Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT): Urinary Screening and Baseline Biochemical and Cardiovascular Assessments

*M. Loredana Marcovecchio,[1] John Woodside,[2] Timothy Jones,[3] Denis Daneman,[4] Andrew Neil,[5] Toby Prevost,[6] R. Neil Dalton,[7] John Deanfield,[2] and David B. Dunger,[1] on behalf of the AdDIT Investigators**

OBJECTIVE

We assessed the association between early increases in albumin excretion and cardiovascular (CV) and renal markers in a large cohort of young people with type 1 diabetes.

RESEARCH DESIGN AND METHODS

As part of preliminary screening for a multicenter, randomized controlled trial of statins/ACE inhibitors, we measured albumin–creatinine ratio (ACR) in six early morning urine samples from 3,353 adolescents (10–16 years of age) and calculated tertiles based on an established algorithm. From those subjects deemed to be at higher risk (upper ACR tertile), we recruited 400 into the intervention study (trial cohort). From those subjects deemed to be at lower risk (middle–lower ACR tertiles), we recruited 329 to the observation cohort. At baseline, vascular measurements (carotid intima-media thickness, pulse wave velocity [PWV], flow-mediated dilatation, digital pulse amplitude tonometry), renal markers (symmetric dimethylarginine, cystatin C, creatinine), and CV disease markers (lipids and apolipoproteins [Apo] A-1 and B, C-reactive protein, asymmetric dimethylarginine) were assessed.

RESULTS

Age- and sex-adjusted PWV was higher in the trial than in the observational cohort (5.00 ± 0.84 vs. 4.86 ± 0.70 m/s; $P = 0.021$). Similarly, non-HDL cholesterol (2.95 ± 0.83 vs. 2.81 ± 0.78 mmol/L; $P = 0.02$) and ApoB–ApoA-1 ratio (0.50 ± 0.14 vs. 0.47 ± 0.11; $P = 0.04$) were higher in the trial cohort. Cystatin C and creatinine were decreased (0.88 ± 0.13 vs. 0.90 ± 0.13 mg/L, $P = 0.04$; 51.81 ± 10.45 vs. 55.35 ± 11.05 μmol/L, $P < 0.001$; respectively) and estimated glomerular filtration rate (137.05 ± 23.89 vs. 129.31 ± 22.41 mL/min/1.73 m^2; $P < 0.001$) increased in the trial compared with the observational cohort.

CONCLUSIONS

Our data demonstrate that in adolescents with type 1 diabetes, the group with the highest tertile of albumin excretion showed more evidence of early renal and CV disease than those in the lower tertiles.

Diabetes Care 2014;37:805–813 | DOI: 10.2337/dc13-1634

[1]*Department of Paediatrics, MRL Wellcome Trust-MRC Institute of Metabolic Science, NIHR Cambridge Comprehensive Biomedical Research Centre and the Cambridge Clinical Trial Unit, University of Cambridge, Cambridge, United Kingdom*
[2]*Institute of Child Health, University College London, London, United Kingdom*
[3]*Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Perth, Western Australia, Australia*
[4]*Department of Paediatrics, The Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada*
[5]*Centre for Diabetes, Endocrinology, and Metabolism, University of Oxford, Oxford, United Kingdom*
[6]*Department of Public Health Sciences, Kings College, London, United Kingdom*
[7]*WellChild Laboratory, Evelina Children's Hospital, St. Thomas' Hospital London, London, United Kingdom*

Corresponding author: David B. Dunger, dbd25@cam.ac.uk.

Received 11 July 2013 and accepted 10 October 2013.

A slide set summarizing this article is available online.

J.D. and D.B.D. contributed equally, and M.L.M. and J.W. are joint first authors.

**A complete list of the AdDIT Investigators can be found in the* APPENDIX.

Microalbuminuria is a marker of early stage renal damage in patients with type 1 diabetes, and an independent risk factor for diabetic nephropathy (DN) and cardiovascular (CV) disease (1,2). In young people with childhood-onset type 1 diabetes, microalbuminuria is associated with CV risk factors, such as dyslipidemia, hypertension, subclinical inflammation, hyperfiltration, decline in renal function (3), and markers of subclinical atherosclerosis, such as endothelial dysfunction and increased carotid intima-media thickness (cIMT) (4), suggesting that microalbuminuria is not only a marker of renal disease but of a generalized "endotheliopathy" (5).

In adolescents with type 1 diabetes, increases in albumin excretion within the normal range during the first years after diagnosis can predict future risk of microalbuminuria (6,7). In the Oxford Regional Prospective Study (ORPS) cohort, an albumin–creatinine ratio (ACR) in the upper tertile of the normal range at the age of 11–15 years independently predicted up to 85% of subjects who later developed microalbuminuria and all of the subjects who developed proteinuria (8). In adults, progressive increases in albumin excretion within the normal range are also associated with an increased risk of CV events (9).

These data suggest that albumin excretion is a continuous risk factor for both renal and CV complications of type 1 diabetes and that early screening and interventions during adolescence may reduce the long-term risk for DN and CV disease. However, there are few data on the association between early increases in albumin excretion and early evidence of CV disease in young people with type 1 diabetes.

Using data collected during the screening phase of the international, multicenter Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT) (10), we aimed to assess whether, during puberty, selecting adolescents with type 1 diabetes by different degrees of urinary albumin excretion would allow the identification of subjects at risk for CV and renal complications.

RESEARCH DESIGN AND METHODS

Study Population

The study population consisted of subjects screened and recruited to AdDIT (10).

AdDIT is a multicenter multinational study involving 32 centers in the U.K., Canada, and Australia that is assessing the efficacy of ACE inhibitors (ACEIs) and statins in high-risk adolescents with type 1 diabetes, defined on the basis of an ACR in the upper tertile of the normal range. The major end point of the study is change in urinary albumin excretion, and secondary end points include markers of CV disease, renal function, retinopathy, and quality of life combined with detailed assessment of compliance and health economic benefits.

Subjects were recruited to AdDIT from a prescreened population of adolescents aged 10 to 16 years with type 1 diabetes diagnosed for ≥1 year or C-peptide negative. The diagnosis of type 1 diabetes was based on the American Diabetes Association criteria (11), insulin dependence since diagnosis, and positivity for one or more diabetes-associated autoantibodies.

At the screening visit, data on chronological age, age at diabetes onset, and duration of diabetes were collected. A blood sample was collected for measuring HbA_{1c} at the local laboratories. Screening also included collection of two sets of three consecutive early morning urine samples for the assessment of ACR at two separate study visits. The three ACR measures at each visit were averaged on the log ACR scale (due to non-normal distribution of ACR values), and the subject's average residual was calculated using an algorithm derived from previous longitudinal studies of the natural history of microalbuminuria (10,12). This algorithm allowed adjustments for age, sex, and duration of diabetes. If the subject's residual was above log 1.2, the patient 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 identified high-risk adolescents who were eligible for AdDIT (trial cohort); the lower and middle tertiles identified low-risk adolescents (observational cohort) who were eligible for the observational arm of the study (10).

Inclusion criteria for subjects recruited to AdDIT were *1*) age 10–16 years, *2*) type 1 diabetes diagnosis for more than 1 year or C-peptide negative, and *3*) centralized assessment of ACR based on six early morning urines in the upper tertile (trial cohort) or in lower and middle tertiles (observational cohort) after adjustment for age, sex, age at diagnosis, and duration of disease. Exclusion criteria for both cohorts were *1*) other types of diabetes, *2*) severe hyperlipidemia and family history data to support diagnosis of familial hypercholesterolemia, *3*) established hypertension unrelated to DN, *4*) prior exposure to the investigational products (Statins and ACEIs), *5*) other comorbidities considered unsuitable by the investigator (excluding treated hypothyroidism and celiac disease), and *6*) proliferative retinopathy. Specific exclusion criteria for the trial cohort were *1*) pregnancy or unwillingness to comply with contraceptive advice and regular pregnancy testing throughout the trial and *2*) breast feeding.

Here we report data from the first 729 adolescents recruited to AdDIT and undergoing CV assessments and the biochemistry data from 554 subjects where results have been processed. It is anticipated that this will represent approximately 99% of all CV disease assessments and approximately 75% of all future data collected and will be representative of the final AdDIT cohort. These data support the rationale for patient recruitment, and the results are of interest independently of the clinical trial results.

Baseline Assessment

Baseline visits for those recruited to both the trial and observational arms of AdDIT included measurement of height, weight, waist circumference (WC), and blood pressure (BP).

Height was measured on wall-mounted stadiometers and weight on electronic scales. WC was measured in triplicate with a flexible tape at the midpoint

between the lower margin of the last palpable rib and the top of the iliac crest following World Health Organization guidelines.

Nonfasting blood samples were collected for HbA_{1c} measurements and centralized assessments of CV and renal markers: lipid profile (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, non-HDL cholesterol, apolipoproteins [Apo] A-1 and B), high-sensitivity C-reactive protein (hsCRP), asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), creatinine, and cystatin C.

On a second day, all recruited subjects attended one of the designated centers for standardized CV assessments, including cIMT and, where facilities allowed, flow-mediated dilatation (FMD), digital pulse amplitude tonometry (PAT), and pulse wave velocity (PWV).

The study conformed to the Declaration of Helsinki and was approved by the Cambridge University Hospitals Research Ethics Committee and other local ethics committees. Written informed consent was obtained from the parents and assent from all study participants.

Vascular Assessments

Ultrasound scanning for cIMT was performed at 11 specialist vascular centers. In a subset of patients ($n = 430$), endothelial function was evaluated by FMD and PAT, and arterial stiffness was assessed by PWV.

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 in running large-scale, vascular phenotyping trials in children (13), and included a 1-week intensive training course in London for all study sonographers. Sonographers were accredited before the study commenced, and intrasonographer reproducibility on five subjects scanned at least 1 week apart for cIMT was $<5\%$. Intrasonographer reproducibility for FMD and PWV on five subjects scanned at least 1 week apart was $<16\%$ and $<10\%$, respectively. A single reader analyzed all cIMT and FMD scans in the core laboratory. Intrareader reproducibility on 50 randomly selected study scans was 2.1% and 8.6% for cIMT and FMD, respectively.

Brachial BP was measured three times [Omron M6 (14), Omron Healthcare, The Netherlands], separated by 5-min interval using the appropriate cuff size. The average of the three measurements is used in the analysis. The cuff was chosen to be of the appropriate size for an adolescent's upper arm. As recommended, an inflatable bladder width that was at least 40% of the arm circumference at a point midway between the olecranon and the acromion and that was as length as to cover 80–100% of the circumference of the arm was used (15). Pulse pressure was calculated as systolic minus diastolic BP.

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° from the midpoint. Images triggered on the *R*-wave of the electrocardiogram (ECG) were recorded in Digital Imaging and Communications in Medicine format as cine loop files for offline analysis (Carotid Analyzer, Medical Imaging Applications, Coralville, IA). The cIMT value was taken as the average of three end-diastolic measurements. cIMT measurements were made on single segment of arterial wall 5–10 mm in length and 10 mm proximal to the bifurcation at the posterior side. Measurements were taken both at the right and left sides, and the mean between the two measurements was calculated. Arterial stiffness was assessed by applanation tonometry (SphygmoCor System, AtCor Medical, Australia) as previously described (16). A pressure tonometer was placed on top of the carotid and femoral arteries, and a pressure waveform was recorded for 10 s. Integral software processes the ECG-synchronized pulsed-pressure waveforms and PWV was calculated as the distance and mean time difference between the carotid-femoral pulse points. The average of two PWV measurements was used in the analysis.

Vascular responsiveness to reactive hyperemia was assessed by FMD in a temperature-controlled room (17). Each participant rested in a supine position for 10 min before an optimized longitudinal, ECG-gated image of the right brachial artery was obtained by high-resolution ultrasound. The test began with 5 min of rest, followed by 5 min of ischemia, which was stimulated by inflation of a pneumatic cuff around the forearm at 200 mmHg. Reactive hyperemia was induced after cuff release and measurements continued for 5 min. The change in diameter of the brachial artery was measured offline using an automatic edge-detection algorithm (Brachial Tools, Medical Imaging Applications). Peak blood flow was measured from the velocity-time integral of the Doppler signal within the first 15 s after cuff deflation. FMD was determined as the relative change in diameter before and after cuff release. FMD corrected for shear stress stimulus was calculated by dividing FMD by the maximal velocity-time integral. Digital PAT was measured simultaneously with the FMD test and is used to evaluate microvascular function. A PAT probe was placed on each index finger and gave a uniform, near-diastolic (70 mmHg) external pressure, providing a measure of changes in pulsatile digital blood volume. The (log scaled) reactive hyperemic index was measured by integral software (EndoPAT, Itamar Medical, Caesarea, Israel) and was calculated as the ratio between the pulse wave amplitude during the 1-min period beginning 60 s after cuff release, compared with the average pulse wave amplitude measured during the 5 min of rest before cuff inflation. The ratio was normalized to the signal from the contralateral control finger.

Biochemistry

All biochemical measurements were performed in a central laboratory (WellChild Laboratory, London). 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 an ELISA. Between-batch imprecision for

the BN Prospec 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 as previously described (18,19). Between-batch imprecision (*n* = 30) was 2.8% at 66.1 μmol/L and 2.5% at 333.3 μmol/L. Plasma ADMA and SDMA were measured simultaneously 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 and for SDMA was 4.4% at 416 nmol/L, 2.9% at 1,426 nmol/L, and 3.1% at 4,371 nmol/L.

Cystatin C and hsCRP were 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 and for hsCRP was 5.8% at 0.89 mg/L and 3.6% at 4.73 mg/L.

ApoA-1 and ApoB were measured by nephelometric immunoassay according to the manufacturer's instructions (Siemens BN Prospec). Between-batch imprecision for ApoA-1 (*n* = 37) was 4.0% at 1.06 mg/L and 5.1% at 2.06 mg/L and for ApoB (*n* = 38) was 7.3% at 0.90 mg/L and 1.9% at 1.50 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 mg/L.

HbA_{1c} was assessed in the local laboratories using Diabetes Control and Complications Trial aligned assays.

Calculations

BMI was calculated as the weight/height^2 and expressed as kg/m^2.

z-scores for anthropometric variables were calculated using the the lambda-mu-sigma (LMS) method. Similarly, z-scores for BP was calculated to allow for the effect of age, sex, and height (15).

Estimated glomerular filtration rate (eGFR) was calculated from plasma creatinine with the following formula: eGFR (mL/min/1.73 m^2) = 42 * height (cm) / plasma creatinine (μmol/L) (20).

Statistical Analysis

Data are summarized as mean ± SD or median (interquartile range) unless otherwise specified. Non-normally distributed variables were log transformed before analysis. Comparisons between groups were performed by unpaired *t* tests for continuous variables and by χ^2 or Fisher's exact test for categorical variable. Analysis of covariance was used to adjust for potential confounders.

P values <0.05 were taken as significant. Analyses were performed using SPSS version 17.

RESULTS

General Characteristics of the Screening Population

Completed screening results from 3,353 adolescents (54.6% boys) were included in our analyses if at least two sets of three early morning urine samples were available for the central laboratory assessment. Based on the ACR results and after applying the previous derived algorithm, 959 (28.6%) subjects have been assigned to the lower, 1,112 (33.2%) to the middle, and 1,282 (38.2%) to the upper tertile of ACR.

The general characteristics of the screening population are shown in Table 1. The mean HbA_{1c} level in the whole screened population was 8.3 ± 1.4% (67.6 ± 14.9 mmol/mol), with a 0.1% increase from the lower to the middle tertile, as well as from the middle to the upper tertile of ACR (*P* = 0.003) (Fig. 1). Seventy-five percent of the screened cohort had an HbA_{1c} ≥7.5% (58 mmol/mol). All screened subjects were on a multiple insulin dose regimen or insulin pumps at the time of the screening visits.

As expected, ACR levels (median [interquartile range]) progressively increased across the generated tertiles: 0.52 (0.43–0.62), 0.71 (0.59–0.85), 1.24 (0.91–1.82) mg/mmol (*P* < 0.001). ACR results from the two screening visits showed a good correlation (*r* = 0.61; *P* < 0.001).

The prevalence of microalbuminuria/macroalbuminuria, based on ACR values >3.5 mg/mmol in boys and >4 mg/mmol in girls, was 2.3–2.6% based on the results from a single screening visit. The majority of these cases represented transient or intermittent microalbuminuria, given that the prevalence of persistent microalbuminuria (ACR in the microalbuminuric range at both study visits), was only 0.7%. All cases with microalbuminuria were within the generated upper tertile (1.7% of all upper tertile cases).

Baseline Results: Trial and Observational Cohorts

The baseline population consisted of subjects recruited to the intervention arm of AdDIT (trial cohort) and those recruited to the observational arm of the study (observational cohort). In total, baseline data were available for 729 recruited participants, 400 from the trial cohort and 329 from the observational cohort. The general characteristics of the participants with available results are shown in Table 1. Adolescents in the trial cohort were slightly younger, had shorter diabetes duration, and were diagnosed at an older age. There was a similar sex distribution between the two groups, whereas BMI and WC tended to be higher in the observational cohort.

Vascular Structure and Function

The results of vascular assessments from 400 adolescents from the trial cohort and 329 from the observational cohort are reported in Table 2.

Table 1—General characteristics of the trial and observational cohorts

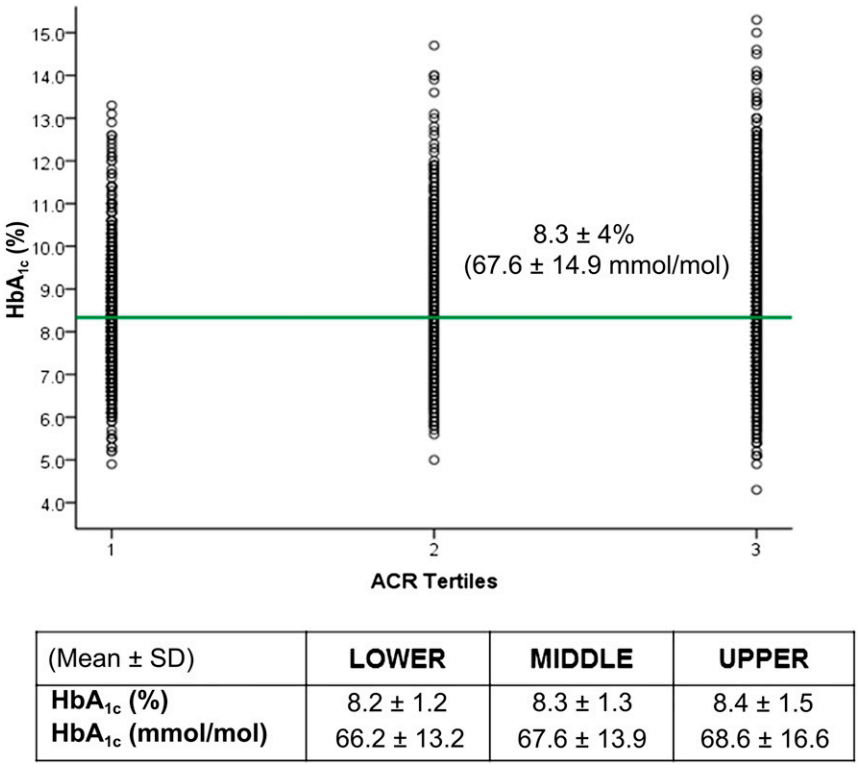
		Cohort		
	Screening population	Trial (upper tertile)	Observational (lower and middle tertiles)	*P* value*
N	3,353	400	329	
Sex (*n* male) (%)	1,831 (54.6)	210 (52.5)	175 (53.2)	0.8
Age (years)	13.0 ± 1.7	13.9 ± 1.6	14.3 ± 1.7	0.006
Age at diagnosis (years)	7.7 ± 3.6	8.1 ± 3.3	6.8 ± 3.5	<0.001
T1D duration (years)	5.3 ± 3.3	5.9 ± 3.1	7.5 ± 3.4	<0.001
HbA_{1c} (%)	8.1 (7.5–9.0)	8.3 (7.6–9.3)	8.2 (7.6–8.9)	0.40
HbA_{1c} (mmol/mol)	65.0 (58.5–74.9)	67.2 (59.6–78.1)	66.1 (59.6–73.8)	
Height (cm)	—	163.5 ± 10.6	163.6 ± 10.3	0.87
Height *z*-score	—	0.49 ± 0.93	0.38 ± 1.00	0.14
Weight (kg)	—	57.7 ± 13.5	59.4 ± 13.8	0.12
Weight *z*-score	—	0.80 ± 0.97	0.82 ± 1.07	0.75
BMI (kg/m^2)	—	21.4 ± 3.7	22.0 ± 3.9	0.04
BMI z-score	—	0.70 ± 1.01	0.82 ± 1.04	0.13
WC (cm)	—	74.7 ± 9.4	76.1 ± 9.9	0.07
WC z-score	—	1.33 ± 1.03	1.42 ± 1.11	0.32

Data are mean ± SD, *n* (%), or median (interquartile range). Missing data were not available at screening. T1D, type 1 diabetes. **P* values are for the comparisons between the trial and the observational cohorts.

Adolescents in the trial cohort had a small but significantly greater age- and sex-adjusted PWV than those in the observational cohort.

Other measures of vascular structure and function (cIMT, FMD, PAT) were not different between the two study groups (Table 2). There were no significant differences in BP between the two study cohorts.

A significant association was found between PWV and systolic BP and diastolic BP z-scores ($r = 0.229$, $P < 0.001$; $r = 0.250$, $P < 0.001$; respectively), whereas no significant association was found between BP measurements and other CV parameters (cIMT, FMD, PAT).



(Mean ± SD)	LOWER	MIDDLE	UPPER
HbA_{1c} (%)	8.2 ± 1.2	8.3 ± 1.3	8.4 ± 1.5
HbA_{1c} (mmol/mol)	66.2 ± 13.2	67.6 ± 13.9	68.6 ± 16.6

Figure 1—HbA_{1c} distribution across tertiles of ACR. *P* value for trend (ANOVA) = 0.003. The horizontal line indicates mean HbA_{1c} in the whole study population.

Biochemical Data

Biomarkers of renal and CV status were available for 307 adolescents recruited to the trial and 254 in the observational cohort (Table 3).

Levels of total cholesterol were higher in the trial than in the observational cohort, although this difference did not reach statistical significance. Levels of non-HDL cholesterol were significantly higher in the trial cohort. In contrast, there were no significant differences in HDL cholesterol, LDL cholesterol, and triglycerides. The ApoB–ApoA-1 ratio was significantly increased in the trial cohort after adjusting for the effect of age and sex (Table 3).

There were no significant differences between the two groups in levels of ADMA and hsCRP (Table 3).

Levels of cystatin C and creatinine were significantly decreased in the trial

Table 2—CV assessment: trial versus observational group

	Trial n = 400	Observational n = 329	P value	Adjusted P value*
cIMT (mm)	0.44 ± 0.05	0.44 ± 0.04	0.92	0.95
FMD (%)	5.38 ± 2.72	5.44 ± 2.87	0.82	0.96
PWV (m/s)	5.00 ± 0.84	4.86 ± 0.70	0.067	0.021
RHI	1.74 ± 0.47	1.81 ± 0.49	0.19	0.28
LnRHI	0.518 ± 0.278	0.555 ± 0.271	0.17	0.24
SBP (mmHg)	111.9 ± 9.1	111.8 ± 9.3	0.92	
SBP z-score	0.06 ± 0.78	0.02 ± 0.81	0.52	
SBP >90th percentile (%)	3.1	2.6	0.41	
DBP (mmHg)	62.1 ± 7.0	63.0 ± 6.2	0.09	
DBP z-score	−0.19 ± 0.63	−0.13 ± 0.58	0.17	
DBP >90th percentile (%)	17.2	14.9	0.41	
Pulse pressure	49.8 ± 9.2	48.9 ± 8.5	0.1	

Data are mean ± SD. RHI, reactive hyperemia index; LnRHI, log-scaled reactive hyperemic index; SBP, systolic BP; DBP, diastolic BP. *Age and sex adjusted.

compared with the observational cohort. This was associated with higher eGFR levels in the former group. In contrast, levels of SDMA were similar between the two groups (Table 3).

CONCLUSIONS

This study shows that adolescents with type 1 diabetes who have raised ACR, but still within the normal range, already have increased arterial stiffening and abnormal lipid profiles and show evidence of hyperfiltration, compared with those with low–moderate ACR levels. The ongoing randomized controlled trial, AdDIT, designed to target these abnormalities, will determine whether early treatment with ACEIs, statins, and/or their combination will have a beneficial effect in these young patients who are at risk for developing not just renal, but also CV complications in later life.

It was recognized from the ORPS study that adolescents with type 1 diabetes and ACR levels within the upper tertile of the normal range are more likely to progress to microalbuminuria (8), which in adults is associated with increased risk of DN and CV disease (1,2). We now show that in the AdDIT trial cohort, even modest elevations in albumin excretion are associated with an increase in age- and sex-adjusted PWV, a composite measure of arterial stiffness. This was in the presence of no differences in other key measures of vascular function and structure, such as cIMT and nitric-oxide-dependent endothelial function. A possible explanation for this early rise in arterial stiffness could be a specific adaptation within the smooth muscle compartment of the arterial wall, whereas more adverse vascular structure and functional changes, including increased cIMT and impaired FMD, might only become evident at a more advanced stage in the progression toward renal disease (21,22). Similarly, previous studies performed in populations of young people with type 1 diabetes mainly showed differences in cIMT or FMD when comparing subjects with and without microalbuminuria (23). Our results are also in line with data from adults with type 1 diabetes, where PWV was one of the first detectable vascular alterations in those with microalbuminuria and macroalbuminuria (24). Increased PWV has emerged as a predictor of future CV events and mortality (24). The vascular reassessment at the end of AdDIT will test the hypothesis that PWV evolution at a very young age can be modified through early treatment with ACEIs and statins, both alone and in combination.

Table 3—Baseline biochemical data: trial versus observational cohorts

	Trial n = 307	Observational n = 254	P value	Adjusted P value*
TC (mmol/L)	4.50 ± 0.86	4.37 ± 0.80	0.06	0.09
HDL cholesterol (mmol/L)	1.54 ± 0.36	1.56 ± 0.37	0.41	0.09
LDL cholesterol (mmol/L)	2.38 ± 0.65	2.31 ± 0.65	0.25	0.21
TG (mmol/L)	0.85 (0.62–1.23)	0.81 (0.62–1.19)	0.37	0.21
Non-HDL cholesterol (mmol/L)	2.95 ± 0.83	2.81 ± 0.78	0.02	0.01
ApoA-1 (g/L)	1.51 ± 0.23	1.53 ± 0.24	0.24	0.06
ApoB (g/L)	0.74 ± 0.20	0.71 ± 0.16	0.35	0.20
ApoB–ApoA-1 ratio	0.50 ± 0.14	0.47 ± 0.11	0.12	0.02
SDMA (nmol/L)	397.88 ± 57.73	396.86 ± 57.73	0.84	0.76
Cystatin C (mg/L)	0.88 ± 0.13	0.90 ± 0.13	0.04	0.04
Creatinine (μmol/L)	51.81 ± 10.45	55.35 ± 11.05	<0.001	0.009
eGFR (mL/min/1.73 m^2)	137.05 ± 23.89	129.31 ± 22.41	<0.001	0.003
ADMA (nmol/L)	467.04 ± 79.66	467.21 ± 77.04	0.56	0.47
hsCRP (mg/mL)	0.49 (0.16–1.20)	0.45 (0.20–0.8)	0.25	0.54

Data are mean ± SD or median (interquartile range). TC, total cholesterol; TG, triglycerides. *P value adjusted for age and sex.

The link between renal and CV disease in type 1 diabetes remains poorly understood. Previous studies have shown an association between albumin excretion and lipid parameters, including total cholesterol, LDL cholesterol, and non-HDL cholesterol (25,26). We found that levels of non-HDL cholesterol were significantly increased in subjects with higher ACR. Non-HDL cholesterol provides a measure of cholesterol contained in all the atherogenic lipoproteins and, in a recent meta-analysis of studies in adults treated with statins, was a better predictor of future CV events than LDL cholesterol (27). We also found a significantly increased ratio between the proatherogenic ApoB and the protective ApoA-1 in subjects with increased ACR, which in adults has consistently been associated with a higher CV risk (28). In the Young Finns

Study, the ApoB–ApoA-1 ratio was directly related to cIMT, FMD, and PWV during adulthood (29), supporting its value as marker for future CV disease development.

In contrast, no differences between novel CV risk markers, such as ADMA and hsCRP, were found between the trial and observational cohorts. Elevated hsCRP has been shown in numerous cohorts with high CV disease risk (30) but this marker is increased in young people with type 1 diabetes only after the onset of microalbuminuria (31). Similarly, in adults with type 1 diabetes, ADMA, an endogenous inhibitor of nitric oxide synthase, has been associated with DN and CV disease (32). Previously, we could not detect any difference in ADMA in young people with and without microalbuminuria (33). Our current findings are in agreement with previous work, which showed that ADMA, like hsCRP, is only elevated in the presence of more advanced stages of DN (34).

There was evidence of hyperfiltration in the trial cohort, as indicated by higher eGFR levels and decreased levels of cystatin C, when compared with the observational group. In recent years, there has been interest in the identification of circulating plasma/serum markers of glomerular filtration rate (GFR), which could be implemented in clinical practice, instead of the cumbersome gold standard infusion techniques. Several studies have reported that cystatin C is a useful circulating marker, inversely related to GFR, both in adult and in pediatric populations (35). Our results on cystatin C and eGFR are consistent with previous observations that hyperfiltration is frequent among adolescents with type 1 diabetes who later go on to develop microalbuminuria (36). Increased GFR has long been recognized as an early stage of DN in young patients with type 1 diabetes, where it is often associated with increased renal size (37). This early stage of hyperfiltration has been shown to be a predictor for later development of microalbuminuria and decline in renal function (37) and has been also associated with endothelial dysfunction and reduced arterial stiffness, suggesting that hyperfiltration is a marker of a more general endothelial dysfunction (38). Therefore, prevention of hyperfiltration during adolescence could be an important target for reduction of future complications. Surprisingly, we could not detect any difference in SDMA, which has previously emerged as another sensitive and reliable circulating marker of GFR in adolescence with established microalbuminuria (39).

Our results on renal and CV markers and vascular measurements were obtained from a subset of the AdDIT population and not from the full cohort, and this could represent a potential study limitation. However, these subjects were representative of the whole cohort in terms of age, diabetes duration, age at diagnosis, and glycemic control.

Although adjustments for diabetes duration, age at diagnosis, and sex were made in the calculation of ACR tertiles, adolescents in the trial group were older at the time of diabetes onset and showed shorter diabetes duration. The shorter diabetes duration could suggest that subjects at higher risk of developing renal and CV complications show increases in ACR earlier in the natural history of diabetes and closer to pubertal years. This might be the result of an interaction between puberty and the individual's genetic predisposition in accelerating the appearance of early signs of complications. Interestingly, this is in line with previous data from the ORPS cohort, where it was found that although the cumulative prevalence of microalbuminuria was unaffected by age at diagnosis, subjects with an early onset of diabetes (i.e., before age 5 years) had a longer "free" interval before the onset of microalbuminuria (40). However, in the current study, the differences in age at diagnosis and duration might also reflect the effect of other environmental factors that we did not explore.

Another potential study limitation is related to the assessment of BP, which did not include a 24-h ambulatory BP monitoring, therefore limiting the possibility of assessing the circadian BP rhythm and potential early BP abnormalities, which were previously linked to microalbuminuria (12,41). In addition, BP assessment was based on an oscillometric instead of the recommended auscultatory technique, although the device used in the current study has been previously validated both in adult and pediatric populations (14).

Inthe current study, the abnormal CV and renal profiles in subjects with increased ACR appeared to be independent of glycemic control, given the small difference (0.1%) in HbA_{1c} between the trial and observational cohorts. However, overall glycemic control in the AdDIT screening population was above the recommended levels for the specific age range, with ≥75% of subjects having values ≥7.5% (58 mmol/mol) (42). Nevertheless, glycemic control was better than that reported in previous population-based studies of cohorts with type 1 diabetes of similar age than the AdDIT screening population, likely reflecting recruitment bias and the beneficial effect of the implementation of more intensive insulin regimens during recent years (26,42). This may also explain differences in age and sex distribution from the previous ORPS/Nephropathy Family Study, from which the tertile algorithm was derived. However, overall, these data emphasize the difficulty of achieving HbA_{1c} less than 7.5% in adolescence due to the combination of specific physiological and psychological characteristics of this period of life.

The AdDIT baseline data for adolescents with type 1 diabetes show that higher-than-average albumin excretion during adolescence is associated with worse measures of DN and CV disease development, independently of HbA_{1c}. This emphasizes the importance of ACR as an early risk marker. Early screening for abnormal ACR and effective interventions during adolescence could reduce lifelong risk of DN and CV disease. The randomized controlled AdDIT will determine the potential benefits of early ACEIs and/or statins treatment on renal and CV disease development.

Acknowledgments. The authors thank the AdDIT Steering Committee for support: S. Marshall (Department of Diabetes and

Metabolism, The Medical School, University of Newcastle upon Tyne), J. Armitage (CTSU University of Oxford), P. Bingley (Diabetes and Metabolism, Department of Clinical Science at North Bristol, University of Bristol), and W. Van't Hoff (Great Ormond Street Hospital, Great Ormond Street, London); the AdDIT Data Monitoring and Ethics Committee for support: C. Baigent (CTSU University of Oxford), Jon Emberson (CTSU University of Oxford), Marcus Flather (Faculty of Medicine and Health Sciences, University of East Anglia Norwich), Rudy Bilous (James Cook University Hospital, Middlesbrough); the study coordinators: Stella Silvester (Department of Paediatrics, University of Cambridge, U.K.), Yesmino Elia (Department of Endocrinology, The Hospital for Sick Children, Toronto, Canada), Barbara Sheil (Telethon Institute for Child Health Research, UWA, Perth, Australia); and Diane Picton, Tracey Stevens, and other members of the Cambridge Clinical Trial Unit (CCTU), University of Cambridge, U.K. The authors also thank all the research nurses involved in the study and all the sonographers who performed the vascular assessments and all participants for their involvement and commitment. The authors acknowledge support from the National Institute for Health Research Cambridge Biomedical Research Centre.

Funding. AdDIT is funded by Diabetes UK, the Juvenile Diabetes Research Foundation, and the British Heart Foundation. The study is also funded in Canada by the Canadian Diabetes Association and the Heart and Stroke Foundation.

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, and approval of the manuscript.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.L.M. analyzed and interpreted data, drafted the manuscript, and performed statistical analysis. J.W. acquired data, analyzed and interpreted data, and drafted the manuscript. T.J., D.D., and J.D. developed the study concept and design, acquired data, critically revised the manuscript for important intellectual content, obtained funding, and supervised the study. A.N. developed the study concept and design, critically revised the manuscript for important intellectual content, and obtained funding. T.P. developed the study concept and design, analyzed and interpreted data, critically revised the manuscript for important intellectual content, obtained funding, and supervised the study. R.N.D. developed the study concept and design, acquired data, critically revised the manuscript for important intellectual content, and obtained funding. D.B.D. developed the study concept and design, acquired data, analyzed and interpreted data, drafted the manuscript, critically revised the manuscript for important intellectual content, obtained funding, and supervised the study. M.L.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix

AdDIT Investigators

U.K. investigators are Carlo Acerini (Cambridge), Fran Ackland (Northampton), Binu Anand (Bury St Edmunds), 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 Hamilton-Shield (Bristol), James Heywood (Cambridge), Nicola Leech (Newcastle upon Tyne), Nick Mann (Reading), Richard Parker (Cambridge), Gerry Rayman (Ipswich), Jonathon Mark Robinson (Wigan), Michelle Russell-Taylor (High Wycombe), Vengudi Sankar (Bolton), Nandu Thalange (Norwich), and Mark Wilson (Cambridge).

Australia investigators are Phil Bergman (Melbourne), Christine Rodda (Melbourne), Fergus Cameron (Melbourne), Andrew Cotterill (Brisbane), Jennifer Couper (Adelaide), Elizabeth Davis (Perth), Kim Donaghue (Sydney), Maria Craig (Sydney), Bruce King (Newcastle), and Charles Verge (Sydney).

Canada investigators are Farid Mahmud (Toronto), Cheril Clarson (London, Ontario), Jacqueline Curtis (Toronto), and Etienne Sochett (Toronto).

References

1. Rossing P, Hougaard P, Borch-Johnsen K, Parving HH. Predictors of mortality in insulin dependent diabetes: 10 year observational follow up study. BMJ 1996; 313:779–784
2. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1982;1:1430–1432
3. Marcovecchio ML, Tossavainen PH, Dunger DB. Status and rationale of renoprotection studies in adolescents with type 1 diabetes. Pediatr Diabetes 2009;10:347–355
4. Krantz JS, Mack WJ, Hodis HN, Liu CR, Liu CH, Kaufman FR. Early onset of subclinical atherosclerosis in young persons with type 1 diabetes. J Pediatr 2004;145:452–457
5. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage. The Steno hypothesis. Diabetologia 1989;32:219–226
6. Schultz CJ, Neil HA, Dalton RN, Dunger DB; Oxforn Regional Prospective Study Group. Risk of nephropathy can be detected before the onset of microalbuminuria during the early years after diagnosis of type 1 diabetes. Diabetes Care 2000;23:1811–1815
7. Stone ML, Craig ME, Chan AK, Lee JW, Verge CF, Donaghue KC. Natural history and risk factors for microalbuminuria in adolescents with type 1 diabetes: a longitudinal study. Diabetes Care 2006;29:2072–2077
8. Dunger DB, Schwarze CP, Cooper JD, et al. Can we identify adolescents at high risk for nephropathy before the development of microalbuminuria? Diabet Med 2007;24: 131–136
9. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, et al. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. Circulation 2004;110:32–35
10. Adolescent type 1 Diabetes cardio-renal Intervention Trial Research Group. Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT). BMC Pediatr 2009;9:79
11. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012;35(Suppl. 1):S64–S71
12. Marcovecchio ML, Dalton RN, Schwarze CP, et al. Ambulatory blood pressure measurements are related to albumin excretion and are predictive for risk of microalbuminuria in young people with type 1 diabetes. Diabetologia 2009;52: 1173–1181
13. Donald AE, Charakida M, Falaschetti E, et al. Determinants of vascular phenotype in a large childhood population: the Avon Longitudinal Study of Parents and Children (ALSPAC). Eur Heart J 2010;31: 1502–1510
14. Stergiou GS, Yiannes NG, Rarra VC. Validation of the Omron 705 IT oscillometric device for home blood pressure measurement in children and adolescents: the Arsakion School Study. Blood Press Monit 2006;11:229–234
15. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004; 114(Suppl. 4th Report):555–576
16. Charakida M, Jones A, Falaschetti E, et al. Childhood obesity and vascular phenotypes: a population study. J Am Coll Cardiol 2012;60:2643–2650
17. Donald AE, Halcox JP, Charakida M, et al. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. J Am Coll Cardiol 2008;51:1959–1964
18. Greenberg N, Roberts WL, Bachmann LM, et al. Specificity characteristics of 7 commercial creatinine measurement procedures by enzymatic and Jaffe method principles. Clin Chem 2012;58:391–401
19. Kilbride HS, Stevens PE, Eaglestone G, et al. Accuracy of the MDRD (Modification of Diet in Renal Disease) study and CKD-EPI (CKD Epidemiology Collaboration) equations for estimation of GFR in the elderly. Am J Kidney Dis 2013;61:57–66

20. Mattman A, Eintracht S, Mock T, et al. Estimating pediatric glomerular filtration rates in the era of chronic kidney disease staging. J Am Soc Nephrol 2006;17:487–496
21. Cé GV, Rohde LE, da Silva AM, Puñales MK, de Castro AC, Bertoluci MC. Endothelial dysfunction is related to poor glycemic control in adolescents with type 1 diabetes under 5 years of disease: evidence of metabolic memory [published correction appears in J Clin Endocrinol Metab 2011;96: 1908]. J Clin Endocrinol Metab 2011;96: 1493–1499
22. Polak JF, Backlund JY, Cleary PA, et al.; DCCT/EDIC Research Group. Progression of carotid artery intima-media thickness during 12 years in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study. Diabetes 2011;60:607–613
23. Järvisalo MJ, Jartti L, Näntö-Salonen K, et al. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. Circulation 2001;104: 2943–2947
24. Theilade S, Lajer M, Persson F, Joergensen C, Rossing P. Arterial stiffness is associated with cardiovascular, renal, retinal, and autonomic disease in type 1 diabetes. Diabetes Care 2013;36:715–721
25. Maahs DM, Maniatis AK, Nadeau K, Wadwa RP, McFann K, Klingensmith GJ. Total cholesterol and high-density lipoprotein levels in pediatric subjects with type 1 diabetes mellitus. J Pediatr 2005;147:544–546
26. Marcovecchio ML, Dalton RN, Prevost AT, et al. Prevalence of abnormal lipid profiles and the relationship with the development of microalbuminuria in adolescents with type 1 diabetes. Diabetes Care 2009;32: 658–663
27. Boekholdt SM, Arsenault BJ, Mora S, et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. JAMA 2012;307:1302–1309
28. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy—a review of the evidence. J Intern Med 2006;259:493–519
29. Juonala M, Kähönen M, Laitinen T, et al. Effect of age and sex on carotid intima-media thickness, elasticity and brachial endothelial function in healthy adults: the cardiovascular risk in Young Finns Study. Eur Heart J 2008;29:1198–1206
30. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension 2004;44:6–11
31. Marcovecchio ML, Giannini C, Widmer B, et al. C-reactive protein in relation to the development of microalbuminuria in type 1 diabetes: the Oxford Regional Prospective Study. Diabetes Care 2008;31:974–976
32. Tarnow L, Hovind P, Teerlink T, Stehouwer CD, Parving HH. Elevated plasma asymmetric dimethylarginine as a marker of cardiovascular morbidity in early diabetic nephropathy in type 1 diabetes. Diabetes Care 2004;27:765–769
33. Marcovecchio ML, Widmer B, Turner C, Dunger DB, Dalton RN. Asymmetric dimethylarginine in young people with Type 1 diabetes: a paradoxical association with HbA (1c). Diabet Med 2011;28:685–691
34. Lin J, Glynn RJ, Rifai N, et al. Inflammation and progressive nephropathy in type 1 diabetes in the diabetes control and complications trial. Diabetes Care 2008;31: 2338–2343
35. Zhang Z, Lu B, Sheng X, Jin N. Cystatin C in prediction of acute kidney injury: a systemic review and meta-analysis. Am J Kidney Dis 2011;58:356–365
36. Amin R, Turner C, van Aken S, et al. The relationship between microalbuminuria and glomerular filtration rate in young type 1 diabetic subjects: The Oxford Regional Prospective Study. Kidney Int 2005;68: 1740–1749
37. 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;52:691–697
38. Cherney DZ, Sochett EB, Lai V, et al. Renal hyperfiltration and arterial stiffness in humans with uncomplicated type 1 diabetes. Diabetes Care 2010;33:2068–2070
39. Marcovecchio ML, Dalton RN, Turner C, et al. Symmetric dimethylarginine, an endogenous marker of glomerular filtration rate, and the risk for microalbuminuria in young people with type 1 diabetes. Arch Dis Child 2010;95:119–124
40. Amin R, Widmer B, Prevost AT, et al. Risk of microalbuminuria and progression to macroalbuminuria in a cohort with childhood onset type 1 diabetes: prospective observational study. BMJ 2008; 336:697–701
41. Lurbe E, Redon J, Kesani A, et al. Increase in nocturnal blood pressure and progression to microalbuminuria in type 1 diabetes. N Engl J Med 2002;347:797–805
42. Rewers M, Pihoker C, Donaghue K, Hanas R, Swift P, Klingensmith GJ. Assessment and monitoring of glycemic control in children and adolescents with diabetes. Pediatr Diabetes 2009;10(Suppl. 12):71–81