HDL-Cholesterol, Blood Pressure, and Asymmetric Dimethylarginine Are Significantly Associated With Arterial Wall Thickness in Children

Julian G. Ayer, Jason A. Harmer, Shirley Nakhla, Wei Xuan, Martin K.C. Ng, Olli T. Raitakari, Guy B. Marks, David S. Celermajer

Objective—Atherosclerosis is found at autopsy in the arteries of adolescents and young adults. Arterial wall thickening may be assessed in vivo by ultrasonography measurement of the carotid intima-media thickness (CIMT), a marker of subclinical atherosclerosis. As the determinants of arterial wall thickness in childhood are unknown, we assessed the influence of cardiovascular risk factors on CIMT in 8-year-old children.

Methods and Results—A community-based sample of 405 children (age 8.0±0.1 years, 49% girls) had anthropometry, family history, blood pressure (BP), and CIMT measured. A blood sample was collected for HDL and non-HDL cholesterol, apolipoproteins A1 and B, high-sensitivity C-reactive protein, bilirubin, and asymmetrical dimethylarginine (ADMA, an endogenous nitric oxide inhibitor). CIMT was significantly associated with systolic BP (r=0.17, P<0.001), diastolic BP (r=0.10, P=0.04), HDL (r=−0.13, P=0.02), and ADMA (r=0.18, P=0.001). CIMT was significantly higher in children with premature parental CHD (0.63±0.07 versus 0.59±0.06 mm, P=0.03). On multivariate analysis, HDL (β coefficient=−0.02, P=0.04), ADMA (β coefficient=0.05, P<0.001), and systolic BP (β coefficient=0.01, P=0.001) were significantly and independently associated with CIMT.

Conclusions—Lower HDL-cholesterol, higher levels of ADMA, and systolic BP are significantly associated with greater arterial wall thickness in early childhood.

Key Words: HDL-cholesterol □ asymmetric dimethylarginine □ pediatric □ intima-media thickness □ blood pressure

Structural evidence of early atherosclerosis is commonly found in adolescents and young adults when their arteries are examined at autopsy. The extent of these lesions increases with age and with the number and severity of traditional cardiovascular risk factors.1,2 B-mode ultrasound examination of the arterial wall, performed during life, produces a characteristic image that correlates with the histological intima-media complex.3 In adults, the carotid artery intima-media thickness (CIMT) is correlated with coronary and carotid atherosclerosis and is a significant predictor of future cardiovascular events.4 For these reasons, CIMT has been used in numerous cross-sectional and intervention studies as a surrogate end point for atherosclerosis.5

Although high-risk children, such as those with familial hypercholesterolemia and type 1 diabetes mellitus, have increased CIMT compared with healthy controls6,7 and risk factors present in childhood are associated with CIMT in adult life,8,9 the factors that influence CIMT in healthy children are largely unknown. We undertook a study of 8-year-old boys and girls to explore the effects of cardiovascular risk factors on arterial wall thickness in early childhood. We hypothesized that the traditional cardiovascular risk factors, such as blood pressure, lipoprotein levels, family history, and relative body weight would have an important effect on arterial wall thickness in healthy children in the first decade of life. We aimed to examine the extent to which markers of inflammation or endothelial dysfunction influenced arterial structural changes in childhood. The effects of prenatal factors, such as birth weight, pregnancy-induced hypertension, and preeclampsia, and maternal cigarette usage were also assessed.

Methods

Setting and Participants

The subjects were healthy 8-year-old children from the western and southwestern areas of Sydney, Australia. They had been enrolled before birth into the Childhood Asthma Prevention Study (CAPS), a randomized controlled trial of house-dust mite avoid-
ance and omega-3 fatty acid supplementation from birth to 5 years. These interventions were tested in a 2x2 factorial design to determine effects on asthma and allergic disease in children at risk for these conditions. The details of the study methods have been published elsewhere. Briefly, pregnant women whose unborn children had at least one parent or sibling with current asthma or wheezing were identified before birth at the antenatal clinics of 6 Sydney hospitals and randomized to 1 of the 4 study groups. Exclusion criteria included babies from multiple births, gestational age less than 36 weeks, birth weight less than 2.5 kg, hospitalization for more than 1 week, or serious illness. Six hundred sixteen subjects were enrolled from September 1997 to December 1999. The children were assessed at 18 months, 3 years, 5 years (the end of the randomized intervention period), and 8 years with measures of growth, lung function, and atopy. At 8 years, families were also invited to participate in a substudy examining the childhood determinants of cardiovascular disease.

Four hundred ten of the original children (67%) consented to participate in the cardiovascular study. Five subjects were excluded (2 with established type 1 diabetes mellitus, 2 who consented for the study but subsequently refused cardiovascular testing, and 1 who was older than the prespecified age range [8.0-0.5 years] at the time of testing), leaving 405 children who are the subject of this report. Although maternal age and education were higher, the children who returned for cardiovascular assessments were otherwise representative of the initial study population for baseline characteristics. This study was approved by the Human Research Ethics Committees of the University of Sydney, the Children’s Hospital at Westmead and Western and South Western Area Health Services. The parent or legal guardian gave written informed consent.

**Measurements**

**Blood Pressure**

Brachial blood pressure (BP) was measured using a validated automated oscillometric device (Welch Allyn Vital Signs Monitor). Supine BP in the left brachial artery was measured after a period of 10 minutes of quiet rest. This was repeated after a further 10 minutes, and a third BP was measured if there was a variance of 10 mm Hg or more; the average of the 2 closest readings was recorded as the brachial BP.

**Carotid Intima Media Thickness**

The right and left carotid arteries were scanned according to a standardized protocol by 1 sonographer, using a portable ultrasound system (Terason 3000, Teratek) with a 5- to 12-MHz linear array transducer and ECG gating. Several 5-second moving-image clips of the distal common carotid artery (CCA) were obtained and stored in a digital format for subsequent offline analysis. Three right and 3 left end-diastolic frames were selected and, for each, the mean IMT of the artery far wall was measured over the region of interest beginning 1 centimeter proximal to the edge of the carotid bulb. Measurements were made by a single observer, who was blinded to each subject’s clinical details, using edge detection software that has previously been validated as accurate and reproducible (Figure 1). The average of the 6 measurements was used in the analysis as mean IMT. To assess interobserver variability, a second observer measured mean IMT in a random selection of 33 subjects, with a 2.3% coefficient of variation (CV).

**Anthropometry**

Measurement of height, weight, and waist and hip circumference were made in a standardized fashion and body mass index (BMI), BMI z-score and waist to height ratio were calculated. Children were divided into “healthy weight,” “overweight,” and “obese” categories based on BMI z-score. Height (or length) and weight were also available for the children at birth, 18 months, 3 years, and 5 years. The change in weight-for-age z-score between 18 months and 3 years and in BMI z-score between 3 years and 8 years were calculated. For additional information on anthropometric measurements and calculations please see the supplemental materials (available online at http://atvb.ahajournals.org).

**Laboratory Tests**

Three hundred twenty-eight (81%) subjects gave consent for collection of a nonfasting venous blood sample. The subjects who had blood collected were representative of the whole cohort with respect to baseline characteristics. Samples underwent centrifugation at 3000 rpm for 5 minutes, and the collected serum was stored at −20°C for less than 2 weeks before total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides (using standard enzymatic procedures) and high-sensitivity C-reactive protein (hs-CRP, using an immunoturbidimetric method) were assayed on a modular autoanalyzer (Roche). Non-HDL cholesterol (total cholesterol minus HDL cholesterol) was calculated for each subject.

Serum was also stored at −80°C and analyzed in a single batch after 1 thaw cycle for apolipoprotein B (apoB, the major protein component of atherogenic lipoproteins) and apolipoprotein A1 (apoA1, the major protein component of HDL) by immunoturbidimetry, bilirubin by colorimetry, and asymmetrical dimethylarginine (ADMA) by enzyme-linked immunosorbent assay (ELISA, Immundiagnostik AG). ADMA was assessed in duplicates (intraassay CV 13%), and the average was recorded as the ADMA level. With ELISA, there is a low cross-reactivity with L-arginine (the precursor of ADMA) and symmetrical dimethylarginine (the stereoisomer of ADMA). For human serum, ELISA shows a low interassay CV, a >95% analytic recovery rate, and close correlation with levels measured by liquid chromatography and gas chromatography mass spectrometry (r>0.98). A study of premature coronary heart disease (CHD, prior to the age of 55 years) in the child’s parents or grandparents was recorded if present. A parental history of hypertension, hypercholesterolemia,
or diabetes was also recorded. The number of maternal and paternal risk factors were added together to give a total number for both parents.

The level of physical inactivity at the age of 8 years was assessed from a standardized parental questionnaire. Parents were asked about the average number of hours per day their children watched television, and physical inactivity was thus assessed in 3 ordered categories: Group 1 (0 to 1 hours per day, n=123), Group 2 (2 to 3 hours per day, n=238), and Group 3 (>3 hours per day, n=42). Information on maternal smoking status during pregnancy, pregnancy-induced hypertension, preeclampsia, and gestational diabetes was collected using a standardized questionnaire that was completed by interview, soon after the delivery of the child.

Statistical Analysis
Continuous variables are expressed as mean±SD, and categorical variables are presented as percentages. Comparison between groups was undertaken by 2 samples t test or 1-way ANOVA. The association between CIMT and other variables were explored using multiple linear regression with forward stepwise selection of covariates. Variables were entered into the model where statistical significance existed in our exploratory analysis, it is minimized by the biological plausibility of the risk factors being tested. Statistical significance was inferred at a 2-sided probability value <0.05. All statistical analyses were performed using SAS Version 9.

Table 1. Baseline Characteristics (n=405) by Gender

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>8.0±0.1</td>
<td>8.0±0.1</td>
</tr>
<tr>
<td>Number (%)</td>
<td>207 (51)</td>
<td>198 (49)</td>
</tr>
<tr>
<td>CIMT, mm</td>
<td>0.59±0.07</td>
<td>0.59±0.06</td>
</tr>
<tr>
<td>Height, cm</td>
<td>129±6</td>
<td>128±6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>29.4±6.7</td>
<td>28.9±6.7</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.57±0.51</td>
<td>3.41±0.44</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.56±1.02</td>
<td>0.48±1.02</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>59.8±7.9</td>
<td>59.1±7.4</td>
</tr>
<tr>
<td>Waist:Height ratio</td>
<td>0.46±0.05</td>
<td>0.46±0.05</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>100±7</td>
<td>101±7</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>59±6</td>
<td>60±5</td>
</tr>
<tr>
<td>Non-HDL cholesterol, mmol/L</td>
<td>2.8±0.7</td>
<td>3.1±0.7</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.5±0.4</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>ApoA1, g/L</td>
<td>1.5±0.2</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.6±0.2</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Triglycerides, * mmol/L</td>
<td>1.3 (0.9–1.7)</td>
<td>1.1 (0.9–1.6)</td>
</tr>
<tr>
<td>ADMA, μmol/L</td>
<td>0.91±0.21</td>
<td>0.91±0.23</td>
</tr>
<tr>
<td>hs-CRP, g/L</td>
<td>0.22 (0.21–0.63)</td>
<td>0.46 (0.21–1.7)</td>
</tr>
<tr>
<td>Bilirubin, * mmol/L</td>
<td>4 (3–6)</td>
<td>5 (3–6)</td>
</tr>
</tbody>
</table>

Values are mean±SD or percentage except *median±IQ range.

Table 2. Bivariate Correlation Analysis for CIMT in Childhood

- ADMA, systolic BP, diastolic BP, non-HDL cholesterol, ApoA1, ApoB, triglycerides, bilirubin
- Variables were entered into the model where statistical significance existed in our exploratory analysis, it is minimized by the biological plausibility of the risk factors being tested. Statistical significance was inferred at a 2-sided probability value <0.05. All statistical analyses were performed using SAS Version 9.

Results
The characteristics of the children are shown in Table 1. There were no important differences in these characteristics between boys and girls. The associations between risk variables and CIMT were similar between the sexes, and thus all subsequent analyses were done with pooled data.

In the whole group the mean, median, and interquartile range for ADMA was 0.91 (0.90, 0.94) to 1.06 μmol/L, respectively. CIMT was significantly positively associated with ADMA (r=0.18, P=0.001). The effect on CIMT of the increasing number of risk factors in parents did not significantly increase CIMT in the children.

Birth weight was not significantly associated with CIMT (Table 2). CIMT was not significantly greater in those children born to mothers who smoked in pregnancy or who had preeclampsia or pregnancy induced hypertension or gestational diabetes (supplemental Table).

Increasing amounts of time spent watching television was not associated with increased CIMT (please see supplemental materials) and CIMT was not significantly different between healthy weight (n=279, 0.59±0.06 mm), overweight (n=68, 0.70±0.07 mm), and obesity (n=4, 0.78±0.06 mm).
0.58 ± 0.06 mm), and obese children (n= 58, 0.60 ± 0.07 mm). In controls, BMI z-score, waist circumference, and waist-height ratio were all not significantly associated with CIMT (Table 2). Weight-for-age z-score change between 18 months and 3 years and BMI z-score change between 3 years and 8 years were similarly not associated with CIMT. Neither increasing hs-CRP group nor bilirubin were significantly related to CIMT.

On multivariable analysis, ADMA, systolic BP, and HDL cholesterol were significantly and independently associated with CIMT (Table 4). A positive parental history for premature CHD entered the model at P = 0.08. Gender, height, and diastolic BP did not significantly contribute to the predictive model for CIMT.

Neither asthma status nor omega 3 fatty acid supplementation randomization group influenced CIMT (data not shown). The association between ADMA and BMI z-score, waist circumference, waist-height ratio, systolic and diastolic BP, non-HDL and HDL cholesterol, triglycerides, and hs-CRP group were not significant (P > 0.05, data not shown).

### Discussion

This study represents the largest analysis to date of the effect of risk factors for cardiovascular disease on arterial wall thickness in healthy young children. We have found that ADMA (an endogenous inhibitor of nitric oxide synthesis), HDL-cholesterol (a circulating lipoprotein with generally atheroprotective functions), and systolic BP are significantly and independently associated with CIMT in the first decade of life. These findings suggest an important role for endothelial function, HDL-cholesterol, and blood pressure in the early arterial structural changes that may lead to atherosclerosis.

### Table 3. Family History and CIMT

<table>
<thead>
<tr>
<th>Family history status</th>
<th>n</th>
<th>CIMT (mm)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No parental premature CHD</td>
<td>389</td>
<td>0.59 ± 0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>Parental premature CHD</td>
<td>14</td>
<td>0.63 ± 0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>No Grandparental premature CHD</td>
<td>326</td>
<td>0.59 ± 0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>Grandparental premature CHD</td>
<td>79</td>
<td>0.60 ± 0.06</td>
<td>0.07</td>
</tr>
</tbody>
</table>

### Table 4. Multivariate Regression Analysis for CIMT

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>β Coefficient*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA, μmol/L</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>-0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Parental premature CHD</td>
<td>0.04</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Model r² = 0.09; excluded variables: gender, height, diastolic BP. *β coefficient represents the change in CIMT in millimeters per unit change in the explanatory variable.

Endothelial dysfunction may be caused by risk factors for atherosclerosis and may be improved by their treatment.16 The critical role of the endothelium in maintaining arterial integrity is largely mediated by endothelium-derived nitric oxide (NO). NO is important as a vasodilator and inhibitor of platelet aggregation, leukocyte adhesion and smooth muscle proliferation. Two studies (1 in 45 diabetic children and 30 controls16 and the other in 2011 young adults17) have found an inverse association between CIMT and brachial artery flow mediated dilatation, suggesting a possible interaction between endothelial dysfunction and greater arterial wall thickness in the young.

ADMA, an endogenous inhibitor of the enzyme that produces NO (NO synthase), has been shown to be an independent risk factor for CHD.18 ADMA levels are associated with cardiovascular risk factors in adults,19,20 and a number of studies have suggested a direct role for ADMA in the pathogenesis of endothelial dysfunction. For example, higher ADMA levels in healthy and at-risk adults correlate with reduced endothelium dependent forearm vasodilatation20–22; intraarterial infusions of ADMA cause vasoconstriction of forearm blood vessels,22,23 and exposure of endothelial cells to ADMA increases their adhesion to monocytes.24,25 The activity of the enzyme responsible for ADMA degradation, dimethylarginine dimethylaminohydrolase (DDAH), is reduced by oxidized LDL and inflammatory cytokines.26 Endothelial cell regeneration occurs more quickly after arterial injury in transgenic mice with increased DDAH activity, compared with wild-type mice,27 implicating DDAH and ADMA degradation as important to intimal integrity.

Our finding of an independent positive association between ADMA levels and CIMT in healthy 8-year-old children is consistent with data from adults19,28 and those with renal disease.29 However, our study highlights that the effect of ADMA on the arterial wall begins early in life, is not merely reflective of vascular degeneration associated with aging, and is independent from the effects of atherogenic lipoproteins. The association of lower HDL-cholesterol with higher CIMT is also consistent with studies in young adults.30,31 In addition to promoting efflux of cholesterol from macrophages ("reverse cholesterol transport"), HDL has potential antiinflammatory and antioxidant effects.32 It is possible that factors other than reverse cholesterol transport mediate the association of HDL with CIMT in prepubertal children, given the relative paucity of lipid in the arterial wall at this age. Our data raise the possibility that HDL-induced modulation of inflammation or NO production might be operative at an early age.

Hypertension is associated with structural changes in small resistance arteries that lead to a reduced lumen size and an increased media-to-lumen ratio. These changes result from remodeling of the arterial wall rather than a net growth of existing structures.33 In hypertensive adults, conduit arteries also demonstrate wall thickening, most likely as a result of medial hypertrophy.34 Our finding of an association between increasing systolic BP and CIMT in childhood may relate to intimal thickening, medial hypertrophy, or both as difference-
The CIMT of adults with at least 1 parent with premature CHD has been shown to be greater than the CIMT of adults without such a family history. Our study supports the influence of premature parental CHD on CIMT and suggests that this familial predisposition may be manifest at an early age. The parents in our study were still relatively young, and thus few of the children studied had a family history of parental CHD. This misclassification would, if anything, tend to underestimate the influence of premature parental CHD on CIMT in offspring. Shared environmental influences, producing both premature parental CHD and greater CIMT in the children, is less likely than a genetic predisposition, given the young age of the children and the lack of an association between increasing parental risk factors and CIMT in their children.

In our study, the absence of an association between certain important cardiovascular risk factors and CIMT highlights that risk factor severity, duration, and timing of exposure may each have an important effect on arterial wall thickness. For example, although apoB-containing lipoproteins are associated with CIMT in healthy young adults, our data suggest that they do not effect CIMT in healthy children in the first decade of life. It is possible that these atherogenic lipoproteins only begin to influence arterial wall thickness after puberty. This is supported by the study of Juonala et al, in which CIMT in 879 young adults (age 31.9 ± 5.0 years) was significantly associated with the apoB levels and the apoB/apoA1 ratio when measured at age 12 to 18 years but not at 3 to 9 years. Additionally, cumulative exposure to LDL cholesterol, when measured from childhood to adulthood, has been shown to be independently associated with higher adult CIMT. The severity of exposure to apoB-containing particles is also likely to be important, as evidenced by case–control studies that demonstrate a higher CIMT in children with familial hypercholesterolemia.

We were also unable to observe an increase in CIMT with higher hs-CRP levels, a finding inconsistent with the data of Jarvisalo et al in 79 healthy children. In this latter study, children were older (10.5 ± 1.1 years) than in our study, and a significant increase in CIMT was only evident in the highest hs-CRP group. This suggests that the level of hs-CRP and the duration of its elevation may interact to increase CIMT. We could only demonstrate a modest nonsignificant association between CIMT and BMI z-score. Moreover, neither waist circumference nor changes in weight status over time were associated with CIMT. In contrast, there are a number of reports of higher IMT in obese children and a reduction in IMT with diet, physical activity, or weight loss. Again, the level of exposure may be important, as most of these studies have been in older children and adolescents and in the significantly obese (BMI z-score > 2.0), who formed a very small proportion of our community-based study population.

Autopsy data from the Pathobiological Determinants of Atherosclerosis Study indicate that an increasing number of risk factors in subjects aged 15 to 24 years is associated with an increasing burden of early (initial and advanced fatty streaks) rather than late (raised fatty streaks and fibrous plaques) atherosclerosis. Therefore, it is possible that our inability to detect significant associations between CIMT and certain risk factors relates to a relative insensitivity of ultrasound in detecting these early lesions. However, Pignoli et al found that high-resolution B-mode ultrasound accurately measured the histological intima plus media complex. Moreover, the IMT of arteries that were normal or had fatty streaks were as accurately measured as in those arteries with more advanced atherosclerosis. Thus, important effects of cardiovascular risk factors on early atherosclerosis are likely to be detectable by high-resolution B-mode ultrasound.

Our study has several potential limitations. The children were originally recruited into an asthma prevention trial, on the basis of a family history of asthma. However, subjects were recruited from unselected antenatal clinics at 6 different general hospitals. The inclusion criteria for entry into the original trial and the cardiovascular substudy ensured selection of subjects who were not at particular risk of cardiovascular disease. Their baseline characteristics (including height, weight, blood pressure, and cholesterol) are very similar to those reported by Garnett et al in an unselected population (n = 255) of 7- to 8-year-old boys and girls from the same geographical area as in our study. Therefore we believe that the distribution of cardiovascular risk factors in our study children is comparable with that found in an unselected community-based population of a similar age. A selection bias is possible because the cardiovascular substudy was based on a proportion (67%) of the original trial subjects. However, as those who participated in the current study had similar baseline characteristics to those in the original trial, we believe that this is unlikely. One of the study inclusion criteria was a birth weight ≥ 2.5 kg. Therefore, although we did not find a significant association between birth weight and CIMT, an effect of very low birth weight cannot be excluded.

Although nonfasting venous blood samples were collected, we examined the effect of non-HDL cholesterol, HDL-cholesterol, apoB, and apoA1, whose levels differ little between the fasting and nonfasted state. The absence of an estimate of glomerular filtration rate is also a potential limitation, as ADMA levels are elevated in subjects with renal failure. None of our subjects had renal failure, and we would expect that the great majority of these healthy children would have had normal renal function. Although serum creatinine has been consistently reported as a strong correlate of SDMA, its association with ADMA levels has been inconsistent. This may relate, at least in part, to enzymatic degradation of ADMA by DDAH occurring in extrarenal as well as renal tissue. As neither creatinine nor creatinine clearance were detectable by high-resolution B-mode ultrasound.
number of hypotheses, does not allow confirmation of the mechanisms underlying early atherosclerosis.

In summary, we have demonstrated that ADMA, HDL cholesterol, and systolic BP are independently associated with CIMT in prepubertal children. These findings highlight the important role of the endothelium in the early changes in the arterial wall that may progress to atherosclerosis. HDL-cholesterol appears to have an effect on arterial wall thickness, at an age when reverse cholesterol transport would not be expected to be important. Our data suggest that the mechanisms that alter arterial structure are present at a very early age and that population-based approaches to reduce the prevalence of cardiovascular risk factors in early childhood may be warranted.

Sources of Funding
D.C. is supported by an Australian Government National Health and Medical Research Council Project Grant (Number 222722). Funding for certain of the blood tests performed was obtained from a Pfizer Australia CVL Grant.

Disclosures
None.

References


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*Arterioscler Thromb Vasc Biol.* published online April 9, 2009;
*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Supplemental Material

Materials and Methods

I. Anthropometry

Height was measured to the nearest 0.5 cm with a portable stadiometer and weight was measured to the nearest 0.1 kg using calibrated electronic scales. Body mass index (BMI) was calculated as weight (kg) /height (m)$^2$. For age 18 months, 3 years, 5 years and 8 years BMI z-score and weight-for-age z-scores were determined by comparison with U.S growth charts from the Centres for Disease Control and Prevention \(^1\). Children were divided into “Healthy weight” (BMI z-score < 1.04 [85\(^{th}\) percentile]), “Overweight” (BMI z-score between 1.04 and 1.64 [85\(^{th}\) to 95\(^{th}\) percentiles]) and “Obese” (BMI z-score > 1.64 [95\(^{th}\) percentile]) categories based upon the measurements at 8 years. Waist circumference was measured at end-expiration, midway between the lower margin of the ribs and the iliac crest. Waist (centimetres) to height (centimetres) ratio was calculated.
## Results

### II. Effect of prenatal factors and physical inactivity at 8-years on CIMT

<table>
<thead>
<tr>
<th>Prenatal Factor</th>
<th>n</th>
<th>CIMT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking in pregnancy</td>
<td>93</td>
<td>0.59±0.06</td>
<td>0.31</td>
</tr>
<tr>
<td>No smoking in pregnancy</td>
<td>312</td>
<td>0.60±0.06</td>
<td></td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>24</td>
<td>0.59±0.06</td>
<td>0.70</td>
</tr>
<tr>
<td>No gestational diabetes</td>
<td>381</td>
<td>0.59±0.06</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia or PIH *</td>
<td>30</td>
<td>0.59±0.06</td>
<td>0.88</td>
</tr>
<tr>
<td>No preeclampsia or PIH *</td>
<td>375</td>
<td>0.59±0.06</td>
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<table>
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<tr>
<th>Television viewing (hours)</th>
<th>n</th>
<th>CIMT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>123</td>
<td>0.59±0.06</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>238</td>
<td>0.59±0.07</td>
<td>0.80</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>42</td>
<td>0.59±0.06</td>
<td></td>
</tr>
</tbody>
</table>

* Pregnancy induced hypertension

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