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Endothelial Dysfunction in Childhood Infection

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Background—Atherosclerosis begins in early life, and endothelial dysfunction is recognized as a key initiating event in the development of atherosclerosis. Although infection has been implicated in endothelial dysfunction and atherogenesis, the impact of acute common childhood infections on the vascular endothelium is unknown.

Methods and Results—We studied 600 children aged 10 years drawn from the Avon Longitudinal Study of Parents and Children. The children were divided into 3 groups: those with current acute infection (AI; $n=135$; 73 boys and 62 girls); a convalescent group with infection in the past 2 weeks ($n=166$; 78 boys and 88 girls), and a healthy control group ($n=299$; 131 boys and 168 girls). Endothelial function was determined in all subjects by high-resolution ultrasound to measure brachial artery flow-mediated dilation (FMD) and was expressed as the percentage change in diameter from baseline after reactive hyperemia. FMD was repeated in 40 children in the AI group and 50 in the control group after a mean interval of 1 year. FMD was lower in both the AI group ($6.3\pm 2.7\%$, mean \pm SD) and the convalescent group ($8.1\pm 3.1\%$) than in the control group ($9.7\pm 2.5\%$; $P<0.001$ for both). The observed differences in FMD remained after adjustment for potential confounding variables. At the repeat visit, FMD was unchanged in controls ($P=0.85$) but improved in the AI group ($P<0.001$).

Conclusions—Acute infection in childhood is associated with impaired endothelium-dependent vasodilation. These findings support a potential role for previously unsuspected extrinsic inflammatory stimuli in the pathogenesis of early atherosclerosis. (*Circulation*. 2005;111:1660-1665.)

Key Words: infection ■ endothelium ■ risk factors ■ population

Atherosclerosis as a clinical disease usually presents in middle and late adulthood, but the pathogenesis of this disease starts very early, often from the first decade of life.¹ Classic risk factors, including hypercholesterolemia, obesity, and diabetes, have been associated with accelerated atherosclerosis in children, which has been linked to cardiovascular morbidity and mortality in later life.^{2,3} It is likely, however, that other influences may be important for initiation and progression of early disease.

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Experimental and clinical studies have led to a dramatic change in our understanding of the pathophysiology of atherosclerosis, providing robust evidence of the importance of vascular inflammation throughout disease development.^{4,5} The endothelium plays a key role as the signal transducer that regulates this process. Although classic risk factors may induce inflam-

mation and endothelial dysfunction, our group has recently shown that even a low-grade inflammatory stimulus such as typhoid vaccination can cause profound, reversible impairment of endothelial function in young healthy subjects.⁶

Infection is the most common inflammatory insult in early life. To date, most clinical studies have focused on the contribution of infection on the advanced stages of atherosclerotic disease in adults, with conflicting results⁷⁻¹⁰; however, the greatest burden of infection occurs in childhood, which raises the possibility that infection may play a more important role during the preclinical phase of atherosclerosis. There has been limited clinical investigation into the role of infection in children; however, a provocative study suggested that a severe infection can accelerate the early progression of structural arterial disease.¹¹ In the present study, we investigated the effect of common mild childhood infections on endothelial function in a large cohort of children.

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Methods

The ALSPAC Population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based study designed to investigate environmental and genetic influences that may affect the health and development of children. The study methods are described in detail elsewhere¹² and on the study's World Wide Web site (<http://www.alspac.bris.ac.uk>). Briefly, 15 541 pregnant women with an expected delivery date between the start of April 1991 and the end of December 1992 were enrolled, which represented 85% of the eligible total general population in 3 health authorities in the Bristol, United Kingdom, area. The cohort of 14 062 liveborn children has been followed up prospectively. Detailed information about the children has been collected from questionnaires administered through childhood, and clinic measurements have been performed on the entire cohort annually since the age of 7 years.

The Vascular Study Population

Our study population consisted of 600 children aged 10 years. These represented consecutive children born between February 2002 and June 2002 who were participating in a vascular study of the entire ALSPAC cohort, provided they did not have asthma or chronic infections and were not taking antibiotics or anti-inflammatory drugs. The protocols were approved by the ALSPAC Law and Ethics committee and by the local research ethics committees. Written informed consent was sought from the parent/guardian, and assent from the child was obtained at the time of the study.

Definition of Infection

Information on the nature, timing, and treatment of the children with acute infection was obtained by questionnaires completed by the parents/guardians of the children at the time of the vascular function measures. Acute infection was defined by clinical symptoms. The commonest were cough, cold, fever, and sore throat. These were reported by the parents in the questionnaires and not confirmed by formal clinical examination or serological evidence. No blood tests were undertaken to document infectious agents or state. The questionnaires inquired about the type and severity of infection and details of any medication or supplement taken at the time of their assessment, or regularly, by the children. Children were then classified into 3 different groups: (1) children who reported acute infection at the time of the study (AI group), (2) children who were convalescing from an infection in the previous 2 weeks but were well at the time of the study (convalescent group), and (3) children without recent illness (controls). None of the illnesses were considered severe enough to warrant medical consultation or prescription of antibiotics.

Endothelial Function

Each child underwent measurement of endothelium-dependent vascular responses of the right brachial artery by high-resolution ultrasound imaging (Aloka 5500, Japan; 7-MHz linear probe and automated vessel-diameter measurements, Brachial Tools, MIA) in a temperature-controlled vascular room in Bristol.¹³ The child lay supine on a couch in a temperature-controlled room (24°C to 26°C), and after 10 minutes' rest, a straight, nonbranching segment of the brachial artery above the antecubital fossa was identified and scanned in a longitudinal fashion. Once depth and gain settings were adjusted, brachial artery diameter was recorded (baseline) for 1 minute. A pneumatic cuff was then inflated to 200 mm Hg on the forearm for 5 minutes, and the segment of the brachial artery was recorded continuously for another 5 minutes. We recorded end-diastolic images at 3-second intervals throughout. Brachial artery diameter was measured offline by an automatic edge-detection system and expressed as a percent change from the baseline diameter.¹⁴ Doppler-derived flow measurements (using a pulsed-wave Doppler signal at a 70° angle) were also obtained continuously. The increase in blood flow after the release of the cuff was expressed as a percentage of the baseline flow.

Potential Confounding Factors

Measures of Social Position

The occupation of both the mother and her partner were recorded in the 32-week antenatal questionnaire. These were used to allocate each to a social class group (which ranged from V=unskilled to I=professional) with the United Kingdom Registrar General's occupational coding. A combined variable was derived that was the highest socioeconomic class of the mother and her partner.

Measures of Cardiovascular Risk Factors

At 10 years, systolic and diastolic blood pressures were measured as the average of the last 2 seated readings with an automated oscillometric device (Dinamap 9301 vital signs monitor) in the left arm. A pediatric cuff was used when arm circumference was <25 cm and a regular adult cuff when arm circumference was >25 cm. Heart rate was measured as the average of the last 2 readings as recorded by the same (Dinamap 9301 vital signs monitor) device. Weight was measured to the nearest 0.1 kg with SECA scales while the child was wearing underwear, and height was measured to the nearest 0.1 cm with a Leicester height meter. From these measurements, body mass index values were calculated (weight/height², with weight in kilograms and height in meters). Nonfasting venous blood samples were collected from each child at age 7 years and analyzed for total cholesterol, HDL, and LDL.

Measures of Previous Infection and Hygiene Practice

Mothers reported, in 4 different questionnaires at 15 months, 15 to 24 months, 24 to 38 months, and 54 months, how often in a normal day their child's face and hands were wiped and also how often hands were wiped before meals. Responses ranged from "not at all" to "5 or more times per day." Mothers were also asked how often their child was given a bath or shower, brushed their teeth, and cleaned their ears. Responses ranged from "hardly ever" to "more than once a day." The score at each time point was the sum of 6 questions that gave values of 0 to 18, with higher values indicating greater hygiene. From these responses, the child's mean hygiene score was derived (ranging from least hygienic to most hygienic).¹⁵ Similarly, a mean infection score was created from questionnaires for the age periods <6 months, 6 to 18 months, 18 to 30 months, 30 to 42 months, and 59 to 81 months, which gave values from 0 to 7. The infection score at each time point was the number of infections (diarrhea, vomiting, cough, high temperature, cold, earache, and ear discharge) reported. In addition an "infection seen by the doctor" score was also derived with individual questions recoded as having visited a doctor with infections versus not having visited a doctor with infection/no infection. With the same time points, a mean antibiotic score was calculated, for which the score at each time point was "0" for no antibiotic use, "1" for only 1 episode of use, and "2" for 2 or more episodes of antibiotic use. For all variables, mean values were only calculated if values were recorded for at least 2 time points.

Follow-Up Study Methods

To assess the long-term effect of the infectious illness on endothelial function, we performed repeat measures. We calculated that 40 children from the AI group would have the power to demonstrate a 25% improvement in the flow-mediated dilation (FMD) response relative to their baseline measurement in the AI group with 90% power and at the 5% significance level. To ensure adequate participation, we invited by letter all 135 children of the AI group and 150 randomly selected control children to attend for an additional vascular assessment (12±6 months after the initial evaluation).

Data Analysis

The data were analyzed with the Stata analysis package, version 8. All vascular function data were normally distributed and are presented as mean and SD for continuous variables or percentages for categorical variables. We examined the effect of infection on endothelial function by linear regression analysis. Multivariable analysis was performed to adjust for other potential confounders. At

TABLE 1. Demographic Characteristics and Clinical Parameters According to Infection Status in Children

	AI Group (n=135)	Convalescent Group (n=166)	Control Group (n=282)	P
Male, %	54.1	47.0	40.4	0.01
Social class I and II, %	67.2	59.7	54.1	0.02
Body mass index @10 years, kg/m ²	16.1 (1.8)	15.8 (1.6)	16.2 (2.2)	0.4
Total cholesterol @7 years, mmol/L	4.4 (0.7)	4.4 (0.7)	4.4 (0.7)	0.9
HDL @7 years, mmol/L	1.5 (0.3)	1.6 (0.4)	1.5 (0.3)	0.1
LDL @7 years, mmol/L	2.2 (0.6)	2.3 (0.5)	2.3 (0.6)	0.5
SBP @10 years, mm Hg	102.2 (8.2)	101.5 (8.9)	100.7 (8.4)	0.1
DBP @10 years, mm Hg	57.1 (6.8)	57.1 (7.2)	56.9 (7.1)	0.8
Heart rate @10 years, bpm	71.2 (11.9)	73.0 (11.5)	71.0 (11.3)	0.6
Infection score: not seen by doctor*	3.7 (0.9)	3.6 (0.9)	3.5 (0.9)	0.1
Infection score: seen by doctor*	1.4 (0.9)	1.3 (0.9)	1.3 (0.9)	0.3
Antibiotic score†	0.8 (0.5)	0.7 (0.5)	0.7 (0.5)	0.1
Hygiene score‡	9.8 (1.8)	9.7 (1.8)	9.8 (2.0)	0.8

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

*Mean number of infections (diarrhea, vomiting, cough, high temperature, cold, earache, and ear discharge) reported at 5 different time points. Individual questions were recoded as "seen doctor with infection" vs "not seen doctor with infection/no infection."

†Mean number of antibiotics used at 5 different time points.

‡Mean of hygiene scores at 4 time points. Hygiene score at each time point was the sum of face washing, hand washing, hand cleaning before meals, bath/shower, and ear hole cleaning values. Higher values indicate greater hygiene.

Values are percent or mean (SD).

the follow-up study, *t* tests were used to compare the differences in endothelial function measures.

Results

Population Characteristics

Of the 600 subjects, 301 children had either an acute infection at the time of the study (AI group, n=135) or were convalescing from an infection in the previous 2 weeks but were well at the time of study. The 299 controls were healthy children without recent illness. The majority of children in both the AI and convalescent groups presented with upper respiratory tract infections (93% and 94%, respectively). The remainder had either ear infections (2% and 2%) or urinary tract infections (5% and 4%). Seventeen children from the control group were excluded from the analysis because their vascular measures could not be linked to the main database. None of the children in the present study had infectious illnesses deemed sufficiently severe to warrant prescription of

antibiotics. The clinical characteristics and risk factor profiles of the children are shown in Table 1.

In the follow-up study, all 43 children who replied from the AI group were studied, but 3 were excluded because they presented with acute infection or with a history of recent infection within the last 2 weeks. Fifty control children had a repeat study performed in an identical manner. None of those children had experienced another infection during the period between the 2 studies.

Associations Between Acute Infection and Clinical Characteristics

Higher socioeconomic position was associated with increased incidence of infection, and there were more boys than girls in the AI group. No other factors were associated with infectious status (Table 1).

Infection and Endothelial Function

Baseline brachial artery diameter, baseline flow, reactive hyperemia flow, and absolute change in flow were similar in

TABLE 2. Vascular Characteristics According to Infection Status

	AI Group (n=135)	Convalescent Group (n=166)	Control Group (n=282)	P
Baseline diameter, mm	2.7 (0.3)	2.7 (0.3)	2.7 (0.5)	0.9
% FMD	6.3 (2.7)	8.1 (3.1)	9.7 (2.5)	0.001
Baseline flow, mL/min	8.6 (3.7)	8.7 (4.1)	9.2 (5.7)	0.6
% Reactive hyperemia flow	492.2 (139.1)	523.2 (138.2)	486.0 (140.9)	0.6
Absolute change in flow, mL/min	31.0 (9.4)	31.3 (16.23)	32.4 (17.6)	0.8

Values are mean (SD); P values were from 1-way ANOVA testing the means across the 3 groups.

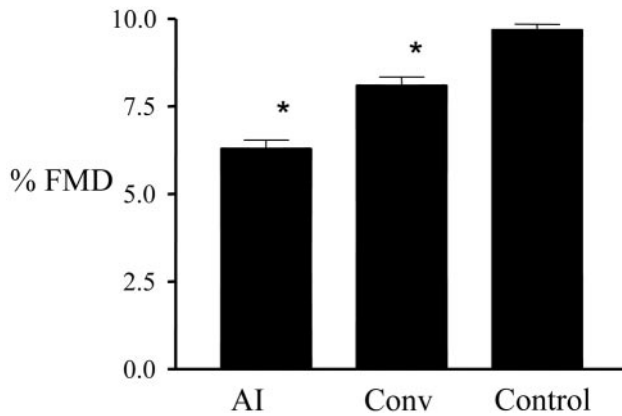


Figure 1. Impact of infection on endothelial function. This figure demonstrates differences in endothelial function (% FMD) between AI group, convalescent (Conv) group, and controls. Values expressed as mean ± SD. * $P < 0.001$ vs control group.

the 3 infection groups (Table 2). Endothelium-dependent FMD was lower in the AI group than in either the convalescent or the control children ($P < 0.001$ for both; Figure 1). In addition, FMD was also lower in the convalescent children than in the controls ($P < 0.001$).

None of the factors mentioned in Table 1, including hygiene and prior infection score, were associated with FMD in this cohort ($P = 0.3$ to $P = 0.9$). However, given the data from the literature, we considered these factors as potential confounders and adjusted for them in the analysis. The impact of AI on FMD was unaltered after multivariable adjustment for gender, baseline brachial artery diameter, total cholesterol, systolic blood pressure, socioeconomic status, body mass index, infection, and hygiene score (Table 3).

Follow-Up Study

The differences in vascular function between the AI and control children who participated in the follow-up study were similar to the findings for the entire cohort. Baseline vessel size was similar in both the AI and the control group at the 2 visits. Baseline and reactive hyperemia flow measurements were similar in the control group at the 2 visits (Table 4). FMD was unchanged in the control children in the 2 visits. In contrast, FMD improved substantially in the children in the AI group (Figure 2; Table 4). At follow-up study, FMD was lower in the AI group than in controls, but this did not reach conventional statistical significance ($P = 0.06$). Thirty-six of the children in the AI group had endothelial function values within the normal range at follow-up. The FMD values of 4 AI children remained 2 SDs below the mean value for the control children. In the control group, 2 children had FMD

values 2 SDs below the mean both at baseline and at restudy. There were no demographic or clinical features that discriminated this small group of AI or control children from the remaining children. Baseline flow measurements in the AI group were higher in the follow-up study than in their index visit. Although this baseline difference in flow resulted in lower reactive hyperemic flow expressed as a percentage, the absolute increase in hyperemic flow was similar to that seen at the first visit (Table 4).

Discussion

This study shows that minor infectious illnesses are associated with impairment in endothelial function in otherwise well children. Vascular function recovered in most but not all children at follow-up study 1 year later. These findings support a potential role for previously unsuspected extrinsic inflammatory stimuli in the pathogenesis of early atherosclerosis.

Most studies of infection and atherosclerosis have examined the late clinical phase of the disease. Infectious agents have been found in atherosclerotic plaques and linked to vascular inflammation.¹⁶ Individual pathogens and total pathogen burden have been associated with structural and functional arterial wall abnormalities and outcome, but no causal relationship has been established.^{17–19} Large antibiotic trials targeting specific infectious agents to treat the established atherosclerotic disease have reported conflicting and mostly disappointing results,²⁰ which suggests at most a limited benefit of treatment in patients with advanced atherosclerotic disease.

Experimental work, however, has suggested that infection may play a more important role in the early phase of atherogenesis and may be amenable to intervention at this stage.^{21–23} We have previously shown that a specific vasculitic disease, Kawasaki disease, can cause long-term vascular dysfunction in the young.²⁴ We now extend these findings to demonstrate an acute vascular effect of minor common infectious illnesses experienced by the majority of children. By studying children, it is possible to minimize the potential confounding but less easily quantifiable effects of lifetime risk factor burden.

We chose to study the effect of these illnesses on endothelial function in conduit arteries because endothelium plays a key role in regulating many of the vascular changes involved in the initiation and progression of early disease. We measured FMD, a technique developed by our group, because this has been shown to be accurate, to be reproducible, to correlate with endothelial function in the coronaries, and to reflect nitric oxide bioavailability.^{13,25,26} Furthermore, endothelial function measured by FMD has been shown to predict cardiovascular outcome in recent reports and to respond to interventions.^{27,28}

We have shown that even mild childhood infections that do not require a visit to the doctor or antibiotic therapy are associated with marked endothelial dysfunction. The impact on FMD was comparable to that seen in previous studies in young adults with risk factors such as diabetes and family history of coronary artery disease.^{29,30} Within this large representative cohort, there was a range of vascular responses

TABLE 3. Associations Between FMD and Acute Infection

	% FMD Unadjusted	% FMD, Adjusted*
AI group	−3.4 (−3.9, −2.8)	−3.1 (−3.9, −2.3)
Convalescent group	−1.7 (−2.2, −1.1)	−1.7 (−2.4, −0.7)

*Adjusted for gender, baseline brachial artery diameter, infection score, hygiene score, total cholesterol, body mass index, social class, and systolic blood pressure.

β (CI) values are from regressions in comparisons with controls.

TABLE 4. Vascular Characteristics of Children in Follow-Up Study

	AI Group (n=40)			Control Group (n=50)			P†
	Visit 1	Visit 2	P*	Visit 1	Visit 2	P*	
Baseline diameter, mm	2.8 (0.3)	2.7 (0.3)	0.2	2.8 (0.9)	2.7 (0.4)	0.5	0.9
% FMD	5.0 (2.3)	8.2 (2.5)	0.001	9.3 (2.4)	9.2 (2.3)	0.8	0.06
Baseline flow, mL/min	8.5 (2.8)	11.6 (6.9)	0.01	10.3 (8.7)	10.3 (4.9)	0.9	0.3
% Reactive hyperemia flow	499.1 (143.2)	432.7 (140.2)	0.05	476.8 (140.5)	477.8 (162.4)	0.9	0.2
Absolute change in flow, mL/min	31.6 (8.9)	33.1 (13.5)	0.8	34.5 (13.9)	33.9 (12.5)	0.6	0.8

Values are mean (SD).

*Visit 1 vs visit 2; †visit 2 of AI group vs visit 2 of control group.

to acute infection. This is unlikely to have been due to variation in cardiovascular risk factor profile in the present study population, because there were similarly low levels of risk factors in all 3 groups. In addition, owing to size and time limitations of the study, blood was not taken at the time of the study. We were therefore unable to define whether specific infectious agents are more likely to produce vascular effects. It was also not possible to quantify the magnitude of the vascular inflammatory response by measuring blood markers of endothelial activation, which have been identified in adult subjects at risk of coronary artery disease. Further studies are required to examine the contribution of specific infectious agents and the potential contribution of parental risk factors, ie, smoking.^{31,32}

A number of potential mechanisms by which infection may damage the arterial wall have been proposed. A direct effect of the virus on the endothelium has been suggested.³³ Alternatively, indirect damage may result from inflammatory cytokines on constitutive endothelial nitric oxide synthase, as well as lipid modifications of both HDL and LDL cholesterol.^{11,34–36}

Animal studies have shown that repeated administration of infectious organisms can lead to progressive endothelial damage.^{37,38} In the present study, we were able to examine changes in FMD over an average follow-up of 1 year. Endothelial responses in most but not all the children in the AI group returned to within the normal range. Thus, the effects of AI at this age may be transient and recoverable. We are unable, however, to determine the potential impact of

recurrent infections, which might be cumulative, as in the animal studies, on the initiation and progression of early atherosclerosis. The AI, convalescent, and control children all had similar reported histories of early childhood infections and hygiene practices. None of the children experienced an infection in the interval between the 2 studies. There were only 4 children from the AI group in whom vascular function remained abnormal at follow-up. This did not allow us to study the factors that might predispose a child to persistent endothelial dysfunction after acute infection. It remains possible that genetic or environmental factors may influence the long-term vascular effects of infection that may take years to become clinically apparent.

Conclusions

We have shown that a minor infectious stimulus in childhood relevant to normal daily life is associated with endothelial dysfunction. This raises the possibility that infection may contribute to mechanisms relevant to the development of atherosclerosis. However, the cumulative impact of individual infectious agents and the interaction with genetic, phenotypic, and other environmental predisposing factors requires prospective evaluation.

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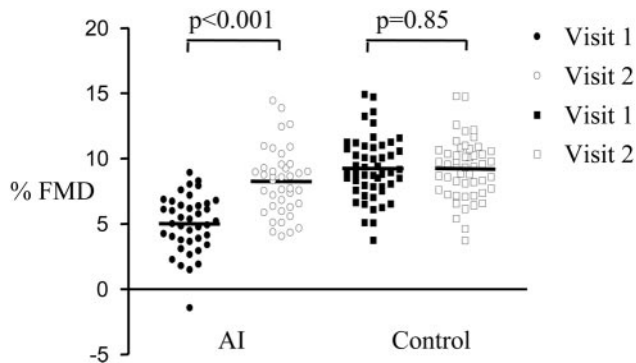


Figure 2. Follow-up study. FMD was reassessed in 40 children in AI group and 50 controls at mean interval of 12 ± 6 months after their initial evaluation. Horizontal lines represent mean values for each group.

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