ORIGINAL RESEARCH

Relation Between Circulating Inflammatory Chemokines and Vascular Characteristics in Healthy, Young Children

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***Background*—**Atherosclerosis begins in childhood with the occurrence of inflammatory vascular wall alterations that are detectable with B-mode ultrasound. Chemokines appear to be involved in the development of these alterations given that they occur early in the atherosclerotic pathway as mediators of vascular inflammation. However, this has not extensively been investigated. Therefore, we studied in healthy young children whether chemokines monocyte chemotactic protein 1 (MCP-1), regulated on activation normal T-cell expressed and secreted (RANTES), and vascular and intercellular adhesion molecules (VCAM and ICAM) related to vascular characteristics of the carotid artery.

***Methods and Results*—**We obtained demography, anthropometry, and overnight fasting plasma of 139 eight-year-old children of the Wheezing Illnesses Study Leidsche Rijn birth cohort. Carotid intima-media thickness (CIMT), distensibility, and Young's Elastic Modulus (YEM) of the common carotid artery were measured sonographically. Chemokine plasma levels were assessed using a multiplex assay. We studied the relation between the chemokines and vascular characteristics using multivariable linear regression analyses with adjustments for sex, systolic blood pressure, homeostasis model assessment of insulin resistance, triglycerides, low-density lipoprotein- and high-density lipoprotein-cholesterol. Of the studied chemokines, RANTES related to common carotid distensibility and YEM. One standard deviation increase in RANTES level related to a 5.45-MPA^{-1} (95% confidence interval [CI], −9.43, −1.39; P=0.01) decrease in distensibility and to a 5.55-kPa increase in YEM (95% CI, 0.40, 10.85; P=0.03). RANTES did not relate to CIMT. MCP-1, VCAM, and ICAM did not relate to any of the studied vascular characteristics.

***Conclusion*—**RANTES appears to be involved in the development of preatherosclerotic inflammatory vascular alterations already in healthy, young children. This may provide further insight into the early-life origins of atherosclerosis. **(*J Am Heart Assoc*. 2015;4: e002346 doi: 10.1161/JAHA.115.002346)**

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Despite improvements in management and prevention of cardiovascular disease (CVD), its medical, social, and economic burden in the aging Western world remains extremely high.[1] Atherosclerosis, the major pathophysiological mechanism underlying CVD, is a systemic inflammatory disease that causes detrimental remodeling of various arterial walls.

Although clinical signs and symptoms usually occur in later adulthood, postmortem studies showed that the atherogenic process already begins in early childhood.[2] Two key processes occurring in early-life atherosclerosis are the increased adherence of leukocytes to the vascular endothelium and their subsequent migration through the vascular wall into the arterial intima.[3–5] Leukocyte adhesion and migration is a multistep process involving a variety of molecules. Leukocytes can be attracted to inflammatory sites by chemokines after a chemotactic concentration gradient. Among others, monocyte chemoattractant protein 1 (MCP-1, CCL2) and regulated on activation normal T-cell expressed and secreted (RANTES, CCL5) have been shown to be involved in the attraction of leukocytes to atherogenic sites. In addition to their chemotactic role, MCP-1 and RANTES also activate leukocytes, inducing adhesion molecule expression on the leukocyte

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surface. When coming in contact with activated endothelium that expresses vascular and intercellular adhesion molecules (VCAM and ICAM), leukocytes firmly adhere and migrate through the vascular wall and into the arterial intima where they initiate the proliferation of smooth muscle cells and the development of lipid deposits and foam cells.[6,7]

Based on their pathogenic role, the aforementioned chemokines are suggested to be involved in early-life atherosclerosis. As such, concentrations in blood of these chemokines may serve as proxies reflecting this early phase of atherosclerosis.[3–5,8–12] This hypothesis is strengthened by various studies that demonstrated that children who have a higher cardiovascular risk burden attributable to obesity, dyslipidemia, diabetes, or hypertension have higher levels of VCAM, ICAM, RANTE,S and MCP-1 as compared to their healthy counterparts.[8,9,11–15]

However, little is known about the relation between these chemokines and established proxies that reflect early-life atherosclerosis, such as sonographically detected preatherosclerotic inflammatory vascular wall alterations (ie, intimal thickening).[16–20] Only ICAM-1 has previously been studied in obese, hypertensive adolescents and appeared to relate to carotid intima-media thickness (CIMT).[10] To the best of our knowledge, there are no previous investigations into the relation between all 4 chemokines and vascular wall characteristics, neither has the relation between cardiovascular risk factors and these chemokines been studied in a healthy, young population.

Therefore, we investigated whether levels of MCP-1, RANTES, VCAM, and ICAM related to vascular wall characteristics and to cardiovascular risk factors in a population of healthy, 8-year-old children.

Methods

Study Design and Population

Our study population comprised 8-year-old children who are currently participating in an ongoing, prospective, population-based birth cohort study that evaluates determinants of wheezing illnesses, the so-called Wheezing Illnesses Study Leidsche Rijn (WHISTLER) study. The WHISTLER study cohort, initiated in December 2001, consists of healthy newborns who live in a residential area near the city of Utrecht, The Netherlands, called Leidsche Rijn. Study design and rationale have been published previously.[21] Children who have neonatal respiratory disease, major congenital abnormalities, or a gestational age <36 weeks were excluded from participation in the WHISTLER study. As from November 2005, the WHISTLER study was extended with cardiovascular measurements (WHISTLER-Cardio). All 5-year-old participants of the WHISTLER study were invited for follow-up measurements at 5 and subsequently at 8 years of age.[18] The current study pertains to those 237 children in whom follow-up examinations at 8 years of age were carried out. In 143 of these 237 children (60.3%), a venous blood sample had successfully been obtained. In 2 children, there was insufficient sodium-heparin plasma available for the measurement of chemokines and in 2 children this measurement failed. Thus, in total, the current study population comprises 139 children (58.6%). The demographic and anthropometric characteristics of the 98 children who did not participate in the current study were similar to those of the children who are participating in the current study (Table S1). Finally, because data on homeostasis model assessment of insulin resistance (HOMA-IR) were missing in 15 participants, complete case analysis was performed in 124 of 139 children (89.2%). The medical ethics committee of the University Medical Center Utrecht (Utrecht, The Netherlands) approved the study. In addition, written informed consent was collected from all parents before study participation.

Measurements

Demographic information

For the WHISTLER study, a detailed questionnaire regarding the development of the child was filled out by the parents of the child at the first (each month), fifth, and eighth year of life of the child. This questionnaire included inquiries on demographic information of the child and on the child's health status. From the questionnaire, it showed that none of the children in our study population had developed a chronic or metabolic illness at 8 years of age.

Anthropometric measurements

Weight, height, waist, and hip circumference were measured in study subjects wearing indoor clothes without shoes and standing with the feet lightly apart. Body height and weight were measured. Hip circumference was obtained twice and measured at the widest level over the major trochanter to the nearest millimeter, whereas waist circumference was also obtained in duplicate and was measured at the level midway between the lowest rib border and the iliac crest. In addition, body mass index (BMI; kg/m^2) was calculated.

Arterial wall characteristics

Characteristics of the right common carotid artery were studied sonographically in all participants using high-resolution echotracking technology (ArtLab, Esaote, Italy), including the use of a 128 radiofrequency line multiarray, with a L10-5, 40-mm linear array transducer.[22] Six-second cineloops were stored without compression (120 MB) for offline analysis. In addition, raw radiofrequency data were analyzed online. This technique has previously been used in children and gives

access to all major mechanical parameters for 4-cm arterial fragments: diastolic diameter *d*, far wall CIMT and the distension, the diameter change as function of time.[18] Diameter and CIMT were measured with 2.1-μm resolution and distension with 1.7-μm resolution after 10 minutes of rest with the subjects placed in a supine position with the head turned to the left.[22] All measurements were repeated a maximum of 4 times during 1 session and performed by a pediatric research nurse and an executive investigator who were both blinded to other characteristics of the study population as well as to possible confounding variables. The sonography examination took ≈30 minutes to complete, during which the children watched a cartoon.

During the sonographic measurements, blood pressure measurements were performed twice at the brachial artery with a semiautomatic oscillometric device with a pediatric cuff (DYNAMAP; Criticon, Tampa, FL). The average of both measurements was used to calculate the common carotid artery local pulse pressure, presuming mean arterial pressure minus diastolic blood pressure constant in the large arterial tree. In order to evaluate the elastic conditions of the artery and of the artery wall, expressed as the cross-sectional distensibility coefficient and the YEM, respectively, we used the averages of the lumen diameter, CIMT, and distension. Table S2 shows the units and formulas used for the various results, which are based on previously performed research.[23] Reproducibility of measurement was assessed for CIMT, distension, and lumen diameter. Measurements were based on multiple assessments per child; they were performed by 1 observer in 10 subjects on 2 different occasions, as published elsewhere.[24] Mean coefficients of variation of CIMT, distension, and lumen diameter in the 8-year-old population were 6.7% and 6.7% and 2.3%, respectively.

Laboratory assessments

The venous blood sample was obtained at a home visit after an overnight fast between 7:00 and 10:30 AM. The sample was collected in 2 tubes, namely in a sodium-heparin tube, that was instantly placed on ice, and in a clotting tube. Subsequently, the sample was transported to the University Medical Center Utrecht and processed at the Laboratory of Translational Immunology of the University Medical Center Utrecht. First, the sample was centrifuged at 1450*g*, at 4°C for 15 minutes. Next, the serum and sodium-heparin plasma were stored at −20°C and −80°C, respectively, within a maximum of 4 hours after the venous blood sample was obtained. Samples remained in storage until they were recollected for further analysis.

In addition, in serum, total and HDL cholesterol levels, triglyceride levels (DxC800/AU5811; Beckman Coulter, Fullerton, CA), and fasting glucose and insulin levels (Modular E170; Roche, Basel, Switzerland) were measured at the laboratory of Clinical Chemistry of the University Medical Center Utrecht. LDL-cholesterol level was calculated using the Friedewald formula.[25] For insulin and triglycerides, it was considered that if levels occurred to be below the detection limit, we used the mean level between zero and the lower detection limit. Finally, we obtained insulin resistance measurements using the HOMA-IR ([fasting insulin (mU/L)×fasting glucose (mmol/L)]/22.5).[26] Cut-off values for insulin resistance were determined in agreement with Kurtoglu's criteria for insulin resistance in prepubertal children, 2.22 and 2.67 for girls and boys, respectively.[27]

A valid method for isolation and protocol for quantification of the specific chemokines in the current study has been evaluated and published previously.[28,29] Measurements of the chemokine levels of MCP-1, RANTES, VCAM, and ICAM were performed using an in-house developed and validated multiplex immunoassay, which is based on Luminex technology (xMAP; Luminex, Austin, TX), of which a detailed description has been published elsewhere.[28,29] In short, from all samples, heteroblock (Omega Biologicals, Bozeman, MT) was used to preabsorb a specific heterophilic immunoglobulins. In addition, Biorad FlexMAP3D (Bio-Rad Laboratories, Hercules, CA) in combination with xPONENT software (version 4.2; Luminex) was used to perform acquisition. Subsequently, the obtained information was analyzed using 5-parametric curve fitting with Bio-Plex Manager software (version 6.1.1; Bio-Rad). For MCP-1, plasma samples were measured undiluted whereas for VCAM and ICAM and RANTES, which naturally occur in high concentrations, the samples were diluted 100 times. All chemokine levels were expressed in ng/mL.

Statistical Analysis

We analyzed demographic characteristics of our study participants by calculating proportions, means with SDs or medians with the 25th and 75th percentiles (Q1 and Q3), respectively.

Additionally, we mutually correlated the chemokines under study using Spearman's rho correlation coefficients (*r*). Spearman's rho was used because RANTES, VCAM, and ICAM were not normally distributed.

Furthermore, multivariable linear regression analyses were used to study the following relations. First, we studied the relation between the vascular characteristics (common CIMT, carotid distensibility, and carotid YEM) and chemokines (MCP-1, RANTES, VCAM, and ICAM), using the vascular characteristics as dependent and the chemokines under study as independent variables. Additionally, we examined the relation between each of the chemokines under study and known classical cardiovascular risk factors, using the chemokines as

dependent variables and the classical cardiovascular risk factors as independent variables.

In the analyses concerning the relation between the vascular characteristics and chemokines under study, we used the natural-logarithm transformed common CIMT, carotid distensibility, and carotid YEM to normalize skewed distributions. This was a necessity in order to meet the mandatory criteria for linear regression models. Similarly, we used the natural-logarithm transformed chemokines RANTES, VCAM, and ICAM in the analyses regarding the relation between the chemokines under study and classical cardiovascular risk factors.

Because various factors can confound the relation between the chemokines under study and the vascular characteristics and classical cardiovascular risk factors under study, we adjusted for these potential confounders using the following method. First, we adjusted our crude model for sex because sex is a key driver for cardiovascular risk (model 1). Subsequently, when adding single covariates changed the regression coefficient of interest by more than 10%, it was included in the fully adjusted multivariable model (model 2). Ultimately, that model contained age (years), sex (reference category: male), systolic blood pressure (SBP; mm Hg), BMI (kg/m^2), LDL-cholesterol level (mmol/L), HDL-cholesterol level (mmol/L), triglyceride level (mmol/L), and HOMA-IR. Of note, we did not adjust our models for age because all children were 8 years old. Finally, because SBP is an important component of carotid distensibility and YEM and one of our confounding variables, we performed an analysis in which we excluded SBP from our fully adjusted model.

After all analyses were performed, we back transformed the log-transformed regression coefficients and confidence intervals (CIs). Conclusions were based on standardized regression coefficients with 95% CIs, corresponding to $P_{2\text{sided}}<0.05$. We used SPSS software (version 20.0; IBM, Armonk, NY) to perform our data analysis.

Results

The characteristics of the study population are presented in Table 1. The 139 children under study had a mean age of 8.1 years (±0.4) and 42% were boys. Mean BMI was 15.9 kg/m^2 (±1.8). Additionally, we observed that Spearman's rho correlation coefficients (r) between the selected chemokines were weak to modest (Spearman r between 0.03 and 0.38; Table 2).

The results of the relation between common CIMT, carotid distensibility, and carotid YEM with the chemokines under study are presented in Table 3. The fully adjusted multivariable model demonstrated a significant inverse relation between RANTES levels and carotid distensibility and a significant positive relation between RANTES and carotid YEM. After full adjustment, a 1 SD increase in RANTES level related to 5.45 MPA^{-1} (95% CI, −9.43, −1.39; P=0.01) decrease in carotid distensibility and to a 5.55-kPa increase in carotid YEM (95% CI, 0.40, 10.85; P=0.03), respectively. Of note, RANTES did not relate to common CIMT; neither did the chemokines MCP-1, VCAM,I and ICAM relate to any of the selected vascular characteristics.

Table 4 displays the results of the relation between each of the chemokines under study and classical cardiovascular risk factors. The fully adjusted model showed a significant positive relation between age and RANTES and VCAM. A 1 SD increase in age related to a 5.33-ng/mL increase in RANTES level (95% CI, 1.41, 9.31; P=0.01) and to a 15.14-ng/mL increase in VCAM level (95% CI, 7.68, 17.12; P<0.001), respectively. Additionally, there was a significant positive relation between triglycerides and ICAM. A 1 SD increase in triglycerides related to a 4.92-ng/mL increase in ICAM level (95% CI, 0.20, 9.97; P=0.04).

Finally, the additional analysis without SBP showed that the exclusion of SBP from our fully adjusted model did not affect our results.

Discussion

The current study demonstrates that in healthy young children, higher plasma levels of RANTES relate to 2 measures of arterial stiffness, namely common carotid distensibility and common carotid YEM. Hence, the relation between RANTES and preatherosclerotic vascular alterations may already exist in healthy, young children.

The 4 chemokines under study are suggested to contribute to early-life preatherosclerotic, inflammatory vascular wall alterations because of their involvement in both leukocyte-endothelial interaction and subsequent transendothelial migration. As such, the expression of these chemokines in plasma may reflect the status of the underlying atherogenic process. Indeed, this has been suggested by others in studies among older participants. Our results agree upon this given that RANTES related to vascular wall characteristics already in healthy, young children.

Evidence on the relation of these chemokines with vascular characteristics and cardiovascular risk factors in children is scarce. Previous studies demonstrated that in children and adolescents with hypertension, obesity, or diabetes mellitus, plasma concentrations of VCAM and ICAM were higher than in healthy controls. In addition, higher levels of VCAM and ICAM related to unfavorable levels of triglycerides, cholesterol levels, and to an increased insulin sensitivity.[8,9,11,30] Furthermore, in prepubertal children, higher levels of MCP-1 have been linked to obesity, whereas RANTES has shown to be

Table 1. Characteristics of Study Population (n=139)

	N	Total Population
Demographic characteristics, child		
Age, mean±SD	139	8.1±0.4
Sex, male (%)	139	59 (42.44)
Height, mean±SD	139	132.5±5.4
Weight, mean±SD	139	28.0±4.4
BMI (kg/m^2), mean±SD	139	15.9±1.8
Waist circumference (cm), mean±SD	138	58.3±50
Systolic blood pressure (mm Hg), mean±SD	139	107±9
Diastolic blood pressure (mm Hg), mean±SD	139	55±6
Smoking parents, no parents (%)	139	86 (73.5)
Glucose metabolism		
Glucose (mmol/L), mean±SD	128	4.61±0.33
Insulin (mIU/L), mean±SD	124	5.66±2.52
HOMA-IR, mean±SD	124	1.18±0.55
Lipid spectrum		
Total cholesterol (mmol/L), mean±SD	128	4.41±0.70
LDL cholesterol (mmol/L), median (Q1, Q3)	125	2.76±0.61
HDL cholesterol (mmol/L), mean±SD	128	1.45±0.27
Triglycerides (mmol/L), mean±SD	127	0.47±0.21
Radiologic characteristics		
Common CIMT (mm), median (Q1, Q3)	139	0.38 (0.35, 0.41)
Common CIMT (ln(mm)), mean±SD*	139	−0.97±0.10
Common carotid distensibility (MPA^{-1}), median (Q1, Q3)	131	85.65 (71.44, 97.71)
Common carotid distensibility (ln(MPA^{-1})), mean±SD*	131	4.43±0.25
Common carotid YEM (kPa), median (Q1, Q3)	131	182.55 (147.89, 221.42)
Common carotid YEM (ln(kPa)), mean±SD*	131	5.19±0.28
Inflammation parameters		
MCP-1 (ng/mL), median (Q1, Q3)	138	0.17 (0.12, 0.23)
RANTES (ng/mL), median (Q1, Q3)	139	49.62 (43.17, 57.08)
VCAM (ng/mL), median (Q1, Q3)	139	899.05 (679.51, 1215.20)
ICAM (ng/mL), median (Q1, Q3)	139	88.46 (79.06, 109.29)

BMI indicates body mass index; CIMT, carotid intima-media thickness; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; ICAM: intracellular adhesion molecule; LDL, low-density lipoprotein; MCP-1, monocyte chemotactic protein 1; Q1, 25th percentile; Q3, 75th percentile; RANTES, regulated on activation normal T-cell expressed and secreted; VCAM, vascular cell adhesion molecule; YEM, Young's Elastic Modulus.
*In this variable, natural logarithmic transformation was performed.

selectively upregulated in children with familial hypercholesterolemia and in children with primary hypertension.[13–15] However, data on the relation between vascular characteristics and chemokines in children, to the best of our knowledge, have previously been assessed solely for ICAM. In this study, Glowinska et al. associated higher levels of ICAM with CIMT in a population of obese, hypertensive children and adolescents.[10]

In the current study, RANTES was the only chemokine relating to unfavorable values of 2 measures for arterial stiffness, namely common carotid distensibility and YEM. In the etiology of arterial stiffness, dysfunctional endothelium plays a pivotal role. The endothelium is of crucial importance for the morphological, functional, and structural regulation of the arterial wall (ie, by secreting endothelin 1 and nitric oxide). Inflammatory responses occurring in the arterial wall may prompt endothelial dysfunction and consequently induce adverse changes in the arterial wall, which may ultimately lead to stiffer arteries. Because of its involvement in the vascular wall inflammation response, RANTES possibly contributes to the development of stiffer arteries. RANTES increases endothelial adherence and permeability, facilitates leukocyte influx into the arterial wall, and promotes macrophage accumulation, neointimal growth and smooth muscle cell proliferation.[31,32]

Our result concurs with previously performed studies. Litwin et al. showed that plasma RANTES levels were higher in children with primary hypertension, a condition closely related to arterial stiffness.[13] Additionally, various experimental studies observed a higher expression of RANTES in early atherosclerotic endothelia or neointimal lesions. The upregulation of other chemokines followed later,[13,33,34] implying that the expression of chemokines depends on the stage of the atherosclerotic process. This may clarify why RANTES is the only chemokine under study that relates to vascular characteristics in healthy, young children in whom the atherogenic process is still in an initial stage.

In our study, age related to RANTES and VCAM. The association of RANTES and VCAM with age was present

Table 2. Mutual Correlations Between Chemokines (n=138)

	MCP-1*	*P* Value*	RANTES*	*P* Value*	VCAM*	*P* Value*	ICAM*	*P* Value*
MCP-1	—	—	−0.12	0.17	−0.21	0.012	0.20	0.017†
RANTES	−0.12	0.17	—	—	0.38	<0.001†	0.03	0.76
VCAM	−0.21	0.012	0.38	<0.001†	—	—	−0.30	<0.001†
ICAM	0.20	0.017†	0.026	0.76	−0.30	<0.001†	—	—

ICAM indicates intracellular adhesion molecule; MCP-1, monocyte chemotactic protein 1; RANTES, regulated on activation normal T-cell expressed and secreted; VCAM, vascular cell adhesion molecule.
*Values are Spearman's rho (*r*) correlation coefficients and *P* values.
†*P*<0.05.

despite that the distribution of age in this study was small. For VCAM and other chemokines, it has been described before in healthy individuals that their plasma levels increase with age, independent of other cardiovascular risk factors.[35] Yet, the age range in these studies was larger than in our study. However, for RANTES, the relation with age has not been described before. Unfortunately, the mechanism underlying this observation remains to be elucidated. Furthermore, it appeared that ICAM related to triglycerides. As such, it appeared that ICAM may be involved in triglyceride metabolism. Various previously performed studies showed that higher triglyceride levels in obese children, children with dyslipidemia, and healthy children indeed associated with higher ICAM levels.[8,9,36,37] It has been hypothesized that triglyceride levels may play a role in ICAM expression and as such possibly contribute to progression of the atherosclerotic process. Furthermore, in contrast to Glowinska et al., we did not observe an association between ICAM and CIMT. This difference is likely attributable to our study population that consists of healthy, young children, whereas the above-mentioned study population comprised children with an increased cardiovascular risk burden. In healthy, young

Table 3. Relation Between Chemokines and Vascular Characteristics (n=124)

	Common CIMT (mm)*	*P* Value	Common Carotid Distensibility (MPA^{-1})*	*P* Value	Common Carotid YEM (kPa)*	*P* Value
MCP-1						
Crude model†	0.50 (−1.19, 2.22)	0.55	1.92 (−2.47, 6.50)	0.40	−2.37 (−7.13, 2.63)	0.34
Model 1†	0.50 (−1.19, 2.22)	0.57	2.63 (−1.69, 7.25)	0.24	−3.34 (−7.96, 1.51)	0.17
Model 2†	0.70 (−−1.09, 2.53)	0.44	−0.60 (−5.07, 3.98)	0.79	−0.30 (−5.45, 5.13)	0.90
RANTES						
Crude model†	−0.60 (−2.27, 1.11)	0.51	−3.25 (−7.23, 0.90)	0.13	2.74 (−2.08, 7.79)	0.27
Model 1†	−0.60 (−2.27, 1.21)	0.52	−4.30 (−8.24, −0.20)	0.04‡	4.19 (−0.70, 9.31)	0.09
Model 2†	−0.70 (−2.47, 1.21)	0.48	−5.45 (−9.43, −1.39)	0.01‡	5.55 (0.40, 10.85)	0.03‡
VCAM						
Crude model†	−0.03 (−1.69, 1.71)	0.97	−3.54 (−7.50, 0.70)	0.10	3.66 (−1.19, 8.87)	0.14
Model 1†	−0.03 (−1.78, 1.71)	0.97	−3.54 (−7.50, 0.60)	0.10	3.67 (−1.09, 8.76)	0.13
Model 2†	−0.50 (−2.18, 1.21)	0.57	−1.88 (−6.01, 2.33)	0.36	2.33 (−2.46, 7.47)	0.35
ICAM						
Crude model†	0.80 (−0.90, 2.53)	0.34	−0.60 (−4.88, 3.98)	0.80	−0.40 (−5.35, 4.71)	0.87
Model 1†	0.90 (−0.90, 2.63)	0.33	−1.00 (−5.26, 3.56)	0.67	0.10 (−4.78, 5.23)	0.97
Model 2†	0.02 (−1.78, 1.92)	0.98	−1.19 (−5.54, 3.36)	0.60	1.21 (−4.02, 6.61)	0.65

BMI indicates body mass index; CIMT, carotid intima-media thickness; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; ICAM, intracellular adhesion molecule; LDL, low-density lipoprotein; MCP-1, monocyte chemotactic protein 1; RANTES, regulated on activation normal T-cell expressed and secreted; SBP, systolic blood pressure; VCAM, vascular cell adhesion molecule; YEM, Young's Elastic Modulus.
*Values are regression coefficients per 1 SD increase in chemokine level with 95% confidence intervals.
†Crude model, model 1: adjusted for sex; model 2: adjusted for sex, SBP, BMI, LDL-cholesterol level, HDL-cholesterol level, triglyceride level and HOMA-IR.
‡*P*<0.05.

Table 4. Relation Between Classical Cardiovascular Risk Factors and Chemokines (n=124)

	MCP-1 (ng/mL)*	*P* Value	RANTES (ng/mL)*	*P* Value	VCAM (ng/mL)*	*P* Value	ICAM (ng/mL)*	*P* Value
Age, y								
Crude model†	−0.01 (−0.02, 0.01)	0.25	4.71 (1.11, 8.44)	0.01‡	15.49 (8.87, 22.51)	<0.001‡	−3.25 (−7.23, 0.80)	0.12
Model 1†	−0.01 (−0.02, 0.01)	0.23	4.81 (1.21, 8.44)	0.01‡	15.49 (8.87, 22.51)	<0.001‡	−3.25 (−7.23, 0.90)	0.34
Model 2†	-3.00×10^{-3} (−0.02, 0.01)	0.66	5.33 (1.41, 9.31)	0.01‡	15.14 (7.68, 17.12)	<0.001‡	−2.66 (−6.85, 1.61)	0.21
Sex (ref: female)								
Crude model†	0.02 (−0.01, 0.05)	0.26	−8.61 (−14.87, −1.88)	0.01‡	1.31 (−10.95, 15.14)	0.85	−3.92 (−12.19, 4.08)	0.33
Model 1†	0.02 (−0.01, 0.05)	0.26	−8.61 (−14.87, −1.88)	0.01‡	1.31 (−10.95, 15.14)	0.85	−3.92 (−12.19, 4.08)	0.33
Model 2†	0.02 (−0.01, 0.05)	0.25	−6.57 (−13.67, 1.01)	0.09	1.21 (−12.63, 17.23)	0.87	0.20 (−8.33, 9.41)	0.97
BMI, kg/m^2								
Crude model†	−0.01 (−0.02, 0.01)	0.24	−0.60 (−4.11, 3.05)	0.72	5.34 (−1.09, 12.19)	0.10	2.02 (−2.27, 6.29)	0.38
Model 1†	−0.01 (−0.02, 0.01)	0.23	−0.60 (−4.02, 3.05)	0.75	5.34 (−1.09, 12.19)	0.10	1.92 (−2.27, 6.29)	0.37
Model 2†	−0.01 (−0.03, 0.01)	0.51	−0.80 (−5.35, 3.98)	0.74	4.29 (−4.40, 13.88)	0.34	1.51 (−3.63, 7.04)	0.56
SBP, mm Hg								
Crude model†	-0.01 (-0.02, 4.00×10^{-3})	0.17	−1.09 (−4.59, 2.53)	0.55	2.63 (−3.63, 9.42)	0.41	2.53 (−1.69, 6.93)	0.24
Model 1†	-0.01 (-0.02, 4.60×10^{-3})	0.18	−1.19 (−4.59, 2.33)	0.50	2.63 (−3.63, 9.42)	0.41	2.53 (−1.69, 6.93)	0.25
Model 2†	-4.00×10^{-3} (−0.02, 0.01)	0.61	−1.49 (−5.35, 2.43)	0.45	0.30 (−6.85, 8.00)	0.94	1.61 (−2.76, 6.29)	0.47
HDL cholesterol, mmol/L								
Crude model†	2.00×10^{-3} (−0.01, 0.02)	0.77	−1.19 (−4.78, 2.53)	0.54	−3.05 (−9.52, 3.77)	0.37	−3.54 (−7.50, 0.60)	0.10
Model 1†	1.00×10^{-3} (−0.01, 0.02)	0.91	−0.60 (−4.21, 3.15)	0.74	−3.25 (−9.70, 3.77)	0.35	−3.34 (−7.41, 0.80)	0.11
Model 2†	-2.00×10^{-3} (−0.02, 0.02)	0.83	−1.88 (−5.82, 2.33)	0.37	−2.27 (−9.52, 5.65)	0.56	−2.66 (−7.13, 2.02)	0.25
LDL cholesterol, mmol/L								
Crude model†	4.00×10^{-3} (−0.01, 0.02)	0.60	1.31 (−2.37, 5.23)	0.48	−0.40 (−7.13, 6.82)	0.91	4.60 (0.20, 9.09)	0.04‡
Model 1†	4.85×10^{-3} (−0.01, 0.02)	0.52	1.11 (−2.66, 4.92)	0.58	−0.40 (−7.13, 6.93	0.92	4.50 (0.10, 8.98)	0.04‡
Model 2†	0.01 (−0.01, 0.02)	0.38	1.21 (−2.86, 5.34)	0.57	−0.60 (−7.78, 7.14)	0.88	3.36 (−1.19, 8.22)	0.15
Triglycerides, mmol/L								
Crude model†	-0.01 (-0.03, 4.00×10^{-3})	0.14	−0.02 (−3.63, 3.77)	0.99	2.02 (−4.78, 9.31)	0.56	5.44 (1.11, 9.97)	0.01‡
Model 1†	-0.01 (-0.03, 4.56×10^{-3})	0.17	−0.40 (−4.02, 3.36)	0.82	2.12 (−4.69, 9.53)	0.55	5.34 (1.01, 9.86)	0.02‡
Model 2†	−0.01 (−0.03, 0.01)	0.31	−0.60 (−4.69, 3.56)	0.76	0.50 (−6.94, 8.65)	0.89	4.92 (0.20, 9.97)	0.04‡

Continued

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Table 4. Continued

	MCP-1 (ng/mL)*	*P* Value	RANTES (ng/mL)*	*P* Value	VCAM (ng/mL)*	*P* Value	ICAM (ng/mL)*	*P* Value
HOMA-IR								
Crude model†	-4.00×10^{-3} (−0.02, 0.01)	0.62	−0.10 (−3.73, 3.67)	0.97	7.25 (0.20, 14.91)	0.04‡	2.63 (−1.78, 7.14)	0.25
Model 1†	-4.00×10^{-3} (−0.02, 0.01)	0.63	−0.10 (−3.73, 3.56)	0.94	7.25 (0.20, 14.91)	0.04‡	2.53 (−1.78, 5.65)	0.25
Model 2†	-1.47×10^{-4} (−0.02, 0.02)	0.99	0.04 (−4.50, 4.81)	0.99	4.50 (−4.21, 14.11)	0.32	−0.30 (−5.35, 5.13)	0.92

BMI indicates body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; ICAM, intracellular adhesion molecule; LDL, low-density lipoprotein; MCP-1, monocyte chemotactic protein 1; RANTES, regulated on activation normal T-cell expressed and secreted; SBP, systolic blood pressure; VCAM, vascular cell adhesion molecule.

*Values are regression coefficients per 1 SD increase in CV risk factor level with 95% confidence intervals.

†Crude model, model 1: adjusted for sex; model 2: adjusted for sex, SBP, BMI, LDL-cholesterol level, HDL-cholesterol level, triglyceride level and HOMA-IR.

‡$P<0.05$.

children, the atherosclerotic process is probably in a more initial stage than in children who already have an increased cardiovascular risk burden because of unfavorable levels of cardiovascular risk factors (ie, obesity and hypertension). Therefore, the associations in our study may be more subtle than in more-diverse populations that also include children who are at higher risk for developing CVD later in life because of an increased cardiovascular risk factor load.

Another possible reason why RANTES is the only chemokine under study relating to vascular characteristics may be that plasma levels of chemokines do not adequately reflect the extent of the inflammatory process occurring in the vascular wall. Although soluble forms of chemokines are involved in inflammation and their expression is regulated by proinflammatory chemokines, the quantity measured in plasma may miss a significant proportion that is also shed, but not available (ie, because of adhesion to other cells that have receptors for these chemokines).[38,39] Specifically, ICAM and VCAM have a resilient affinity for ligand-bearing cells. Consequently, their plasma levels may not reflect the total quantity that is actually shed. Thus, the relation between cardiovascular risk factors and soluble ICAM may exist, because cardiovascular risk factors precede the occurrence of vascular wall alterations, yet relations with vascular characteristics may not. Indeed, a previous study showed that blood concentrations of ICAM did not associate to the extent of endothelial impairment in healthy and hypercholesterolemic adults, implying that circulating ICAM does not serve as a direct substitute for the extent of endothelial impairment in early atherosclerosis. The researchers suggest that soluble ICAM mainly reflects an increased expression of ICAM located on the surface of endothelial cells. As such, soluble ICAM does reflect the occurrence of an inflammatory response and activation of endothelial cells, a phase of early atherosclerosis, yet soluble ICAM does not serve as a biochemical marker for the extent of endothelial impairment.[40] Unfortunately, we were unable to relate the chemokine levels under study to their cell-bound counterparts. Therefore, this remains to be elucidated.

This study has several strengths. The WHISTLER birth cohort is a unique cohort because it comprises healthy, 8-year-old children, sampled from the general population. In addition, these children are of a prepubertal age. Therefore, we excluded possible effects of pubertal hormones and of confounding lifestyle characteristics (ie, smoking) on the levels of the selected chemokines and on the values of the vascular wall characteristics. Moreover, common CIMT, distensibility, and YEM are established markers of vascular wall alterations and thus of cardiovascular risk evaluation already in young children.

On the other hand, we realize that our results are of subtle strength given that they have been evaluated in a

small sample of healthy, young children, in whom the vascular changes are still in an initial stage. However, previous studies with even smaller sample sizes observed a relation between the other chemokines under study and cardiovascular risk factors, between RANTES and primary hypertension and between RANTES and other risk factors for CVD. Therefore, we do believe our results are of importance. Additionally, previous studies performed in the WHISTLER study cohort observed relations between cardiovascular risk factors and the vascular characteristics under study, implying that the distribution of vascular wall characteristics is large enough to observe relations regarding cardiovascular risk. Secondly, because evidence on the relation between chemokines and cardiovascular risk factors is scarce, it may be that the confounding variables we used actually serve as intermediates in the pathway of the relation between chemokines and vascular alterations and not as confounding variables. If they were indeed intermediates instead of confounding variables, our results may have been affected. However, we selected our confounding variables based on the literature that was available and based on a rule of thumb and created unadjusted, partially adjusted and fully adjusted models. Therefore, we do not expect that our results have been affected. Moreover, associations observed in cross-sectional studies, such as the current study, do not necessarily provide evidence of causality. Additionally, because the majority of our study population comprised children with a relatively high socioeconomic and Dutch ethnic background, the generalizability of our results is restricted to groups with a similar socioeconomic and ethnic background. Finally, residual confounding remains an issue, yet can unfortunately never be excluded in cohort studies such as this.

We feel that the observation we found in the current study requires validation in larger, more heterogeneous cohorts. Discovering proxies that accurately reflect preatherosclerotic, inflammatory, vascular alterations already in early life may not only further unravel the very early pathophysiology of atherosclerosis, but it may also allow for the detection of high-risk populations already early in life and as such enable early initiation of preventive strategies.

In conclusion, the chemokine RANTES appears to be involved in the development of preatherosclerotic inflammatory vascular alterations already in healthy, young children.

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Disclosures

None.

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