Renal and cardiovascular risk according to tertiles of urinary albumin-creatinine ratio:

the Adolescent Type 1 Diabetes cardio-renal Intervention Trial (AdDIT)

M. Loredana Marcovecchio[1], MD; Scott T. Chiesa[2], PhD; Jane Armitage[3], MD; Denis Daneman[4], MD; Kim C. Donaghue[5], MD; Timothy W. Jones[6], MD; Farid H. Mahmud[4], MD; Sally M. Marshall[7], MD; H. Andrew W. Neil[8], DSc; R. Neil Dalton[9], PhD; John Deanfield[2], MD; David B. Dunger, MD[1,10]; on behalf of the Adolescent type 1 Diabetes cardio-renal Intervention Trial (AdDIT) study group.

[1]Department of Paediatrics, University of Cambridge, Cambridge, UK
[2]National Centre for Cardiovascular Prevention and Outcomes, University College London, London, UK
[3]Medical Research Council Population Health Research Unit, Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK
[4]Department of Paediatrics, The Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada
[5]Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead and University of Sydney, New South Wales, Australia.
[6]Telethon Kids Institute, University of Western Australia, Perth, Australia
[7]Institute of Cellular Medicine (Diabetes), Faculty of Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne, UK
[8]Oxford Centre for Diabetes, Endocrinology& Metabolism, University of Oxford, Oxford, UK
[9]Guy's and St Thomas' NHS Foundation Trust, London, UK
[10]Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK.

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Corresponding author:
Professor David B Dunger
University Department of Paediatrics
Level 8, Box 116, Addenbrooke's Hospital
Hills Road, Cambridge CB2 0QQ, UK
Tel.: +44 (0) 1223 336886
Fax: +44 (0) 1223 336996
Email: dbd25@cam.ac.uk

ABSTRACT

OBJECTIVE Baseline data from the Adolescent Type 1 Diabetes cardio-renal Intervention Trial (AdDIT) indicated that tertiles of urinary albumin-creatinine ratios (ACR) in the normal range at age 10-16 years are associated with risk markers for diabetic nephropathy (DN) and cardiovascular disease (CVD). We aimed to determine whether the top ACR tertile remained associated with DN and CVD risk over the 2-4 year AdDIT study.

RESEARCH DESIGN AND METHODS 150 adolescents (mean[SD] age: 14.1[1.6]years) with baseline ACR in the upper tertile (high-ACR group) recruited to the AdDIT trial, who remained untreated, and 396 (age: 14.3[1.6]years) with ACR in the middle and lower tertiles (low-ACR group), who completed the parallel AdDIT observational study, were evaluated prospectively with assessments of ACR, renal and CVD markers, combined with carotid intima-media thickness (cIMT) at baseline and end of study.

RESULTS After a median follow-up of 3.9 years, the cumulative incidence of microalbuminuria was 16.3% in the high- vs 5.5% in the low-ACR group (log-rank p<0.001). Cox models showed independent contributions of the high-ACR group (hazard ratio [95%CI]: 4.29[2.08; 8.85]) and HbA1c (1.37[1.10; 1.72]) to microalbuminuria risk. cIMT change from baseline was significantly greater in the high vs low-ACR group (mean difference: 0.010[0.079]mm, p=0.006). Changes in estimated GFR, systolic blood pressure and hs-C-Reactive Protein were also significantly greater in the high-ACR group (p<0.05).

CONCLUSIONS ACR at the higher end of the normal range at the age of 10-16 years is associated with an increased risk of progression to microalbuminuria and future CVD risk, independently of HbA1c.

The prognosis of young people with type 1 diabetes remains poor. Recent data indicate that for a 20-year old person with type 1 diabetes, life expectancy is reduced by 10-13 years compared to the respective background population (1,2). Diabetic nephropathy (DN) and cardiovascular disease (CVD) are the main contributors to morbidity and mortality, and diabetic retinopathy is a major cause of vision loss (3).

Microalbuminuria has been recognized for a long time as a hallmark of DN and a predictor of CVD in people with type 1 diabetes (4,5). However, recent evidence indicates that increases in urinary albumin excretion even within the normal range, and thus below the specific cut-off for the definition of microalbuminuria, may predict renal and cardiovascular risk in adults with diabetes as well as in the general population (6–8).

In young people with type 1 diabetes, early increases in urinary albumin excretion during the first years after diagnosis are predictive of future risk of DN (9). In longitudinal cohorts of adolescents with type 1 diabetes, tertiles of urinary albumin-creatinine ratio (ACR) adjusted for age, diabetes duration and sex, at the age of 11-16 years, were highly predictive of those individuals who went on to develop microalbuminuria and macroalbuminuria after puberty (10).

The Adolescent Type 1 Diabetes cardio-renal Intervention Trial (AdDIT) was primarily designed to explore the potential cardio-renal protection provided by angiotensin-converting enzyme (ACE) inhibitors and statins in adolescents with type 1 diabetes at increased risk of vascular complications based on an ACR in the upper tertile of the normal range (11,12). The study protocol also included a group of adolescents with an ACR in the lower and middle tertiles who participated in a parallel observational study (11).

At baseline, AdDIT participants with an ACR in the upper tertile showed evidence of glomerular hyperfiltration, increased lipid levels and pulse wave velocity, and impaired autonomic function (11,13,14), despite similar HbA1c values across ACR tertiles. These data

supported the hypothesis that risk stratification using ACR tertiles during early adolescence may be helpful for the early identification of patients at risk of developing vascular complications.

We have now evaluated whether early stratification based on tertiles of ACR in adolescents aged 10-16 years participating in the AdDIT study could predict those at higher risk of developing renal and cardiovascular complications during a 2-4 year follow up period.

RESEARCH DESIGN AND METHODS

Study design and participants

The study population consisted of adolescents with type 1 diabetes screened and recruited into the AdDIT Trial and the parallel Observational study (11). These screened population of 4,407 adolescents (age between 10 to 16 years) with type 1 diabetes from the UK, Canada and Australia provided 2 sets of 3 consecutive early morning urine samples for the assessment of urinary ACR. The three ACR measures at each visit were averaged on the log ACR scale and the subject's average residual was calculated using an algorithm derived from previous longitudinal studies of the natural history of microalbuminuria (10). This algorithm allowed adjustments for age, sex and type 1 diabetes duration. If the residual was above log 1.2 the participant was assigned to the upper tertile of ACR. Values between 0.8 and 1.2 identified the middle ACR tertile, whereas values below 0.8 identify the lower ACR tertile. The upper tertile of ACR was used to classify adolescents at higher risk for vascular complications based on previous findings (10), who were eligible for the AdDIT trial (Trial cohort), whereas the lower and middle tertiles identified adolescents with a lower risk, who were eligible for the Observational arm of the AdDIT study (11). Further details of the inclusion and exclusion criteria were previously reported (11,12).

Between 2009 and 2013, 443 adolescents (10-16 years) with ACR in the upper tertile of the normal range were randomized to an ACE inhibitor (Quinapril) or matching placebo, and

separately to a statin (Atorvastatin) or matching placebo in a 2x2 factorial design over a 2-4-year treatment period, until March 2016 (12). For the present analysis, only data collected from adolescents randomized to the placebo ACE inhibitor/and placebo statin were used (n=109). These 109 adolescents and an additional 41 adolescents with baseline ACR in the upper tertile screened for AdDIT, who declined to take part into the Trial but agreed to be followed up, represent the 'high-ACR group' (n 150). 404 screened adolescents with an ACR in the middle or lower tertiles were recruited to the parallel AdDIT Observational study. 8 of them withdrew from the study before the baseline visit and the remaining 396 represent the 'low-ACR group' (Supplementary Figure S1). Both groups underwent similar baseline and follow up assessments, following a standardized protocol (11).

The study sponsor was the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust. The study protocol conformed to the Declaration of Helsinki and was approved by the Cambridge University Hospitals and participating research ethics committees. Parents of participants provided written informed consent, and study participants were asked to provide their assent, until they reached an age when they could consent to study follow up.

Procedures

Baseline Assessment. Baseline visits for the high- and low-ACR groups included measurement of height, weight, waist circumference and arterial blood pressure. Non-fasting blood samples were collected for local HbA1c measurements and central assessments of CVD and renal biomarkers. These included a lipid profile (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol), other CVD markers (high sensitivity C-reactive protein (hsCRP), and asymmetric dimethylarginine (ADMA)), renal markers (creatinine, cystatin C).

All study participants also attended one of the designated centres for standardized cIMT assessment.

Follow up visits. Every 6 months: 3 early morning urines were collected for central assessment of ACR; height, weight, waist circumference, pubertal stage, blood pressure, smoking status were recorded; blood samples were taken for local HbA1c. Annual blood samples were collected for centralized measurements of renal and CVD markers as at baseline.

Throughout the trial treating physicians were encouraged to strive for optimal glycemic control, but targets and methods of insulin delivery were not stipulated in the protocol.

Biochemistry

HbA1c was assessed locally, using DCCT aligned methods, whereas all other biochemical measurements, were performed in a central laboratory (WellChild Laboratory, Evelina Children's Hospital, London), using standardized methods.

Urine albumin was measured using nephelometric immunoassay according to the manufacturer's instructions (Siemens BN Prospec). Urine albumin concentrations below the limit of quantitation of nephelometry, typically <2.1 mg/L, were measured using ELISA. Between-batch imprecision was 3.7% at 4.16 mg/L (n= 51), 2.9% at 19.0 mg/L (n= 55), and 2.9% at 144 mg/L (n= 54). Between batch imprecision on the ELISA at <2.1 mg/L was <15%. Urine creatinine was measured using a chromatographic stable isotope dilution electrospray mass spectrometry–mass spectrometry (MSMS) method on an AB SCIEX API5000. Between-batch imprecision (n= 48) was 2.6% at 6.89 mmol/L and 3.3% at 17.4 mmol/L. Plasma creatinine was measured using a reference stable isotope dilution electrospray MSMS. Between-batch imprecision (n= 30) was 2.8% at 66.1 μmol/L and 2.5% at 333.3 μmol/L. Cystatin C was measured by particle-enhanced nephelometric immunoassay

according to the manufacturer's instructions (Siemens BN Prospec). Between-batch imprecision (*n*= 38) for cystatin C was 3.5% at 0.87 mg/L and 3.6% at 4.64 mg/L. Plasma ADMA was measured using a chromatographic stable isotope dilution fragmentation-specific electrospray MSMS. Between-batch imprecision (*n*= 30) for ADMA was 2.5% at 401 nmol/L, 2.7% at 917 nmol/L, and 2.7% at 2,413 nmol/L. hsCRP was measured by particle-enhanced nephelometric immunoassay according to the manufacturer's instructions (Siemens BN Prospec). Between-batch imprecision (*n*= 38) was 5.8% at 0.89 mg/L and 3.6% at 4.73 mg/L. Total cholesterol (second-generation formulation), HDL cholesterol (third-generation formulation), LDL cholesterol, and triglycerides were measured colorimetrically on a COBAS Integra 400 plus according to the manufacturer's instructions. Between-batch imprecision for total cholesterol (*n*= 35) was 2.6% at 4.71 mmol/L and 2.1% at 8.62 mmol/L, for HDL-cholesterol (*n*= 35) was 3.1% at 0.86 mmol/L and 3.9% at 1.49 mg/L, for LDL-cholesterol (*n* = 36) was 3.1% at 3.07 mmol/L and 2.5% at 4.92 mmol/L, and for triglycerides (*n* = 35) was 2.9% at 1.47 mmol/L and 2.8% at 4.82 mmol/L.

Vascular Assessments

Ultrasound scanning for cIMT was performed at 11 specialist vascular centres at baseline and at the end of follow-up. Vascular sonographers were accredited before the study commenced. Sonographer training was conducted through the Vascular Physiology Unit at University College London, which has extensive experience of undertaking large scale, vascular phenotyping trials in children, and included a one-week intensive training course in London for all study sonographers. Sonographers were accredited before the study commenced and intra-sonographer reproducibility on 5 subjects scanned at least one week apart was <5%. A single reader analyzed all cIMT scans in the core laboratory. Intra-reader reproducibility on 50 randomly selected study scans was 2.1%.

Common carotid artery ultrasound images were acquired using a standardized protocol. The artery was scanned in the ear-ear plain with the head rotated to 45 degrees from the mid-point. Images triggered on the R-wave of the ECG were recorded in DICOM format as cine loop files for offline analysis (Carotid Analyser, Medical Imaging Applications, Coralville, Iowa). The cIMT value was taken as the average of 3 end-diastolic measurements. cIMT measurements were made on a single segment of arterial wall 5-10 mm in length at least 10 mm proximal to the bifurcation.

Calculations

Estimated GFR (eGFR) was calculated from plasma creatinine with a modified Schwartz formula, based on our previous study in type 1 diabetes adolescents (15): eGFR $(ml/min/1.73m^2) = 42 \times height$ (cm)/plasma creatinine (μmol/l) (16), and the Zappitelli equation (age <18 years): $43.82 \times (1/cystatin\ C)^{0.635} \times (1/creatinine)^{0.547} \times 1.35^{height}$ (17).

Study outcome measures

Primary outcome measure: incidence of microalbuminuria in relation to baseline ACR tertiles.

Microalbuminuria was defined as an ACR >3.5mg/mmol (in males) or 4mg/mmol (in females) in at least 2 out of 3 early morning urines at any study visit.

Cases with microalbuminuria at screening were excluded from this analysis.

Time to first incidence of microalbuminuria was analysed as a time-to-event variable using the calendar date when microalbuminuria was observed or censoring at the final visit if microalbuminuria was never observed.

Secondary outcomes measures include: changes in cIMT between baseline and the end of study; trends in cardiovascular risk factors during the study period: blood pressure, lipid

levels, hsCRP, and ADMA; trends in renal markers: creatinine, eGFR; trends in glycemic control, as measured by HbA1c.

Statistical analysis

Data are summarized as mean±SD or median [interquartile range (IQR)] unless otherwise specified. Non-normally distributed variables were log-transformed before analysis. Between-group baseline comparisons were performed by unpaired t-tests for continuous variables and by χ^2 or Fisher's exact test for categorical variables.

For the primary endpoint, the assessment of the effect of the baseline ACR group (low vs high) was performed by Kaplan-Meier estimation of survival curves and compared by log-rank test, and by Cox proportional hazards models and results are expressed as hazard ratios (HR). The univariate association between baseline and longitudinal clinical and laboratory parameters (ACR category, HbA1c, age, age at diagnosis, duration, blood pressure, lipids, and eGFR) and microalbuminuria was assessed in univariate cox regression models. Variables with p<0.05 were then included in a multivariate model.

For the analysis of cIMT, assessed only at baseline and follow-up, an ANCOVA model was used to estimate the effect of ACR categories (low- vs high ACR group). The final cIMT measurement was used as dependent variable in the model, and adjustments were made for baseline cIMT, age, sex, HbA1c, blood pressure, cholesterol.

Linear mixed models, adjusted for age and sex, were used to assess overtime differences in the high- vs low-ACR group in the secondary continuous endpoints assessed at multiple time-points during the study period.

We considered p<0.05 as significant for the primary outcome, for secondary and exploratory outcomes p values are not adjusted but should be interpreted cautiously.

RESULTS

- **Cohort description**

The study population consisted of all participants completing the Observational arm of the AdDIT study (low-ACR group, n 396) and the 150 with ACR in the upper tertile unexposed to the active RCT drugs (high-ACR group) for a total of 546 participants (Supplementary Figure S1). The duration of follow up was similar in the low- and high-ACR groups: 3.9 (3.3-4.1) vs 3.9 (3.1-4.1) years.

- **Baseline characteristics**

The general characteristics of the study participants at baseline are shown in Table 1. Adolescents in the high-ACR group were slightly older at the time of diabetes diagnosis and had shorter diabetes duration than those in the low-ACR group. There was a similar sex distribution and no differences in anthropometric parameters (height, weight, BMI, waist circumference) between the two groups. Biochemical data showed similar HbA1c values between the two groups whereas ACR levels were, by definition, higher in the high-ACR group (Table 1). No significant differences were found in cholesterol and triglycerides levels, ADMA, hsCRP, whereas creatinine levels were lower in the high-ACR group and this was associated with a higher eGFR (Table 1).

- **Incidence of Microalbuminuria**

At baseline, 10 of the 546 (1.8%) participants had ACR in the microalbuminuria range, all in high-ACR group. These study participants were excluded from the analysis of incident cases of microalbuminuria.

During the median follow of 3.9 years of follow up, 31 study participants developed microalbuminuria. The cumulative incidence of microalbuminuria was significantly higher in the high- than in the low-ACR group (16.3% vs 5.5%, log-rank p<0.001) (Figure 1). The univariate associations between baseline clinical and laboratory parameters (ACR group, HbA1c, age, age at diagnosis, duration, LDL-cholesterol, blood pressure, eGFR) and microalbuminuria were assessed in univariate cox regression models (Table 2). Variables with p<0.05 (ACR group and HbA1c) were then included in a multivariate model, which showed that the ACR group (low vs high: HR (95% CI): 4.29 (2.08-8.85)) and HbA1c (1.37 (1.10-1.72)) were independent factors associated with future risk of microalbuminuria (Table 2).

Similar results were obtained when post-baseline mean values of predictors were included in the Cox regression models (Supplementary Table S1).

The independent effect of HbA1c and baseline ACR on the cumulative incidence of microalbuminuria during follow up was further explored by dividing the study population into tertiles of mean HbA1c. This analysis showed a progressive increase in microalbuminuria from the lower to the upper tertile of HbA1c both in the low- and high-ACR groups (Supplementary Figure S2).

- **Carotid intima media thickness**

cIMT was assessed at baseline in 149 adolescents from the high-ACR group and 370 from the low-ACR group. cIMT was then re-assessed at the final visit in 126 high- and 266 low-ACR participants. At baseline mean cIMT values were comparable between the high- and low-ACR groups (0.444 [0.051] vs 0.440 [0.041] mm, p=0.38) (Figure 2). In contrast, at the end of the follow up period cIMT was significantly higher in the high- than in low-ACR group (0.448 [0.050] vs 0.434 [0.043]mm, p=0.009).

The change in cIMT from baseline differed significantly between the high- and low-ACR groups (mean difference [SD]: 0.010 [0.079], p=0.006), and this remained significant even after adjusting for age, sex, blood pressure, HbA1c, LDL-cholesterol (p=0.008). Similar results were obtained when mean levels of HbA1c, blood pressure, LDL cholesterol during the study period where included in ANCOVA model for the adjustments instead of baseline values (p=0.014).

- **Other secondary outcomes**

Linear mixed models were used to assess changes over time in the other key renal and cardiovascular markers assessed in the two study groups (Table 3).

No significant differences were found in HbA1c, which showed a small increase in both groups. Similarly, there were no significant differences in lipid levels. Significant differences were found in eGFR (assessed by both the Schwartz and Zappitelli formulas), which was greater over time in the high- vs low-ACR group (mean difference [SE]): 4.84 [1.49], p=0.01), reflecting greater rates of hyperfiltration, as well as in systolic blood pressure (2.08 [1.00], p=0.03) and hsCRP (0.85 [0.32], p=0.008) (Table 3).

CONCLUSIONS

This study showed that, in a cohort of around 546 adolescents with type 1 diabetes, assessed at the age of 10-16 years, a higher ACR even within the normal range was associated with a greater risk of progression to microalbuminuria, a higher eGFR and a worse cardiovascular profile, as indicated by a thicker cIMT and greater blood pressure and hsCRP values during a 2-4 year follow-up period.

Variation in urinary albumin excretion has long been adopted as a screening tool for the detection of DN, where levels in the microalbuminuric range are indicative of incipient nephropathy and risk of progression to macroalbuminuria and end-stage renal disease (5,18).

However, the predictive value of these observations has been partly put in doubt by the high rates of reversal from microalbuminuria to normoalbuminuria over subsequent years (19), and the observation that some individuals may show decline in GFR in the absence of microalbuminuria (20). Nevertheless, the links between early evidence of DN, including microalbuminuria, and CVD risk in type 1 diabetes remain robust (21,22).

In the normal population associations between variations in ACR within the normal range and future CVD risk have been observed (8,23), perhaps suggesting that increases in albumin excretion may be a continuous marker for both CVD and DN risk in individuals with type 1 diabetes.

During the screening phase of the AdDIT study, we observed that young people (10-16 years) with an ACR in the highest tertile, although still within the normal range, already showed evidence of increased risk for CVD, as indicated by greater arterial stiffness and signs of impaired cardiac autonomic function, as well as higher rates of hyperfiltration, compared to adolescents in the lower/middle tertiles of ACR (11,13,14). The current longitudinal data from the AdDIT high- and low-ACR groups support these initial observations.

The rate of progression to microalbuminuria was much greater in participants in the higher than in those in the lower and middle tertiles of ACR at baseline. As expected HbA1c was also an independent risk factor for the development of microalbuminuria in both the low- and high-ACR groups.

Of interest the higher eGFR levels associated with ACR in the upper tertile at baseline, persisted during the follow-up period, although baseline eGFR was not an independent predictor of incident microalbuminuria. However, the persistence of hyperfiltration during the study period could be an additional risk factor for progression towards more advanced stages of DN and long-term development of CVD (24,25).

The higher rates of microalbuminuria in the high-ACR group may not necessarily predict future risk of DN, as in adolescent cohorts we have observed high rates of reversal at the end of puberty, particularly in those with good glycemic control (26), but both intermittent and persistent microalbuminuria did predict all future cases of macroalbuminuria (26). This is also in line with recent data from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Complications studies highlighting the predictive role of even intermittent microalbuminuria for future renal and CVD outcomes (22).

The cardiovascular outcomes in the high- and low-ACR groups also supported the baseline observations. The measured cardiovascular surrogate outcome cIMT demonstrated worsening over time in the high-ACR group. This was supported by longitudinal changes in hsCRP and systolic blood pressure, perhaps reflecting increased CVD risk in those with the highest ACR. hsCRP is a well-known cardiovascular risk marker (27), and we previously found that its levels tend to increase in parallel with the development of microalbuminuria (28). Whereas hsCRP increased in the whole study population, its levels were consistently higher in the high-ACR group. Similarly, although there were no differences at baseline in blood pressure, over time levels of systolic blood pressure were higher in the high-ACR group. Higher levels of cardiovascular risk factors, such as blood pressure, inflammatory markers throughout adolescence could contribute through a cumulative exposure to the lifetime risk of CVD, which still remains the leading cause of morbidity and mortality among individuals with type 1 diabetes (29).

Thus, AdDIT provides evidence that variations in albumin excretion in individuals with type 1 diabetes as young as 10-16 years old may partially predict future DN and CVD risk, but the extent to which this adds to prediction based on HbA1c and other risk factors still needs to be determined. The large sample size of the study cohort and the well-standardized methods for collection and analysis of clinical, biochemical and vascular data support the validity of the

study findings. However, the present findings were derived from a very selective study population, recruited into a clinical trial and parallel observational study. Indeed, there is a need for replication and validation of the study findings in other cohorts of adolescents and older subjects with type 1 diabetes. There also remains a need to assess the value of the ACR tertiles in predicting long-term complications during early adulthood. Over the next 3-4 years AdDIT participants will be entering the second/third decade of type 1 diabetes and that critical post-pubertal period when the first direct evidence of vascular complications is observed (30). Thus, ongoing follow up of the cohort could help in answering this relevant question.

In conclusion, these data support the concept that risk stratification using ACR during early adolescence may be valuable for the early identification of patients at risk of developing renal and cardiovascular complications, and to guide the implementation of preventive and treatment strategies to reduce the burden associated with vascular complications of diabetes.

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Authors Contributions

MLM and DBD are the garantors of this work and, as such have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

MLM was involved in the literature review, data acquisition, data interpretation, statistical analysis, and wrote the first draft of the manuscript. STC was involved in data collection and analysis and critical discussion. JA, SMM and AHN were involved in the study supervision, data interpretation and critical discussion. DBD, DD, JD, RND, TJ were involved in the study concept and design, data interpretation, critical discussion, study supervision. FHM and KCD were involved in data acquisition and interpretation, critical discussion. MLM and DBD drafted the first version of the manuscript. All authors contributed to a critical revision of the manuscript and approved the final version

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Duality of interest

None related to the present work.

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Table 1. Baseline characteristics of the study population

	LOW-ACR GROUP	HIGH-ACR GROUP	p
N	396	150	
Sex, male (%)	211 (53.3)	77 (51.3)	0.95
Age (years)	14.3 (1.6)	14.1 (1.6)	0.17
Age at diagnosis (years)	6.9 (3.6)	8.2 (3.1)	0.001
Duration of diabetes (years)	7.3 (3.4)	5.9 (3.1)	<0.001
Height (cm)	163.5 (10.6)	162.1 (10.1)	0.17
Weight (Kg)	59.0 (13.9)	57.1 (12.9)	0.15
Waist circumference (cm)	72.5 (16.0)	73.6 (9.0)	0.41
BMI (Kg/m^2)	21.9 (3.8)	21.6 (3.6)	0.38
Systolic blood pressure (mmHg)	115.6 (11.3)	116.4 (13.3)	0.52
Diastolic blood pressure (mmHg)	67.1 (7·7)	65.6 (7.8)	0.05
HbA1c (%)	8.4 (1.3)	8.5 (1.3)	0.72
HbA1c (mmol/mol)	68.8 (14.2)	69.3 (14.6)	0.72
Total Cholesterol (mmol/l)	4.36 (0.84)	4.28 (0.90)	0.38
HDL Cholesterol (mmol/l)	1.57 (0.42)	1.55 (0.36)	0.71
LDL Cholesterol, mmol/l	2.31 (0.66)	2.28 (0.66)	0.62
Triglycerides (mmol/l)	0.83 (0.64-1.19)	0.88 (0.62-1.20)	0.72
hsC-Reactive Protein (mg/l)	0.49 (0.19-1.14)	0.59 (0.19-1.20)	0.21
ADMA (nmol/l)	471.2 (74.7)	483.7 (93.2)	0.16
Albumin-creatinine ratio, mg/mmol	0.63 (0.53-0.74)	1.23 (0.99-1.83)	<0.001
Cystatin C (mg/dl)	0.86 (0.13)	0.84 (0.13)	0.22
Creatinine (µmol/l)	55.1 (11.1)	52.0 (10.0)	0.009
eGFR (Schwartz) (ml/min/1.73m^2)	128.5 (21.2)	135.2 (24.6)	0.007
eGFR (Zappitelli) (ml/min/1.73m^2)	104.9 (16.2)	109.2 (17.5)	0.02
Carotid intima-media thickness (mm)	0.440 (0.041)	0.444 (0.051)	0.38

Data are mean (SD), median (interquartile range) or n (%); ADMA: asymmetric; BMI: body mass index; eGFR: estimated glomerular filtration rate; dimethylarginine; hs: high sensitivity.

Table 2. Cox regression models for incident microalbuminuria

UNIVARIATE MODELS			
	HR	**95% CI**	**p value**
ACR group (low vs high)	4.52	2.20-9.33	<0.001
HbA1c, per %	1.39	1.11-1.73	0.003
Sex (Female vs male)	0.61	0.30-1.25	0.18
Age at diagnosis, per year	0.98	0.88-1.08	0.64
Duration at baseline, per year	0.99	0.71-1.13	0.87
Age at baseline, per year	0.89	0.71-1.13	0.35
Systolic BP, per mmHg	0.99	0.97-1.03	0.73
Diastolic BP, per mmHg	0.99	0.95-1.04	0.99
LDL-cholesterol, mmol/l	0.75	0·41-1·37	0.35
eGFR, per ml/min/1.73m^2	1.00	0·98-1·03	0.81
MULTIVARIATE MODEL			
ACR group (Low vs high)	4.29	2.08-8.85	<0.001
HbA1c, per %	1.37	1.10-1.72	0.006

Results are hazard ratios (HR), 95% confidence intervals (CI) and p values. BP: blood pressure; eGFR: estimated glomerular filtration rate

Table 3. Mean over time changes in cardiovascular and renal markers: high- vs low-ACR group

	β-Estimate (SE)	P
Total cholesterol (mmol/l)	-0.11 (0.08)	0.20
HDL cholesterol (mmol/l)	-0.05 (0.03)	0.11
LDL cholesterol (mmol/l)	-0.07 (0.07)	0.29
Triglycerides (mmol/l)	0.10 (0.06)	0.09
ADMA (nmol/l)	2.07 (5.80)	0.72
hsCRP (mg/l)	0.85 (0.32)	0.01
Systolic blood pressure (mmHg)	2.08 (1.00)	0.03
Diastolic blood pressure (mmHg)	-0.06 (0.58)	0.92
eGFR (Zappitelli) (ml/min/1.73m^2)	4.84 (1.49)	0.01
eGFR (Schwartz) (ml/min/1.73m^2)	5.90 (1.71)	0.01
HbA1c (%)	0.08 (0.14)	0.54

Results from linear mixed models, adjusted for age and sex

Data are reported as β-Estimate and standard error (SE). β-Estimate are equal to the mean difference between the high- and low-ACR groups

Figures Legends

Figure 1. Cumulative incidence of microalbuminuria in the low- and high-ACR groups (Time is calculated from the beginning of the study; ACR: albumin creatinine ratio)

Figure 2. Comparison of carotid intima-media thickness at baseline and follow up between the high- and the low-ACR groups. (Data are means and 95% CI. Baseline: p=0.38; Final: p=0.009)

Figure 1. Cumulative incidence of microalbuminuria in the low- and high-ACR groups
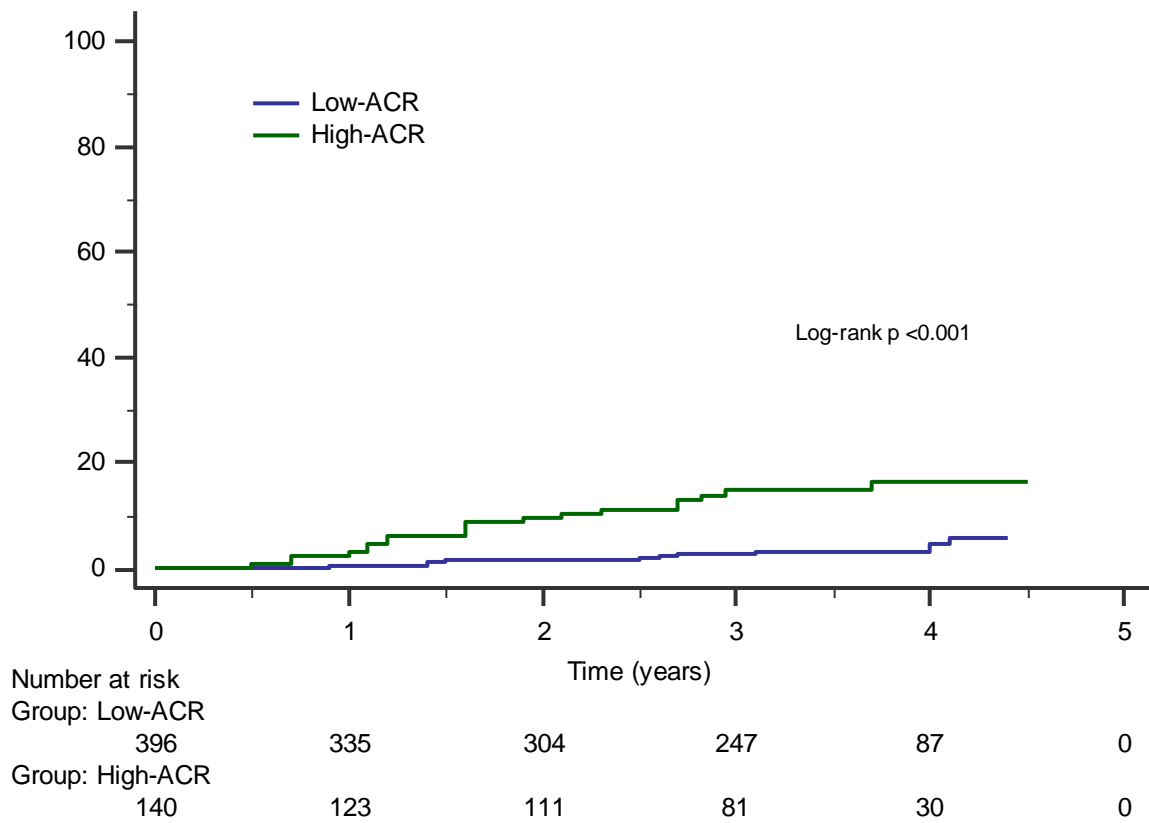
Time is calculated from the beginning of the study.
ACR: albumin creatinine ratio

Figure 2. Comparison of carotid intima-media thickness at baseline and follow up between the high- and the low-ACR groups
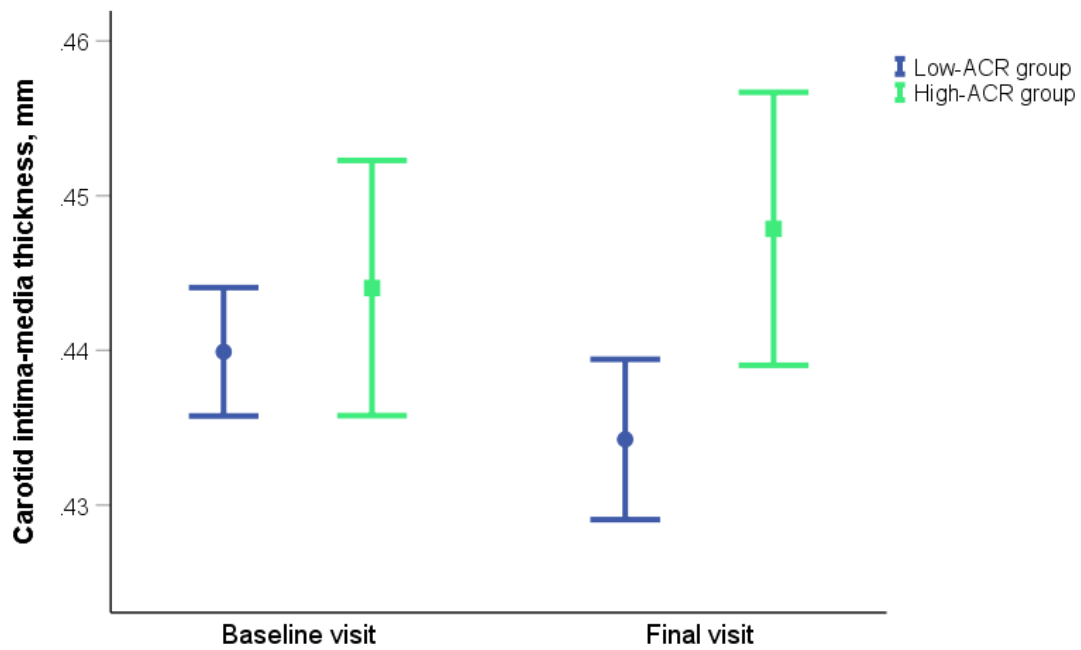
Data are means and 95% CI. Baseline: p=0·38; Final: p=0·009

SUPPLEMENTARY TABLES AND FIGURES

Table S1. Cox regression models for incident microalbuminuria with post-baseline parameters

UNIVARIATE MODELS			
	HR	**95% CI**	**p value**
Mean HbA1c, per %	1·56	1·24-1·98	<0·001
Mean Systolic BP, per mmHg	0·85	1·00-1·03	0·85
Mean Diastolic BP, per mmHg	1·03	0·96-1·09	0·42
Mean LDL-cholesterol, mmol/l	0·75	0·41-1·37	0·35
Mean eGFR, per ml/min/1.73m^2	1·02	1·00-1·04	0·12
MULTIVARIATE MODEL			
ACR group (Low vs high)	4·20	2·03-8·67	<0·001
Mean HbA1c, per %	1·51	1·19-11·91	0·001

Results are hazard ratios (HR), 95% confidence intervals (CI) and p values. BP: blood pressure; eGFR: estimated glomerular filtration rate

Figure S1. Flow of study participants

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                    ┌─────────────────────────────────────────────────────┐
                    │                    Screening                        │
                    │  4407 Adolescents (10-16 yr) with T1D across UK,     │
                    │            Canada and Australia                     │
                    └─────────────────────────────────────────────────────┘
                                          │
                  ┌───────────────────────┴───────────────────────┐
                  │                                                │
        ┌───────────────────────┐                    ┌───────────────────────┐
        │     Trial cohort      │                    │  Observational cohort:│
        │  Upper ACR tertile:   │                    │  Lower and Middle ACR │
        │        N 443          │                    │     tertile: N 404    │
        └───────────────────────┘                    └───────────────────────┘

┌──────────────────────┐   ┌──────────────────────┐   ┌──────────────────────┐
│ Excluded: 334        │   │ Upper tertile ACR    │   │ 8 participants       │
│ participants exposed │←  │ subjects declining   │  →│ withdrew after       │
│ to ACE Inhibitors    │   │ Trial participation  │   │ consent and before   │
│ and statins          │   │ N 41                 │   │ the baseline visit   │
└──────────────────────┘   └──────────────────────┘   └──────────────────────┘

        ┌───────────────────────┐                    ┌───────────────────────┐
        │    High-ACR group     │                    │     Low-ACR group     │
        │        N 150          │                    │        N 396          │
        └───────────────────────┘                    └───────────────────────┘

Follow up: 3.9 (3.1-4.1)years                    Follow up: 3.9 (3.3-4.1) years

        ┌─────────────────────────────────────────────────────────────────┐
        │   Assessed at baseline and every 6 months following a similar    │
        │                          protocol                                │
        └─────────────────────────────────────────────────────────────────┘
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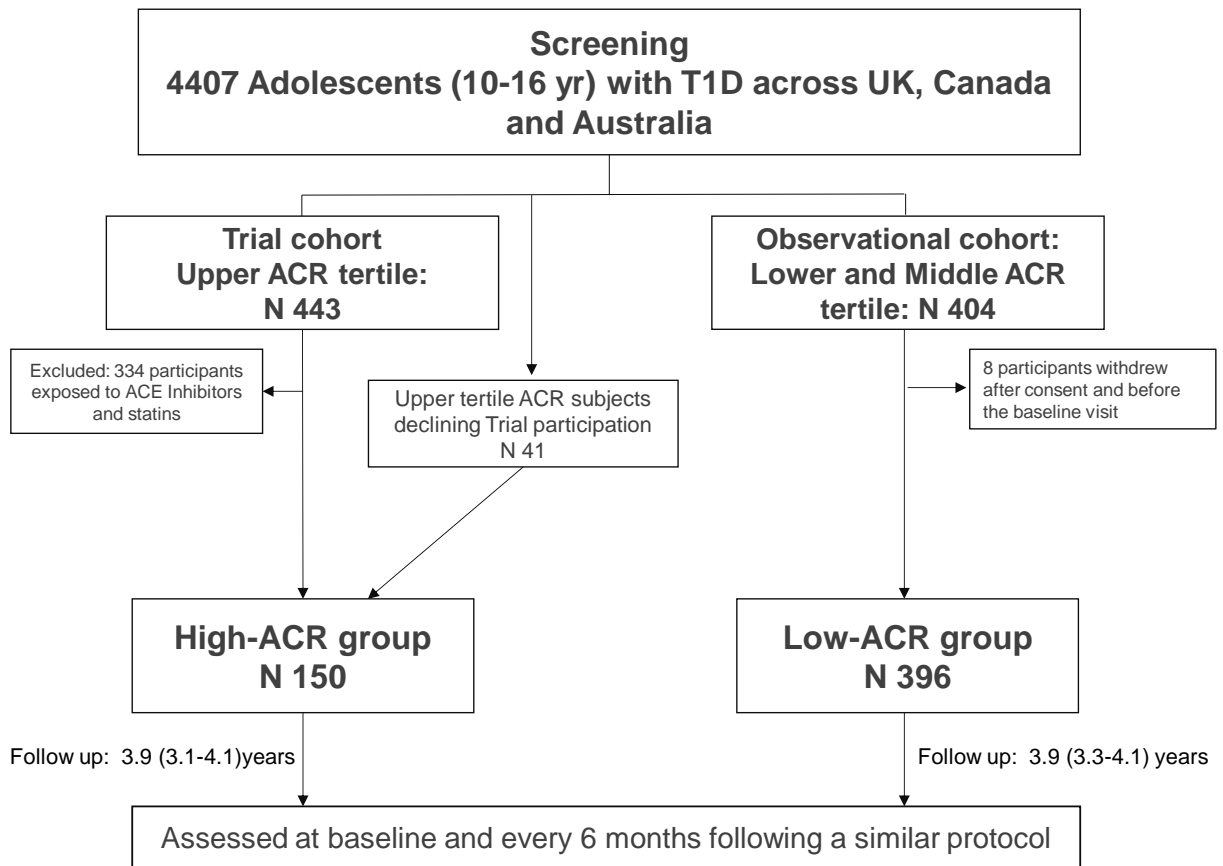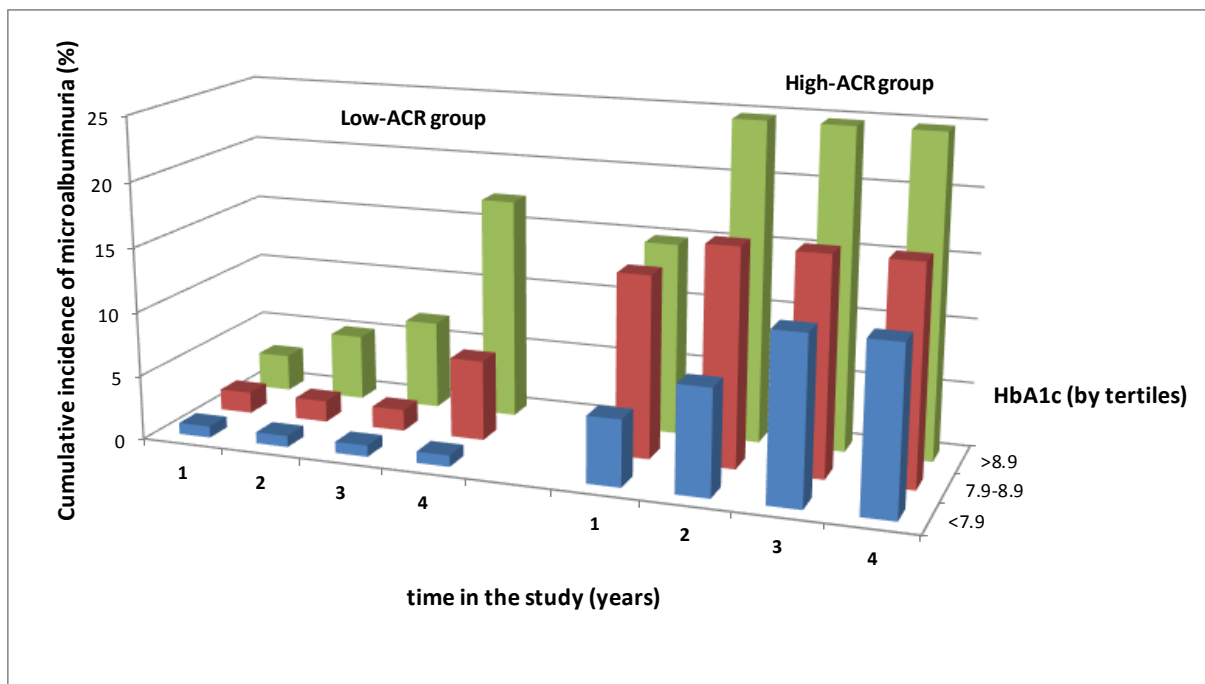
Figure S2. Cumulative incidence of microalbuminuria by tertiles of mean HbA1c

Bars represents cumulative incidence by years in the study (x-axis) and tertiles of mean HbA1c (z-axis) in the low-ACR group (right) and high-ACR group (left). Cumulative incidences by years in the study were calculated by life table method