Early Atherosclerosis Relates to Urinary Albumin Excretion and Cardiovascular Risk Factors in Adolescents With Type 1 Diabetes: Adolescent Type 1 Diabetes cardio-renal Intervention Trial (AdDIT)

OBJECTIVE
The origins of cardiovascular and renal disease in type 1 diabetes begin during childhood. We aimed to evaluate carotid (cIMT) and aortic intima-media thickness (aIMT) and their relationship with cardiovascular risk factors and urinary albumin excretion in adolescents with type 1 diabetes in the Adolescent Type 1 Diabetes cardio-renal Intervention Trial (AdDIT).

RESEARCH DESIGN AND METHODS
A total of 406 adolescents with type 1 diabetes, who were 14.1 ± 1.9 years old with type 1 diabetes duration of 6.7 ± 3.7 years, and 57 age-matched control subjects provided clinical and biochemical data and ultrasound measurements of vascular structure (cIMT and aIMT). Vascular endothelial and smooth muscle function was also measured in 123 of 406 with type 1 diabetes and all control subjects.

RESULTS
In type 1 diabetic subjects, mean/maximal aIMT ($P < 0.006; <0.008$), but not mean/maximal cIMT, were greater than in control subjects. Mean/maximal aIMT related to urinary albumin-to-creatinine ratio (multiple regression coefficient [SE], 0.013 [0.006], $P = 0.03; 0.023 [0.007], P = 0.002$), LDL cholesterol (0.019 [0.008], $P = 0.02; 0.025 [0.011], P = 0.02$), and age (0.010 [0.004], $P = 0.004; 0.012 [0.005], P = 0.01$), independent of other variables. Mean/maximal cIMT was greater in males (0.023 [0.006], $P = 0.02; 0.029 [0.007], P = <0.0001$), and mean cIMT related independently to systolic blood pressure (0.001 [0.001], $P = 0.04$). Vascular smooth muscle function related to aIMT and cIMT but not to urinary albumin excretion.

CONCLUSIONS
aIMT may be a more sensitive marker of atherosclerosis than cIMT in type 1 diabetes during mid-adolescence. Higher urinary albumin excretion, even within the normal range, is associated with early atherosclerosis and should direct clinical attention to modifiable cardiovascular risk factors.
Children with type 1 diabetes are at greatly increased risk for the development of both renal and cardiovascular disease in later life (1,2). Evidence is accumulating that these two complications may have a common pathophysiology, with endothelial dysfunction a key early event.

Microalbuminuria is a recognized marker of endothelial damage (3) and predicts progression to proteinuria and diabetic nephropathy, as well as to atherosclerosis (4) and increased cardiovascular risk (5). It is, however, rare in adolescents with type 1 diabetes who more often have higher urinary albumin excretion rates within the normal range, which are associated with later progression to microalbuminuria and proteinuria (6). Renal decline may, however, precede microalbuminuria, at least in adults with type 1 diabetes (7,8), and the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study irrefutably confirms the importance of intensive therapy to reduce risk at each stage of the progression to proteinuria (9).

The Adolescent Type 1 Diabetes cardiorenal Intervention Trial (AdDIT) (10) is designed to examine the impact of minor differences in albumin excretion in adolescents on the initiation and progression of cardiovascular and renal disease. The primary cardiovascular end point in AdDIT is carotid intima-media thickness (cIMT). Subclinical atherosclerosis can be detected noninvasively using high-resolution ultrasound to measure the intima-media thickness (IMT) of the carotid arteries, which predicts cardiovascular morbidity and mortality (11,12). Whereas cIMT is increased by late adolescence and during early adulthood in type 1 diabetes (13), this finding is inconsistent in earlier childhood, when the first changes of atherosclerosis begin. Autopsy studies in children show that the earliest atherosclerotic changes (fatty streaks) occur in the distal abdominal aortic wall (14); aorta IMT (aIMT) is measurable and increased in children and adolescents with type 1 diabetes (15,16). It is also possible that drivers of atherosclerosis differ between different vascular beds.

The primary aim of this study was to examine the relationship of increased urinary albumin excretion and cardiovascular risk factors in adolescents with type 1 diabetes with structural arterial wall changes. We hypothesized that even minor increases in albumin excretion would be associated with early atherosclerosis but that this would be detectable only in the abdominal aorta. Secondary aims were to compare cIMT and aIMT in type 1 diabetes and control subjects and in a subgroup examine the relationship of vascular endothelial and smooth muscle function with IMT and urinary albumin excretion.

### RESEARCH DESIGN AND METHODS

#### Subjects and Study Design

A total of 406 adolescents, aged 10–16 years, with type 1 diabetes for more than 1 year, recruited in five centers across Australia, were enrolled in this cross-sectional study (Table 1). Some participants (n = 337) had been screened for urinary albumin excretion as part of AdDIT, a multicenter multinational (U.K., Canada, and Australia) randomized controlled trial (clinical trial reg. no. ISRCTN91419926) (10). An additional 69 participants were not screened for the AdDIT study but were recruited using the same inclusion criteria and investigations, except for having one early morning urinary albumin-to-creatinine ratio (ACR) only.

The AdDIT screening measured ACR in two sets of three consecutive early-morning urine samples. The two screening visits showed a close correlation, and the median of the six urinary ACR measurements was used in analysis (17).

Exclusion criteria were other types of diabetes without detected islet autoantibodies at diagnosis, severe hyperlipidemia and/or familial hypercholesterolemia, hypertension, exposure to ACE inhibitors or statins, other comorbidities considered unsuitable by the investigator (excluding treated hypothyroidism and celiac disease), and proliferative retinopathy.

Healthy age- and sex-matched control subjects (n = 57) were recruited at the central site, Adelaide, South Australia, from relatives and school friends of the participants with type 1 diabetes. They had the same clinical, biochemical, and ultrasound assessments. They were compared with 167 adolescents with type 1 diabetes for IMT and 123 adolescents with type 1 diabetes for flow-mediated dilatation (FMD) and glyceryl trinitrate-mediated dilatation (GTN), and all had their images measured on the same ultrasound machine in Adelaide.

The study was approved by the Human Research Ethics Committee of each of the five participating centers in Australia. Written informed consent was obtained from all parents/guardians and the study participants.

#### Clinical Assessments

All participants were required to be well without fever, intercurrent infection, or

<table>
<thead>
<tr>
<th>Table 1—Characteristics of adolescents with type 1 diabetes and control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>BMI z score</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
</tr>
<tr>
<td>Mean SBP (mmHg)</td>
</tr>
<tr>
<td>Mean DBP (mmHg)</td>
</tr>
<tr>
<td>HbA₁c (%), mmol/mol</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
</tr>
<tr>
<td>Urinary ACR (mg/mmol)</td>
</tr>
<tr>
<td>Race (% European/Asian/other)</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated.
ketosis on the days of investigation. Data on age, duration of diabetes, anthropometry (weight, height, and waist circumference), pubertal (Tanner) stage, and blood pressure were collected. Height was measured on wall-mounted stadiometers and weight on electronic scales. Waist circumference 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.

Brachial blood pressure was measured three times (Omran M6 Blood Pressure Monitor; Kyoto), separated by 5-min intervals using the appropriate cuff size. The average of the three measurements was used in the analysis. The cuff was chosen to be of the appropriate size for the adolescents’ upper arm, with a bladder width that was at least 40% of the arm circumference at a point midway between the olecranon and the acromion and a bladder length to cover 80–100% of the circumference of the arm.

A blood sample was collected for HbA1c and lipid profile (total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol) measurement at the local laboratories.

**Ultrasound Assessment of Vascular Structure and Function**

Ultrasound images of vascular structure (aIMT and cIMT) were collected at five Australian vascular centers by accredited vascular sonographers. All images were read at the central site in Adelaide. Reproducibility and quality control among the measurements of IMT in each center and between centers were ensured as follows: 1) sonographer training conducted through the Vascular Imaging Unit at the Women and Children’s Hospital, Adelaide, which has extensive experience over 15 years in running vascular health studies in children; 2) intrasonographer reproducibility on scans produced on two separate occasions in the same five subjects for aIMT was 2.8% and on scans produced on two separate occasions in the same 13 subjects for cIMT was 3.6%; 3) three trial images were sent and assessed for quality before the sonographer was accredited to be part of the study; 4) all images were read by one of two independent observers (O.M. and M. La Forgia) at the central site who were blinded to the subjects’ clinical characteristics; and 5) intraobserver coefficient of variation (CV) in 33 subjects, within this cohort, was 0.6% for cIMT and 1.4% for aIMT, and interobserver CV in 20 subjects, within this cohort, was 1.8% for cIMT and 2.3% for aIMT.

Ultrasound images of left and right common carotid arteries and abdominal aorta were acquired using our standardized protocol (16). For cIMT, images of the posterior wall of the distal 10-mm-long arterial segment of the common carotid artery, just 1 cm proximal to the carotid bulb, were recorded. For aIMT, images of the straight, most distal 10 mm of the abdominal aorta just before the bifurcation were recorded. A minimum of three images at end diastole, triggered on the R wave of the electrocardiogram (ECG), for each of the common carotid arteries and abdominal aorta was taken and digitally stored for later analysis. The greatest distance between the lumen-intima interface and media-adventitia interface was measured at a minimum of 100 points using a semiautomated edge detection and measurement computer software package (B Bailey; Royal Prince Alfred Hospital, NSW, Australia). Three best quality images for cIMT (right and left carotids) and aIMT were selected and analyzed for mean and maximal IMT. The mean of the readings was recorded to give the final result for each subject.

Ultrasound assessments of vascular endothelial function measured by FMD and vascular smooth muscle function measured by GTN were performed at the central site only. FMD and GTN were assessed as previously described (18). Brachial artery diameter was measured in a longitudinal section 2–15 cm above the elbow using B mode ultrasound with a 17-MHz linear array transducer (Philips IU22; Philips, Bothell, WA). An ECG was recorded simultaneously with the ultrasound images. Each study included four scans: 1) a resting scan, after which reactive hyperemia was induced by occluding arterial blood flow using a sphygmomanometer inflated to 250 mmHg for 4 min; 2) an FMD scan recorded between 45 and 75 s after cuff deflation; 3) a control scan 10–15 min later; and 4) the last scan, taken 4 min after the sublingual administration of the GTN spray (400 µg; Nitrolingual Pump spray, Sanofi Aventis). For each scan, measurements were made over four consecutive cardiac cycles, incident with the R wave on the ECG, by observers blinded to the subject group using ultrasonic calipers. Measurements were averaged and expressed as percentages of the resting vessel diameter. Interobserver CV between 20 subjects studied on two occasions was 3.9% for FMD and 4.0% for GTN (18).

**Biochemical Assessments**

In AdDIT participants, urinary biochemical assessments were performed in a central laboratory (WellChild Laboratory, London, U.K.). Urine albumin was measured using nephelometric immunoassay (Siemens BN Prospec). Urine albumin concentrations below the limit of quantitation of nephelometry, <2.1 mg/L, were measured using 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 electrospay mass spectrometry–mass spectrometry 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 mass spectrometry–mass spectrometry.

In those 69 of 406 subjects who were not screened for AdDIT, urinary albumin was measured by an immunoturbidimetric assay and urinary creatinine by an enzyme colorimetric method (Roche Cobas C501; Hitachi) at the Adelaide site. There was a close correlation between measurements performed in London and Adelaide in 106 samples (r = 0.99 [P < 0.0001], 0.81 [P < 0.0001], and 0.99 [P < 0.0001] for albumin, creatinine, and ACR, respectively). Lipids were measured using commercial enzymatic assays on Roche Hitachi cobas C systems. HbA1c was assessed in the five local laboratories using a Vantage analyzer (Siemens Diagnostics, Camberley, U.K.), or a Variant analyzer (Bio-Rad Laboratories, Hercules, CA), both of which showed high correlations with DCCT-standardized control subjects (r = 0.98)
Calculations
BMI was calculated as weight in kilograms divided by the square of height in meters, and z scores were calculated using the Centers for Disease Control and Prevention, National Center for Health Statistics 2000 growth charts, U.S. (19). Estimated glomerular filtration rate (eGFR) (milliliters per minute per 1.73 meters squared) was calculated as 42 × height (centimeters)/plasma creatinine (micromoles per liter).

Statistical Analysis
Data are summarized as mean ± SD. Clinical characteristics (Table 1) were compared between groups using independent-sample t tests and χ² tests as appropriate. Univariable linear regression models were used to identify predictors of each IMT outcome in adolescents with type 1 diabetes. Variables that were statistically significant (P < 0.05) on univariable analysis and that had data available on >75% of observations (all except for GTN and eGFR) were entered into multivariable models for each IMT outcome in order to identify independent predictors of the respective outcome. To avoid potential issues with multicollinearity, BMI (correlated with waist circumference) and total cholesterol (correlated with LDL cholesterol) were not considered for inclusion into multivariable models. Comparisons of the four IMT outcomes (mean/maximal cIMT and mean/maximal aIMT) between the adolescents with type 1 diabetes and control subjects were performed using simple linear regression models.

Two-tailed P values <0.05 were considered statistically significant. Statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, NC).

RESULTS

Subject Characteristics
A total of 406 adolescents (211 boys) with type 1 diabetes (47 from New South Wales, 35 from Queensland, 167 from South Australia, 75 from Victoria, and 82 from Western Australia) and 57 healthy adolescents (26 boys) from South Australia were included in the analysis (Table 1). There were no significant clinical or biochemical differences between the type 1 diabetic subjects screened for AdDIT (n = 337) and the rest of the type 1 diabetic group (n = 69) (data not shown).

Table 2—Multivariable models for aIMT in type 1 diabetes

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>Mean aIMT Coefficient (SE)</th>
<th>P</th>
<th>Maximal aIMT Coefficient (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.010 (0.004)</td>
<td>0.004</td>
<td>0.012 (0.005)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes duration</td>
<td>0.001 (0.002)</td>
<td>0.70</td>
<td>0.002 (0.002)</td>
<td>0.38</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.001 (0.001)</td>
<td>0.41</td>
<td>0.001 (0.001)</td>
<td>0.28</td>
</tr>
<tr>
<td>Mean SBP</td>
<td>0.001 (0.001)</td>
<td>0.12</td>
<td>0.002 (0.001)</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean DBP</td>
<td>−0.001 (0.001)</td>
<td>0.38</td>
<td>−0.001 (0.001)</td>
<td>0.71</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.019 (0.008)</td>
<td>0.02</td>
<td>0.025 (0.011)</td>
<td>0.02</td>
</tr>
<tr>
<td>Urinary ACR</td>
<td>0.013 (0.006)</td>
<td>0.03</td>
<td>0.023 (0.007)</td>
<td>0.002</td>
</tr>
<tr>
<td>Center*</td>
<td>−0.0001</td>
<td></td>
<td>&lt;−0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Comparing all five centers simultaneously.
that the pathogenesis of cardiovascular and renal progres,
Early Atherosclerosis in Type 1 Diabetes

Diabetes Care

HbA1c independently related to cIMT analysis. Our study demonstrates that not measured and urinary albumin excretion and early adulthood, and of mixed ethnic origins (13). aIMT was not measured and urinary albumin excretion was not included in their analysis. Our study demonstrates that atherosclerosis can be detected by early adolescence after a shorter duration of disease. In SEARCH CVD, only HbA1c independently related to cIMT (13). As in SEARCH CVD, the majority of our type 1 diabetic subjects (78%) did not achieve target metabolic control of HbA1c <7.5% (<58 mmol/mol), consistent with a recent audit of Australian pediatric diabetes clinics (25), representing >80% of children with type 1 diabetes in Australia, including the five centers participating in this study. Metabolic control is an established risk factor of cIMT in type 1 diabetics (26), but at this early stage of atherosclerosis, we emphasize the additional importance of other cardiovascular risk factors.

The study is not without its limitations. The relatively small number of control subjects provided comparative data for IMT and vascular function only, as measured on the same machine by the same sonographer. However, our primary purpose was to examine the relationship between IMT and risk factors in type 1 diabetes. We would require very large numbers of control subjects to examine a relationship between ACR and IMT in the normal childhood population.

In conclusion, atherosclerosis is detectable from early adolescence in type 1 diabetes. Its early independent associations are male sex, age, systolic blood pressure, LDL cholesterol, and, importantly, urinary albumin excretion. Changes appear to occur first in the aorta in type 1 diabetes, as has been detected in normal childhood. Early rises in urinary albumin excretion during adolescence not only are important for determining risk of progression to microalbuminuria and diabetic nephropathy but also may alert the clinician to increased risk of cardiovascular disease. Their detection during adolescence should prompt extra attention to modifiable cardiovascular risk factors in addition to efforts to maximize metabolic control.

Acknowledgments. The authors thank Dr. Jennifer Harrington and Dr. Jemma Anderson, Adelaide, South Australia, for contributions to recruitment of subjects. The authors thank David Celemajer, Jason Harmer, and Dr. Jim Ramsay for supervision of training and collection of IMT images. The authors are thankful for the support of the AdDIT research nurses (Meredith Krieg, Alison Pryke, Julie Kendall, Claire Bingley, Julienne Wilson, Alison Roberts, and Julie Dart) and sonographers (Melissa La Forgia [lead], Yukari Newman, Katie Maslin, Jane Koleff, Amanda Crowe, Sinh Le, and Rachel Tarte) in this study. The authors thank all participants for their involvement and commitment.

Funding. The study was funded by the National Health and Medical Research Council, Australia, no. 632521; Diabetes UK; the JDRF; and the British Heart Foundation. The funders had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

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

Author Contributions. O.M. acquired data, analyzed and interpreted data, and critically revised the manuscript. A.S.P. developed the study concept and design, acquired data, critically revised the manuscript, and supervised the study. T.S. analyzed and interpreted data, critically revised the manuscript, and performed statistical analysis. T.W.J., K.C.D., and E.D. developed the study concept and design, acquired data, critically revised the manuscript, and obtained funding. F.I.C. acquired data and obtained funding. A.C. and M.E.C. acquired data, critically revised the manuscript, and obtained funding. R.G. acquired data and supervised the study. N.D. developed the study concept and design and analyzed and interpreted data. D.Da. developed the study concept and design. D.Du. developed the study concept and design, critically revised the manuscript, and obtained funding. J.D. developed the study concept and design and critically revised the manuscript. J.J.C. contributed to the study concept and design, acquired data, critically revised the manuscript, and performed the statistical analysis. O.M., T.S., and J.J.C. are the guarantors of this work and, as such, had 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.

References

1. Kavey RE, Gallada V, Daniels SR, et al.; American Heart Association Expert Panel on Population and Prevention Science; American Heart Association Council on Cardiovascular Disease in the Young; American Heart Association Council on Epidemiology and Prevention; American Heart Association Council on Nutrition, Physical Activity and Metabolism; American Heart Association Council on High Blood Pressure Research; American Heart Association Council on Cardiovascular Nursing; American Heart Association Council on the Kidney in Heart Disease; Interdisciplinary Working Group on Quality of Care and Outcomes Research. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism, High Blood Pressure Research, Cardiovascular Nursing, and the Kidney in Heart Disease; and the Interdisciplinary Working Group on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. Circulation 2006;114:2710–2738