progression of renal disease in adults (5; 6). Power spectral analyses of heart rate variability allow more sensitive detection of cardiac autonomic changes than the conventional Ewing battery of tests based on dynamic cardiovascular manoeuvres (5; 7; 8). Recent studies utilising these modern methods have demonstrated that adverse changes in heart rate variability may be detected during adolescence in people with type 1 diabetes, and are primarily associated with poor glycemic control (1; 9; 10). However, there is a paucity of data on whether cardiac autonomic function is associated with early renal dysfunction, prior to the onset of microalbuminuria.

Nephropathy risk in type 1 diabetes begins with a progressive increase in albumin excretion during adolescence, before the threshold for microalbuminuria is reached (11). Albumin excretion phenotype in children aged between 11-15 years, defined by albumin:creatinine ratio (ACR) tertile adjusted for age, gender and diabetes duration, has been shown to stratify into nephropathy risk (11). Since autonomic neuropathy is a risk factor for progression of renal disease in adults (5; 6), we hypothesised that adolescents with type 1 diabetes at higher risk of nephropathy would have an adverse cardiac autonomic profile compared to those at lower risk of nephropathy, independent of glycemic control. We therefore examined heart rate variability in young adolescents with type 1 diabetes stratified for nephropathy risk based on these previously validated ACR tertiles.

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Research Design and Methods

Adolescents with type 1 diabetes duration greater than one year (n=445, 49% female, aged 10-17 years) were recruited as part of the nephropathy screening to determine eligibility for the Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT). AdDIT is a multicentre randomised controlled trial across the United Kingdom, Australia and Canada, where adolescents at risk of diabetic nephropathy, indicated by upper tertile ACR, are randomised to an angiotensin-converting-enzyme (ACE) inhibitor and/or 'statin' (HMG-CoA reductase inhibitor) or placebo (12). Data for the current study were collected prior to any intervention. This study was approved by the Human Research Ethics Committees of each participating centre. Informed consent was obtained from participants and their families.

Participants underwent a 10-minute continuous ECG recording in supine position using the LabChart-Pro (ADInstruments, Sydney Australia). Measurements were taken in a quiet room, after the patients were rested for 10 minutes. All traces were reviewed and analysed by a single operator masked to patients' clinical status. Traces were checked to ensure R-waves were adequately identified from artefacts and ectopic beats. The term "NN" is used in place of RR to emphasize the fact that the processed beats are normal sinus rhythm (ie. every QRS complex was preceded by a p-wave). Heart rate variability refers to the variations of heart rate and successive cardiac cycles, under the control of the autonomic nervous system. Time-domain measures of overall heart rate variability included: mean heart rate, standard deviation of mean NN intervals (SDNN) and root mean squared difference of successive NN-intervals (RMSSD). Geometric-domain analysis was performed using triangular index (TI), total number of all NN intervals divided by the height of the histogram of all NN intervals measured on a discrete scale with bins of 7.8125 ms

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(1/128 seconds). Frequency-domain measures included: low frequency (LF), defined as >0.04Hz and <0.15Hz, and high frequency (HF) components, defined as >0.15Hz and <0.4Hz, and the LF:HF ratio, considered to be an estimate of the relative sympathetic and parasympathetic balance (13). Age matched community controls (n=62; 45% female, age range 10-17 years) were contemporaneously recruited from healthy Sydney school-aged adolescents.

All urine samples were analyzed centrally at The WellChild laboratory at The Evelina Children's Hospital, London. Samples were stored at −70°C prior to shipping. Urine albumin was measured using laser immunonephelometry (Siemens BN Prospec) and for concentrations <2.1 mg/l by an enzyme linked immunoabsorbent assay (ELISA) (14). Urine creatinine was measured using a chromatographic stable isotope dilution electrospray mass spectrometry-mass spectrometry (MSMS) method. For each participant, two time-point ACR measures (mg/mmol), each based on three consecutive early morning samples at two separate visits, were averaged on the log ACR scale and the average residual calculated using age, gender and duration and the coefficients from the previously described linear regression model in the Oxford Regional Prospective Study of Childhood Diabetes (ORPS) cohort (11). Upper ACR tertile was assigned to residual value above 1.2, middle ACR tertile to values between 0.8 and 1.2, and lower ACR tertile to values below 0.8. For this study, the lower two tertiles were combined for analysis.

HbA1c was analysed locally at each centre, using DCCT aligned methods: HbA1c Variant analyser (Bio-Rad Laboratories, CA, USA), Adams Arkray Inc, (Kyoto, Japan), Vantage analyser, Siemens Diagnostics, Camberley UK or DCA 2000.

Estimated glomerular filtration rate (eGFR) was calculated from the formula: eGFR (ml/min/1.73m^2) = 42*height (cm)/plasma creatinine (µmol/l)(12). Lipid profile measurements (cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) were measured using routine laboratory methods.

Height and weight were measured to 0.1 cm and 0.1 kg. Height, weight and body mass index (BMI) standard deviation scores (SDS) were calculated according to the LMS method (15). Blood pressure was measured using Omran M6 blood pressure (all centres) and/or Dinamap (NSW) monitor using an appropriate sized cuff. Age and gender related percentiles and SDS for systolic and diastolic blood pressures were calculated according to published standards (16). Systolic hypertension was defined as systolic blood pressure above the 95th percentile for age and gender.

Statistical analysis

Descriptive data are summarised as mean (± standard deviation) for parametric data. Heart rate and heart rate variability parameters were normally distributed and analyzed as continuous variables. Urine albumin excretion groups (upper vs lower two tertiles) were analyzed as categorical variables. Mean differences between groups were compared using independent samples T-tests (diabetes versus

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controls, ACR group upper vs lower tertiles). Marginal means for the ACR groups (upper vs lower tertiles) were evaluated at 11, 13, 15 and 17 years of age, and compared using ANOVA. Linear regression was used to model the association between ACR group and continuous ACR and heart rate or heart rate variability outcomes. Multivariable regression analysis was used to examine the association between ACR group and other clinical variables (age, HbA1c, BP, BMI SDS and lipids) with heart rate/heart rate variability outcomes. All statistical analyses were conducted using SPSS version 21.

Results

Participant characteristics

Of the 445 participants who underwent heart rate variability testing, 217 (48%) were in the lower two tertiles for ACR (mean age 14.6 ±1.5 years) (Table 1). Adolescents in the high risk (upper tertile) group were slightly younger, and had shorter diabetes duration than those in the low risk (lower tertiles) group. There was no significant difference in gender, HbA1c or BMI SDS between the two groups. HDL-cholesterol and systolic blood pressure SDS were marginally higher in the upper tertile group. Of the 55/445 adolescents with systolic hypertension, 73% were in upper tertile whilst 27% were in the lower tertiles (p<0.001).

Standardised ACR as a continuous variable was significantly associated with HR (β=1.5, CI 0.4 to 2.7, p<0.01) and RMSSD – a measure of overall heart rate variability (β=-4.4, CI -8.4 to -0.3, p=0.03), which remained significant after adjusting

for age and HbA1c. Systolic and diastolic blood pressures were not significant variables in the model.

Comparison of heart rate variability in the diabetes subgroups: upper ACR tertile versus lower ACR tertiles

Compared to the lower tertiles, the upper tertile group had significantly faster HR and lower HRV (SDNN, RMSSD, TI) (Table 2). ACR group remained significantly associated with HR, SDNN and RMSSD in multivariable analysis (Table 3). ACR group was a significant explanatory variable, independent of HbA1c, for heart rate and two measures of overall heart rate variability (SDNN and RMSSD); and independent of age and HbA1c for heart rate and RMSSD (Table 3).

The marginal means of heart rate and RMSSD for the two ACR groups at four arbitrary age points are graphically represented in Figure 1 (a) and (b), demonstrating the decrease in heart rate and heart rate variability with age.

Compared with controls, adolescents with diabetes had faster heart rate, lower overall heart rate variability (SDNN, RMSSD and TI) and higher sympathetic tone (LF:HF ratio) in comparison to age-matched controls (Appendix: Table S1) (10).

Conclusions

In this multicentre study of young adolescents with type 1 diabetes, we found significant differences in cardiac autonomic profile according to albumin excretion phenotype. Adolescents with a greater risk of later nephropathy also had lower

overall heart rate variability and higher resting heart rate, compared to those at lower risk of nephropathy; indicating that autonomic dysfunction is associated with future risk of nephropathy prior to the development of microalbuminuria. Furthermore, it is possible that the autonomic dysregulation contributes to renal dysfunction.

This is the first large-scale study examining heart rate variability in relation to risk of nephropathy in adolescents with type 1 diabetes who have not yet developed microalbuminuria. Reduced heart rate variability has been associated with microalbuminuria in older adolescents (4; 9). In the SEARCH study, lower SDNN (one measures of overall heart rate variability) was associated with microalbuminuria in older adolescents with type 1 diabetes (mean age 18.8 years and mean duration 9.8 years) (9). In this current study, we found significantly lower SDNN, RMSSD, (measures of overall heart rate variability) and faster heart rate in younger adolescents at risk of nephropathy (mean age 14.1 years, mean duration 6.4 years). Adult studies in type 1 diabetes demonstrate an early disturbance in cardiac autonomic function prior to onset of microalbuminuria. Higher 24 hour ambulatory blood pressure and reduced heart rate variability were detected in normoalbuminuric adults with type 1 diabetes with high-normal albumin excretion (above the median of 4.2mcg/min) compared to those with low-normal albumin excretion (17). These changes occurred in the context of significantly worse glycemic control in the group with high-normal albumin excretion; in contrast our findings were independent of HbA1c.

It is clear that glycemic control contributes to both autonomic dysfunction and other microvascular complications (18). Our study nevertheless demonstrated significant

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differences in heart rate variability between the two risk groups despite similar mean HbA1c. Furthermore, heart rate, SDNN and RMSSD remained significant after adjustment for HbA1c in multivariable analysis. However, the association between ACR group and another measures of overall heart rate variability (TI) was weakened after adjustment for HbA1c, suggesting that HbA1c does modify the relationship between some measures of cardiac autonomic dysfunction and albumin excretion.

Obesity has been associated with reduced parasympathetic activity and relative increase in sympathetic activity in adolescents with and without diabetes (10; 19). In contrast, we found was no significant difference in BMI SDS in the ACR groups to explain HRV differences between upper and lower ACR tertiles. The lack of difference in BMI SDS in the two groups helps exclude the confounding effect of obesity on autonomic function and albumin excretion in this cohort.

Although systolic and diastolic blood pressures were significantly higher in the upper tertile ACR group in our study, the majority of adolescents in both ACR groups had blood pressure within the normotensive range for age and gender. Whilst adolescents with type 1 diabetes with microalbuminuria have higher blood pressure than those with normoalbuminuria (20; 21), there are mixed data on the detection of higher incident blood pressure prior to elevation in albumin excretion. In the T1D Exchange Clinic Registry, higher diastolic blood pressure in adolescents was a risk factor for microalbuminuria (22), whereas another study showed that higher incident blood pressure did not precede the onset of increasing albumin excretion (23). High

daytime blood pressure may therefore be a relatively late clinical finding in the progression of abnormal albumin excretion.

Ambulatory blood pressure may better predict nephropathy risk in adolescent diabetes than incident blood pressure (21; 24). Furthermore, increase in nocturnal systolic blood pressure preceded the progression to microalbuminuria, and loss of nocturnal dip in blood pressure predicted the development of microbuminuria in adolescents with type 1 diabetes (25). As elevation in nocturnal systolic blood pressure and loss of nocturnal blood pressure dip can signify autonomic dysfunction (26), we therefore speculate that autonomic dysfunction may even precede the elevation of ACR into the higher risk tertile in this young population with type 1 diabetes.

It has been proposed that autonomic dysfunction plays a causative role in renal disease, through changes in nocturnal glomerular function and renal hemodynamics (6; 27) or a loss of protective effect on the kidneys leading to progression of renal disease (6; 28; 29). Animal data support the role of increased sympathetic activity in the pathogenesis of renal disease (30). A recent study of adults with type 2 diabetes found that cardiac autonomic dysfunction was associated with increased albuminuria and lower estimated glomerular filtration rate (eGFR) at baseline, and also predicted subsequent decline in eGFR (31). Clinical studies in adults with type 1 diabetes have been less clear on the role of cardiac autonomic function on renal decline (28; 29; 32). In adolescents with type 1 diabetes, there is evidence for autonomic dysregulation occurring early in the pathogenesis of microvascular complications.

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We previously found that abnormal pupillary response, a marker of autonomic dysfunction, preceded development of microalbuminuria and diabetic retinopathy 12 years later (33).

A limitation of this study is its cross-sectional nature; thus it cannot examine causative and temporal relationships between albumin excretion and the autonomic function. Autonomic dysfunction and abnormal albumin excretion may also share common etiology not formally measured in this study, such as insulin resistance (34; 35) and biochemical mediators of inflammation (36-38). Strengths of this study include the large participant recruitment and standardised methodology, heart rate variability analysis by one operator, and careful clinical characterisation for eligibility into a follow up randomised controlled trial (AdDIT).

This study elucidates the adverse heart rate variability profile already present in young adolescents with type 1 diabetes identified to be at higher risk of nephropathy. Longitudinal analysis of these patients will determine possible early predictive value of heart rate variability in addition to albumin excretion phenotype on renal and cardiac outcomes.

Acknowledgments

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Appendix

AdDIT INVESTIGATORS: Australia: Phil Bergman (Victoria), Christine Rodda (Victoria), Bruce King (New Lambton), Charles Verge (Sydney). UK: Carlo Acerini (Cambridge), Fran Ackland (Northampton), Binu Anand (West Suffolk), Tim Barrett (Birmingham), Virginia Birrell (Middlesbrough), Fiona Campbell (Leeds), Tim Cheetham (Newcastle Upon Tyne), Chris Cooper (Stockport), Ian Doughty (Manchester), Atanu Dutta (Stoke Mandeville), Julie Edge (Oxford), Julian

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Dysfunction And Inflammation In Type 1 Diabetic Patients: Effect Of Beta-Blockade.

Figure Legend

Abbreviations: RMSSD root mean squared difference of successive NN-intervals

Legend: black circles = lower ACR tertiles; black squares = upper ACR tertile

Table 1: Participant characteristics stratified by urine albumin:creatinine ratio (ACR) tertiles

	Lower ACR tertiles	**Upper ACR tertile**	**P value**
N (% male)	217 (54%)	228 (50%)	0.45
Age (years)	14.6 (1.5)	14.1 (1.5)	<0.01
Height SDS	0.38 (1.06)	0.38 (0.97)	1.00
Weight SDS	0.77 (0.77)	0.67 (0.89)	0.21
BMI SDS	0.67 (0.80)	0.55 (0.90)	0.14
Duration (years)	7.6 (3.4)	6.4 (3.0)	<0.01
HbA1c (%)	8.3 (1.2)	8.5 (1.4)	0.07
HbA1c (mmol/mol)	67 (13)	69 (15)	0.07
eGFR	122 (22)	132 (25)	<0.01
Mean SBP (mmHg)	114 (11)	118 (13)	<0.01
Mean DBP (mmHg)	64 (20)	67 (8)	0.09
SBP SDS	0.36 (0.86)	0.78 (1.03)	<0.01
DBP SDS	0.20 (1.88)	0.40 (0.77)	<0.01
Cholesterol (mmol/L)	4.5 (0.9)	4.5 (0.9)	0.64
HDL-cholesterol (mmol/L)	1.45 (0.34)	1.54 (0.35)	0.02
Triglycerides (mmol/L)	1.1 (0.7)	1.2 (0.8)	0.75
LDL-cholesterol (mmol/L)	2.5 (0.8)	2.4 (0.7)	0.31

Abbreviations: ACR standardized albumin:creatinine ratio; SDS standard deviation scores; BMI body mass index; eGFR estimated glomerular filtration rate; SBP systolic blood pressure; DBP diastolic blood pressure; HDL-cholesterol high-density lipoprotein cholesterol; LDL-cholesterol low-density lipoprotein cholesterol. Data are presented as mean (standard deviation)

Table 2: Heart rate variability (HRV) in adolescents with type 1 diabetes stratified by urine albumin:creatinine ratio (ACR) tertiles

HRV Parameter	Lower ACR tertiles	Upper ACR tertile	*P* value
Heart rate (bpm)	73 (12)	76 (13)	<0.01
SDNN (ms)	76 (36)	68 (30)	0.02
RMSSD (ms)	71 (49)	63 (43)	0.04
LF Power	1839 (2282)	1363 (1237)	<0.01
HF Power	2439 (3751)	2076 (3229)	0.27
LF:HF ratio	1.3 (1.2)	1.5 (1.3)	0.14
TI	17 (7)	15 (6)	0.04

Abbreviations: HRV heart rate variability; HR heart rate; SDNN standard deviation of mean NN intervals; RMSSD root mean squared difference of successive NN-intervals; LF low frequency; HF high frequency; TI triangular index; ACR standardized urine albumin:creatinine ratio.

Data are presented as mean (standard deviation)

Table 3: Multivariable analysis of heart rate variability (HRV) outcomes and ACR tertiles

HRV parameter	Factor*	B (95% CI)	*P* value
HR	Upper ACR tertile[+]	2.5 (0.2 to 4.8)	0.03
	Age	-1.2 (-1.9 to -0.4)	<0.01
	HbA1c	1.9 (1.0 to 2.8)	<0.001
SDNN	Upper ACR tertile	-6.6 (-12.7 to -0.4)	0.04
	HbA1c	-3.8 (-6.2 to -1.4)	<0.01
RMSSD	Upper ACR tertile	-9.5 (-18.2 to -0.8)	0.03
	Age	-3.1 (-6.0 to -0.3)	0.03
	HbA1c	-3.9 (-7.2 to -0.5)	0.02

Abbreviations: HRV heart rate variability; HR heart rate; SDNN standard deviation of mean NN intervals; RMSSD root mean squared difference of successive NN-intervals; ACR standardized albumin:creatinine ratio.

*Diabetes duration, BMI SDS, systolic or diastolic BP SDS were not significant explanatory variables in the above models

+Reference group is lower tertiles