

Adiposity is Associated with Endothelial Activation in Healthy 2-3 Year-old Children

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ABSTRACT

Adiposity is associated with C-reactive protein level in healthy 2-3 year-old children and with other markers of endothelial activation in adults, but data are lacking in very young children. Data from 491 healthy Hispanic children were analyzed. Mean age was 2.7 years (SD 0.5, range 2-3 years); mean body mass index (BMI) was 17.2 kg/m² (SD 1.9) among boys and 17.1 kg/m² (SD 2.1) among girls. E-selectin level was associated with BMI (R = 0.11; p <0.02), ponderal index (p <0.02), waist circumference (p = 0.02), fasting insulin (p <0.02), and insulin resistance (p ≤0.05); these associations remained significant after adjustment for age, sex and fasting glucose. sVCAM was also associated with BMI (R = 0.12; p <0.05). These observations indicate that adiposity is associated with inflammation and endothelial activation in very early childhood.

KEY WORDS

children, adiposity, E-selectin, sICAM, sVAM

INTRODUCTION

Obesity and hyperinsulinemia or insulin resistance are associated with higher triglyceride level, lower level of HDL-cholesterol, and higher blood pressure, even in the absence of diabetes mellitus. This cluster of metabolic characteristics has been termed syndrome X, the insulin resistance syndrome, and the multiple metabolic or metabolic syndrome¹⁻⁴. The currently accepted term is metabolic syndrome, which has been defined in publications by the World Health Organization⁵ and the National Cholesterol Education Program⁶. With increases in the prevalence of obesity among children and adults in the US, the importance of the metabolic syndrome in the development of atherosclerosis has received greater emphasis^{6,7}. Diabetes mellitus in adults is an established risk factor for atherosclerosis and cardiovascular disease (CVD)⁸. Studies in adults without diabetes mellitus but with impaired glucose tolerance, elevated insulin level, or both, have also shown increased risk of CVD^{9,10} and of subclinical atherosclerosis¹¹⁻¹³.

Obesity and hyperinsulinemia are also associated with increased levels of inflammatory factors. Recent data indicate that macrophages accumulate in visceral adipose tissue¹⁴, that macrophage content of adipose tissue correlates with body mass index (BMI)^{14,15}, and that fat tissue macrophage-related inflammatory factors contribute to insulin resistance¹⁵. Pro-inflammatory cytokines, specifically interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), are produced by macrophages in

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Abbreviations: BMI = body mass index; IL-6 = interleukin 6; sICAM = soluble intercellular adhesion molecule; sVCAM = soluble vascular cell adhesion molecule.

fat tissue as well as by adipocytes^{14,16}. IL-6 and TNF- α are pluripotent cytokines with many activities including the stimulation of production of acute phase proteins in the liver, activation of the innate and adaptive immune systems, and possibly interference with insulin signaling, thereby contributing to insulin resistance¹⁶. Data in prediabetic adults indicate that inflammatory activation is related to increased insulin resistance, rather than diminished insulin secretion¹⁷, and activation of inflammatory factors is now widely considered to be part of the metabolic syndrome¹⁸⁻²⁰. Studies of these emerging aspects of the metabolic syndrome in very young children are almost entirely lacking.

Adhesion molecules modulate the adhesion and migration of leukocytes at sites of inflammation and include selectins, integrins, and intercellular adhesion molecules (ICAMs)²¹. Selectins are transmembrane glycoproteins that, together with their receptors, participate in rolling, which is the initial stage of leukocyte adhesion to vascular endothelial cells²². Integrins and ICAMs participate in trans-endothelial migration and cell adhesive interactions with the extracellular matrix²¹. E-selectin is expressed on endothelial cells that have been stimulated by inflammatory cytokines²². Macrophage antigen, the principal neutrophil membrane integrin, binds to endothelial cells via its ligand, ICAM-1^{21,23}. Evidence in mice²² and humans²⁴⁻²⁶ supports the role of adhesion molecules in the pathogenesis of atherosclerosis, albeit with some inconsistency in human studies, possibly related to differences in measures of atherosclerosis. Elevated triglyceride level, low HDL-cholesterol level, or both, may lead to elevated levels of adhesion molecules²⁷⁻²⁹. In general, blood levels of E-selectin, ICAM-1 and VCAM-1 are considered markers of endothelial activation, usually associated with increased inflammation³⁰.

In an earlier report of data from the same group of 2-3 year-old children whose data are described here, we reported that measures of adiposity were positively correlated with fasting insulin level, and also that adiposity and fasting insulin level were positively correlated with C-reactive protein³¹. We therefore hypothesized that measures of adiposity, fasting insulin level, or both, would be associated

with higher levels of pro-inflammatory cytokine mediators, specifically IL-6 and TNF- α , and the adhesion molecules E-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1), in this same group of very young children.

METHODS

Study population

Children were recruited at the Columbia University Medical Center's (then Columbia-Presbyterian Medical Center) ambulatory general pediatric practices and affiliated community-based pediatrics practices in northern Manhattan, New York, between 1992 and 1995, for a randomized trial of a diet moderately reduced in saturated fat content. After entry into the study but before randomization, the 524 study children attended four visits over 6 months at which dietary and other measurements were obtained. We included in the present study 491 children whose pre-randomization blood sample and anthropometric data were collected either on the same day ($n = 368$) or on different days but at visits within 30 days of each other ($n = 123$). The 33 children whose visits were more than 30 days apart were excluded from the analysis. All children were Hispanic. The study was approved by the Institutional Review Boards at Columbia University Medical Center and Teachers College.

Measurement procedures

Height and weight were measured using precision stadiometers and balance scales to the nearest 0.1 cm and 0.1 lb, respectively. BMI was calculated as weight in kilograms divided by the square of the height in meters. Ponderal index was calculated as weight in kilograms divided by the cube of the height in meters. Four skinfolds were measured with Lange calipers (Cambridge, MD) on the right side of the body: subscapular, triceps, waist (abdominal), and hip (supra-iliac)³² as described in previous publications^{31,33}. The values for the four sites were summed to provide a single score for sum of skinfolds. Circumference of the waist (level of the umbilicus) was measured with a tape measure to the nearest 0.1 cm³². Of the 491 children, 100 (20.4%) were 'overweight' (equal to

or greater than 95th percentile), 96 (19.6%) were 'at risk of overweight' (85th to less than 95th percentile), 275 (56.0%) were 'healthy weight' (5th percentile to less than the 85th percentile), and 20 (4%) were 'underweight' (less than 5th percentile) according to the NHANES-CDC (Feb 2007)³⁴.

Biochemical analyses

Children were instructed to fast after dinner the night before the interview, except for water, and blood samples were obtained at the start of the clinical assessment. Plasma samples were prepared from blood drawn into EDTA tubes. Samples were stored in a freezer at -70°C. Serum insulin levels were measured using a double antibody radioimmunoassay with 60-70% cross reactivity with pro-insulin³⁵. Serum glucose levels were measured using standardized enzymatic procedures (Hitachi 704; Hitachi, Tokyo, Japan). The fasting insulin resistance index was calculated as [fasting glucose (mM/l)][fasting insulin (μU/ml)] / 22.5^{36,37}.

IL-6 was measured by ultrasensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). The laboratory analytical coefficient of variation (CV) for this assay was 6.3%. TNF-α was measured by an ultrasensitive, solid-phase sandwich ELISA using a monoclonal antibody specific for TNF-α (Quantikine HS Human TNF-α Immunoassay; R&D Systems, Minneapolis, MN). The lower detection limit is 0.06-0.32 pg/ml with a detection range of 0.5-32 pg/ml (normal circulating levels are reported to be in the 10-80 pg/ml range). Intra-assay CVs ranged from 5.3-8.8% and interassay CVs from 10.8-16.7%. sICAM-1 was measured by an ELISA assay (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). The minimum detectable level is <0.35 ng/ml with an assay range of 2.73-49.55 ng/ml. The laboratory CV was 5.0%. sVCAM-1 was measured by solid-phase sandwich ELISA using a monoclonal antibody specific for sVCAM-1 (Quantikine Human sVCAM-1 Immunoassay; R&D Systems, Minneapolis, MN). The lower detection limit is 0.17-1.26 ng/ml with a detectable range for the assay of 0-200 ng/ml. Intra-assay CVs ranged from 2.3-3.6% and interassay CVs from 5.5-7.8%. Soluble E-selectin, also known as endothelial leukocyte adhesion

molecule-1 (ELAM-1) and CD62E, was measured using a high sensitivity quantitative sandwich enzyme (Parameter Human sE-Selectin Immunoassay; R&D Systems, Minneapolis, MN). The minimum detectable level of E-selectin is typically <0.1 ng/ml and the assay range is 0.47-10.52 mg/ml. Intra-assay and interassay CVs ranged from 4.7-5.0% and 5.7-8.8%, respectively. C-reactive protein (CRP) was measured by nephelometry as described in an earlier report³¹. IL-6, TNF-α, sICAM-1, sVCAM-1, E-selectin, and CRP were measured at the Laboratory for Clinical Biochemistry Research, University of Vermont (Burlington, VT).

Statistical analysis

Values for fasting insulin that were below the detection limit of the assay (1.0 μU/ml) were recorded as 1.0 μU/ml (n = 85). The distributions of continuous variables were assessed for normality and the natural log transformations of skewed variables were used in subsequent analysis. Sum of skinfolds, insulin, insulin resistance, IL-6, TNF-α, E-selectin, s-VCAM and s-ICAM levels were positively skewed and therefore transformed using the natural logarithm of the value in analyses. Descriptive statistics are presented as the mean ± standard deviation (SD) or median and interquartile range of untransformed values.

Differences between two groups were assessed by Student's two sample t-test and Wilcoxon test for continuous normal distributed and skewed variables, respectively. Pearson correlation coefficients were calculated between serum inflammatory markers and adiposity measures and fasting insulin level after natural logarithm transformation of the skewed variables. Partial correlation coefficients were calculated between serum inflammatory markers and adiposity measures and fasting insulin level after adjustment by age and gender and additionally after adjusting for these variables and fasting insulin level.

RESULTS

Characteristics of the study children

The final sample for analyses consisted of 491

children, of whom 52% (255) were boys and 48% (236) girls. Age (mean \pm SD) in boys was 32.5 ± 6.0 months and in girls was 33.6 ± 6.0 months ($p < 0.04$) (Table 1). There were no differences in height, weight, BMI, ponderal index and waist circumference between boys and girls. Girls had significantly higher sum of skinfolds than boys ($p = 0.01$). There was no difference in levels of glucose or insulin resistance index between boys and girls. Medians and interquartile ranges of fasting serum insulin were $5.0 \mu\text{U/ml}$ (2.0, 9.0) for girls and $4.0 \mu\text{U/ml}$ (2.0, 8.0) for boys ($p < 0.05$). Among the overweight children, median serum insulin levels and insulin resistance index were significantly higher compared with those among the non-overweight children (data not shown), consistent with an earlier publication of data in these children³¹.

The medians and interquartile ranges of IL-6 levels were 1.3 pg/ml (0.9, 2.6) for boys and 1.5 pg/ml (0.9, 2.6) for girls. There was no difference in IL-6 levels between boys and girls. The medians and interquartile ranges of TNF- α levels were 9.3 pg/ml (6.9, 12.5) for boys and 7.9 pg/ml (6.0, 11.4) for girls ($p < 0.01$). In the whole group, mean sVCAM level was $1078 \pm 349 \text{ ng/ml}$, mean sICAM level was $356 \pm 95 \text{ ng/ml}$, and mean E-selectin level was $103 \pm 34 \text{ ng/ml}$. There was no difference in the levels of E-selectin, sVCAM-1, or sICAM-1 between boys and girls (Table 1).

Correlations of adiposity measures with markers of inflammation and endothelial activation

In the whole group of children, sVCAM level was correlated with BMI ($R = 0.09$; $p < 0.05$) and ponderal index ($R = 0.10$; $p < 0.05$), and E-selectin level was correlated with BMI ($R = 0.12$; $p \leq 0.01$), ponderal index ($R = 0.15$; $p < 0.001$) and fasting serum insulin ($R = 0.09$; $p < 0.05$) (Table 2). Among boys, sVCAM level was correlated with BMI ($R = 0.21$; $p < 0.001$), ponderal index ($R = 0.16$; $p \leq 0.01$), sum of skinfolds ($R = 0.23$; $p < 0.001$), and waist circumference ($R = 0.20$; $p \leq 0.01$). These correlations were not significant among girls. Among boys, E-selectin level was correlated with BMI ($R = 0.17$; $p \leq 0.01$), ponderal index ($R = 0.16$; $p \leq 0.01$), and waist circumference

($R = 0.14$; $p < 0.05$). Among girls, ponderal index ($R = 0.14$; $p < 0.05$) was significantly correlated with E-selectin level. The correlations between adiposity measures and IL-6 and sICAM were not significant in either boys or girls. There were no significant correlations between fasting insulin level and inflammatory markers, except between E-selectin and fasting insulin level ($R = 0.09$, $p < 0.05$). As previously reported³¹, essentially all of the variability in the insulin resistance index was explained by variability in the fasting insulin level, and essentially none by variability in fasting glucose level, so that analyses using the insulin resistance index did not add additional information to those using the fasting insulin level.

Partial correlation coefficients of adiposity measures with markers of inflammation and endothelial activation, after adjustment for age and gender and for fasting insulin level

As shown in Table 3, E-selectin remained significantly associated with BMI, ponderal index, and waist circumference after adjustment for age and gender. E-selectin was also correlated with fasting insulin and insulin resistance after adjustment for age and gender. sVCAM remained significantly correlated with waist circumference after adjustment for age and gender.

As shown in Table 4, E-selectin also remained significantly correlated with BMI, ponderal index, and waist circumference after adjustment for age, gender and additional adjustment for fasting insulin. sVCAM remained significantly associated with BMI and waist circumference after adjustment for age, gender and fasting insulin.

DISCUSSION

The main finding of this study is that adiposity is associated with higher levels of E-selectin and sVCAM in healthy children as early as 2-3 years of age, suggestive of adverse effects of adiposity on endothelial function and health. We examined four anthropometric measures of adiposity - BMI, ponderal index, sum of four skinfolds, and waist circumference - and found results that were consistent across these measures. In addition to E-

TABLE 1

Characteristics (mean \pm SD) of the 491 2-3 year-old children in the Hispanic Children's Health Study, New York: total children and by gender

Characteristics	Boys n = 255	Girls n = 236	Total n = 491
Age (months)	32.5 \pm 5.9	33.6 \pm 6.2*	33.0 \pm 6.1
Anthropometric variables			
Weight (kg)	14.9 \pm 2.4	14.8 \pm 2.7	14.9 \pm 2.6
Height (cm)	93.2 \pm 5.4	92.7 \pm 5.4	92.9 \pm 5.4
Body mass index (kg/m ²)	17.2 \pm 1.9	17.1 \pm 2.1	17.2 \pm 2.0
Ponderal index (kg/m ³)	18.6 \pm 2.4	18.5 \pm 2.5	18.6 \pm 2.4
Sum of skinfolds (mm)	30.1 \pm 9.2	32.8 \pm 12.1	31.4 \pm 10.8
Waist circumference (cm)	50.3 \pm 3.7	50.3 \pm 3.8	50.3 \pm 3.8
Metabolic variables			
Glucose (mg/dl)	79.2 \pm 11.8	77.4 \pm 10.8	78.3 \pm 11.4
Insulin (μ U/ml)	6.4 \pm 7.8	7.6 \pm 8.9	6.9 \pm 8.4
Insulin resistance index	1.4 \pm 2.2	1.5 \pm 2.2	1.5 \pm 2.2
Inflammatory markers			
CRP (mg/l)	0.7 \pm 1.4	0.8 \pm 1.4	0.8 \pm 1.4
IL-6 (pg/ml)	1.3 (0.9, 2.6) 2.3 \pm 2.6	1.5 (0.9, 2.6) 2.6 \pm 2.9	1.4 (0.9, 2.4) 2.4 \pm 2.8
TNF- α (pg/ml)	9.3 (6.9, 12.5) 10.2 \pm 7.2	7.9 (5.9, 11.4) 9.5 \pm 9.6	8.7 (6.3, 11.9) 9.8 \pm 8.4
sVCAM (ng/ml)	1004.0 (840.8, 1239.6) 1082.7 \pm 377.6	1027.6 (856.2, 1205.6) 1072.1 \pm 315.5	1018.5 (848.9, 1233.4) 1077.6 \pm 348.9
sICAM (ng/ml)	362.8 (314.9, 411.3) 353.4 \pm 96.9	365.9 (313.8, 409.3) 359.7 \pm 93.1	364.6 (314.8, 410.4) 356.4 \pm 95.1
E-selectin (ng/ml)	98.5 (76.3, 121.6) 101.9 \pm 34.2	99.3 (77.8, 125.9) 103.8 \pm 33.7	98.9 (77.1, 124.1) 102.8 \pm 33.9

Results are means \pm SD or medians (interquartile range).

p-value based on t-test or Wilcoxon test at 0.05.

* p = 0.04.

TABLE 2

Pearson correlation coefficients between adiposity measures and IL-6, TNF- α , sVCAM, sICAM, and E-selectin levels in 491 2-3 year-old children in the Hispanic Children's Health Study, New York

	BMI (kg/m ²)	Ponderal index (kg/m ³)	Sum of skinfolds (mm)	Waist circumference (cm)	Fasting insulin (mU/ml)	Insulin resistance index
Total	(n = 490)	(n = 490)	(n = 398)	(n = 484)	(n = 491)	(n = 491)
IL-6 (pg/ml)	0.05	0.08	0.01	-0.01	0.07	0.06
TNF-α (pg/ml)	0.02	0.07	0.01	0.01	-0.07	-0.07
sVCAM (ng/ml)	0.09 [‡]	0.10 [‡]	0.07	0.07	0.00	-0.01
sICAM (ng/ml)	0.03	0.03	0.04	0.02	0.01	-0.00
E-selectin (ng/ml)	0.12 [†]	0.15 [*]	0.09 [§]	0.08	0.09 [‡]	0.08
Boys	(n = 254)	(n = 254)	(n = 207)	(n = 252)	(n = 255)	(n = 255)
IL-6 (pg/ml)	0.07	0.12	0.07	0.01	0.05	0.05
TNF-α (pg/ml)	0.09	0.13 [‡]	0.10	0.06	-0.05	-0.05
sVCAM (ng/ml)	0.21 [*]	0.16 [‡]	0.23 [*]	0.20 [†]	0.04	0.03
sICAM (ng/ml)	0.04	0.05	0.00	-0.02	0.07	0.07
E-selectin (ng/ml)	0.17 [†]	0.16 [†]	0.13	0.14 [‡]	0.09	0.08
Girls	(n = 236)	(n = 236)	(n = 191)	(n = 232)	(n = 236)	(n = 236)
IL-6 (pg/ml)	0.02	0.06	-0.06	-0.03	0.08	0.07
TNF-α (pg/ml)	-0.05	0.02	-0.04	-0.04	-0.07	-0.07
sVCAM (ng/ml)	-0.05	0.03	-0.09	-0.08	-0.04	-0.05
sICAM (ng/ml)	0.03	0.01	0.07	0.07	-0.07	-0.09
E-selectin (ng/ml)	0.07	0.14 [‡]	0.06	0.01	0.09	0.08

* p < 0.001; † p ≤ 0.01; ‡ p < 0.05; § p = 0.06; || p = 0.07.

selectin and sVCAM, we examined other markers of inflammation and endothelial activation, including IL-6, TNF- α , and sICAM, but we found no significant associations between adiposity measures or fasting insulin level and these measures of inflammatory activation in children in this age group.

In bivariate analyses performed separately in boys and girls, we found some differences between the sexes in whether these associations were

statistically significant. We did not have data on the physical activity levels of the children in our study, or on sex hormone levels, both factors known to influence endothelial activation^{38,39}. It is possible that either or both contributed to the observed differences between boys and girls. We did not have a prior hypothesis that there would be differences between boys and girls at this very early age, and sampling variability in smaller samples is also a possible explanation.

TABLE 3

Partial correlation coefficients between adiposity measures and IL-6, TNF- α , sVCAM, sICAM, and E-selectin levels after adjusting by age and gender in 491 2-3 year-old children in the Hispanic Children's Health Study, New York

	IL-6 (pg/ml)	TNF-α (pg/ml)	sVCAM (ng/ml)	sICAM (ng/ml)	E-selectin (ng/ml)
BMI (kg/m ²)	0.04	0.00	0.08 [§]	0.04	0.11*
Ponderal index (kg/m ³)	0.05	0.00	0.08	0.05	0.11*
Sum of skinfolds (mm)	0.00	0.03	0.07	0.03	0.10 [‡]
Waist circumference (cm)	0.01	0.05	0.09 [†]	0.02	0.11*
Fasting insulin (μ U/ml)	0.08	-0.04	0.01	0.00	0.11*
Insulin resistance index	0.08	-0.04	0.01	-0.01	0.10 [†]

* p < 0.02; [†] p \leq 0.05; [‡] p = 0.06; [§] p = 0.07.

TABLE 4

Partial correlation coefficients between adiposity measures and IL-6, TNF- α , sVCAM, sICAM, and E-selectin levels after adjusting by age, gender and fasting insulin in 491 2-3 year-old children in the Hispanic Children's Health Study, New York

	IL-6 (pg/ml)	TNF-α (pg/ml)	sVCAM (ng/ml)	sICAM (ng/ml)	E-selectin (ng/ml)
BMI (kg/m ²)	0.03	0.00	0.08 [‡]	0.04	0.09*
Ponderal index (kg/m ³)	0.04	0.01	0.07	0.05	0.10*
Sum of skinfolds (mm)	-0.00	0.04	0.07	0.03	0.08
Waist circumference (cm)	-0.00	0.05	0.09 [†]	0.02	0.09*

* p < 0.05; [†] p = 0.05; [‡] p = 0.07.

Previously we reported data from this same group of 2-3 year-old children showing that measures of adiposity were associated with fasting insulin level³¹. In multivariate analyses, adiposity and fasting insulin level were associated with higher level of C-reactive protein³¹, a marker of inflammatory activation⁴⁰⁻⁴⁴. The associations reported here of adiposity with E-selectin and sVCAM levels extend these earlier observations and support the hypothesis that the metabolic syndrome, including some aspects of the inflammatory response and endothelial activation, is phenotypically expressed at a very early age in

childhood and at relatively low levels of adiposity. Our data in a much younger age group are consistent with the finding in adolescent boys and girls that CRP is inversely related to endothelial function and health as assessed by flow-mediated brachial artery responsiveness⁴⁵.

The mean height and weight of the children in our study were well within the normal range for healthy children in the US⁴⁶. Mean height and weight for boys in our study were 93.2 cm and 15.0 kg, compared to medians for 3 year-old boys of 96 cm and 14.3 kg. Mean height and weight for girls in our study were 92.7 cm and 14.8 kg compared to

medians for 3 year-old girls of 95 cm and 13.8 kg. These comparisons suggest that the children in our study were slightly more obese than national averages at the time of the study. The children in our study were recruited following procedures designed to obtain a sample of normal, healthy children who were free of chronic diseases and who lived in the community surrounding our site. Thus, the relationships we observed appear to be present in normal, healthy children at a very early age and earlier in life than has previously been appreciated.

The roles of adiposity and fasting insulin level in accounting for the several expressions of the metabolic syndrome that we have observed in this group of very young children appear to be complex. Adiposity was associated with higher fasting insulin level in our data, but the correlations were modest ($R = 0.16$ for boys and $r = 0.14$ for girls;

$p < 0.05$ for both)³¹, consistent with findings in older children⁴⁷. Fasting insulin level was independently associated with CRP level in our earlier study³¹ and here was found to be associated with E-selectin, independent of adiposity. Adiposity was also associated with E-selectin level, independent of fasting insulin level, and adiposity but not fasting insulin level was associated with sVCAM. These observations are consistent with two, or more, pathways, one acting through insulin resistance or hyperinsulinemia, and the other through a direct action of adipose tissue products on inflammatory response and endothelial health. It is also possible that more accurate measures of body composition, insulin resistance, or both, may lead to better dissection of this issue.

The use of frozen samples is widespread and widely accepted. However, we recognize that the study samples were frozen at -70°C for approximately 10-12 years. While all of the specific protein assays used in this study have not been studied, Lewis *et al.* reported a high level of stability for frozen samples for nine coagulation, fibrinolysis, and inflammatory factors, albeit over a shorter period of 7-59 months⁴⁸. We note that even if the samples were not completely stable during storage, the effects would be random and could not explain the positive associations observed, but

would rather lead to an underestimation of the true relationships.

We interpreted the levels of circulating E-selectin and s-VCAM as biomarkers of endothelial cell activation^{22,30}. We note nonetheless that the processes that lead to soluble forms of what are normally insoluble integral membrane proteins in endothelial cells are not well understood, and that this interpretation is made with caution.

Several additional limitations may affect the interpretation of our findings. First, the study children were not randomly sampled. However, it is highly unlikely that selection bias arising from non-random sampling or from subject characteristics associated with study participation influenced

the observed associations between adiposity and inflammatory markers. The Hispanic families in our study originated almost entirely from the Caribbean basin. Additional studies in very young children of other race/ethnicity would be useful in confirming the relationships we observed. Secondly, direct measurement of insulin resistance was not a feature of the study protocol. However, fasting insulin level has been shown in adults to correlate well with direct measurement of insulin resistance in normoglycemic subjects⁴⁹. Adiposity was assessed using anthropometric methods rather than through measurement of body composition. We did not seek to measure the possible effects of the higher levels of inflammatory factors on vascular structure or function in the children in our study. Finally, the differences we have observed might be considered small, or moderate at most; however, we note that their occurrence at such an early age, with the potential for considerable lifetime exposure to these elevated levels, raises concern.

In summary, while the associations between adiposity and E-selectin and sVCAM levels were modest in magnitude, and associations with IL-6, sICAM, and TNF- α were not observed, these observations nonetheless add to the evidence that adiposity is associated with inflammatory activation in very young children.

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