The Relationship between Body Fat Mass Percentiles and Inflammation in Children

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Objective: Obesity has been associated with markers of increased systemic inflammation in both human and animal studies. Increased inflammation is linked to metabolic and cardiovascular disease. The objective of this study was to evaluate the association between percentile body fat and inflammation in a nationally representative sample of US children.

Methods: 6,950 children 8-18 years of age between 1999 and 2004 were studied. Measurement of body fat percentage was measured by dual-energy X-ray absorptiometry scan and converted to an age- and sex-adjusted percentile. The main outcome measures were abnormal c-reactive protein (CRP > 1.0 mg/dl) and absolute neutrophil count (ANC > 6,600).

Results: Children with higher levels of body fat (≥70th percentile) had a higher odds of having elevated CRP (OR 2.88-10.69) and elevated ANC (OR 2.14-3.24) compared with children with body fat <70th percentile.

Conclusions: The link between inflammation and body fat in children warrants further longitudinal research to understand the temporal relationship between overweight/obesity and inflammation in the pediatric obese population and its implications for chronic disease risk.

Introduction

Studies in both animals and humans suggest that visceral adipose tissue is a major source of pro-inflammatory signals, including cytokine production and macrophage activation (1,2). This obesity-induced inflammation contributes to the pathogenesis of cardiovascular disease, hypertriglyceridemia, and insulin resistance (3-5), and may additionally be linked to increased risks of cancer (6), autoimmune diseases, and sepsis (7).

Epidemiologic studies in adults and children (8) have shown that elevated body mass index (BMI) is associated with elevated systemic markers of inflammation including C-reactive protein (CRP) and peripheral leukocyte counts (9). There have been few clinical studies that have investigated pediatric obesity and inflammation using CRP and other inflammatory cytokines such as TNFα and IL6 (10). However, these studies have had a relatively small sample size (11-13) or have used BMI as a surrogate marker of obesity status (9). BMI correlates fairly well with body fat in adult populations; nonetheless it is an imperfect measure of adiposity especially in the pediatric population (14,15). Given that BMI does not directly correlate to body fat (16), additional studies are needed to directly evaluate the relationship between body fat and inflammation in children in the context of a national epidemic of childhood obesity.

Our objective was to assess whether excess body fat, as measured by a more robust standard method for measuring body fat dual energy X-ray absorptiometry (DEXA), is associated with elevations in clinical markers of inflammation, including abnormal levels of CRP and absolute neutrophil count, in a nationally representative US pediatric population ($N = 6,950$).

Methods

We used data from the National Health and Nutrition Examination Survey (NHANES) 1999-2004, a cross-sectional, nationally representative examination study of the US civilian noninstitutionalized population (17) which oversamples individuals 12-19 years, as well as minority populations. In the survey, a subset of individuals had inflammatory markers performed, including high sensitivity CRP levels (measured by latex-enhanced nephelometry) (18) and absolute neutrophil counts (19) (see references for assay details).

NHANES captured measures of body fat using whole body DEXA scans in a subset of individuals 8 years and older using a Hologic QDR 4500 fan-beam densitometer (Hologic, Bedford, MA). Scans were conducted in the fast mode by trained radiology technologists and were analyzed using Hologic DOS software version 8.26:a3)

TABLE 1 Descriptive characteristics of total population by race

	Overall population	White	Black	Hispanic	Other
N	5,627	1,543 (62.4%)	1,678 (13.4%)	2,199 (18.4%)	207 (5.8%)
Male	3,331 (59.2%)	887(58.7%)	989 (58.8%)	1,337 (59.8%)	118 (62.7%)
Age (mean)	13.1	13.2	13.0	13.0	13.3
BMI					
Underweight (<5th percentile)	130 (2.7%)	43 (2.8%)	28 (1.7%)	52 (2.6%)	7 (4.1%)
Normal weight (5-84th percentile)	3,260 (60.7%)	975 (62.8%)	904 (53.5%)	1,251 (56.6%)	130 (67.3%)
Overweight	1,008 (17.6%)	257 (17.1%)	318 (18.9%)	405 (19.6%)	28 (15.0%)
Obese	1,207 (19.0%)	263 (17.3%)	422 (25.9%)	481 (21.2%)	41 (13.6%)
Body Fat Percentile					
<5th	-	-	-	-	-
5th-69th	3,819 (69.6%)	1,091 (70.6%)	1,216 (72.0%)	1,362 (62.1%)	150 (77.2%)
70th- 84th	874 (15.1%)	223 (14.5%)	206 (12.7%)	417 (19.9%)	28 (11.5%)
85th-89th	341 (5.8%)	87 (5.8%)	93 (5.4%)	150 (6.5%)	11 (4.8%)
≥ 90th	593 (9.5%)	142 (9.1%)	163 (10.0%)	270 (11.5%)	18 (6.6%)
Abnormal CRP	192 (2.8%)	40 (2.7%)	65 (3.8%)	86 (3.1%)	1 (0.1%)
Abnormal ANC	389 (7.5%)	127 (8.1%)	43 (2.7%)	207 (9.6%)	12 (6.0%)

(17). Detailed documentation of the procedures for the DEXA scans is available online (17).

Our main outcome variable of interest was an elevated CRP, which was defined as a level > 1.0 mg/dl, which is consistent with definitions used for assessing inflammation in the pediatric population (9). This is based on previous studies that have shown an association between a CRP > 1.0 mg/dl and increased cardiovascular and metabolic disease risk (20) and has previously been used in pediatric studies (21). We also assessed a secondary marker of inflammation, defined as an absolute neutrophil count (ANC) > 6,600, a threshold that has been used by other studies for defining the presence of inflammation and is the 95th percentile for this population (9).

Our independent variable was total percentage body fat, which was converted to an age- and sex-adjusted percentile for percentage body fat recently published by the CDC (22). The definition of "normal" percentage body fat adjusted for age and sex is not clear; therefore, similar to previous studies, (14) we defined thresholds at the 70th, the 85th, and the 90th percentile of percentage body fat, as these thresholds captured similar proportions of children in the overweight (31.9% have a BMI ≥ 85th percentile), obese (16.3% have a BMI ≥ 95th percentile), and severely obese (11.3% have a BMI ≥ 97th percentile) categories, respectively (23). We used "normal" percentage body fat (5-69th percentile) as our referent group for our analyses. We eliminated those under 5th percentile to eliminate underweight children who may be categorized as failing to thrive and are likely to have underlying disease or illness and hence CRP or ANC that may be falsely elevated.

For the CRP analysis, of 6,950 children aged 8-18 years of age with DEXA data, we excluded 646 children with missing CRP data, 320 children with an incomplete scan, one child with a CRP >10, and 356 children were excluded for a percentage body fat less than 5th percentile, leaving a sample size of 5,627.

Compared with the children excluded from our study for missing CRP, children in our sample were older (13.1 years vs. 12.2 years, $P = 0.001$), but there were no differences by sex, body fat, BMI, or race.

For the ANC analysis, of 6,950 children 8-18 years of age with DEXA data, we excluded 579 children with a missing ANC data, 327 children with an incomplete scan, 64 children with a CRP > 10 and 356 children with a percentage body fat less that 5th percentile, leaving a sample of 5,624 children. Compared with the children excluded from our study for missing ANC, children in our sample were older, with a mean age of 13.1 vs 12.1 years ($p = 0.001$). However, there were no significant differences by sex, body fat, BMI, or race.

Statistical Analysis

We performed bivariate analyses using simple logistic regression to examine the association between abnormal CRP or ANC across the different categories of body fat percentiles. We then performed multivariable logistic regression models predicting these outcomes, which adjusted for age, sex, and race, and used the 5-69th percentile group as the reference category. We tested for race by body fat percentiles (White, Black, Mexican-American), age by body fat percentile and, sex by body fat percentile interactions. In additional sensitivity analyses, we conducted logistic regression using body fat percentile as a continuous variable predicting abnormal CRP or ANC, and we performed linear regression using body fat percentile as a continuous variable predicting CRP as a continuous variable. Finally, we also conducted additional analyses using total body fat mass and its association with CRP and ANC as continuous or dichotomous outcomes, adjusting for total mass, age, race, and gender.

The NHANES surveys use a complex sample survey design and weighting methodology (17). We used STATA 11.0 statistical

software, which incorporates appropriate sampling weights to adjust for the complex sample design. NHANES provides the DEXA data as multiply imputed datasets because of nonrandom missing data. Therefore, we performed analyses on each of the multiple imputation datasets and combined results using STATA.

This study was considered exempt by the University of Michigan Institutional Review Board.

Results

Table 1 shows the overall population characteristics as well as the characteristics based on ethnicity. There were no differences by age or gender, but there were differences by race, with a greater percentage of elevated CRP for Mexican and non-Hispanic Black children ($P = 0.0006$). Overall 2.8% ($n = 192$) of children had an abnormal CRP and 7.5% ($n = 389$) of children had an elevated ANC. As shown in Table 1 we created categories of body fat percentiles to match population distributions according to weight status. Based on

BMI, 17.6% of children were overweight and 19% were obese. Accordingly, 15.1% of children were between 70th and 84th percentile body fat (to match the overweight category), and 5.8% and 9.5% were in the 85-89th and the ≥90th percentile categories (to overall match the obese category).

Table 2 shows the results of the multivariable logistic regression models. With increased body fat, there was an increasing odds ratio of having abnormal CRP. For example, compared with children with body fat in the 5-69th percentile, those with body fat 70-89 percentile had approximately three times the odds of abnormal CRP, and children with body fat ≥90th percentile had approximately 10 times the odds of abnormal CRP. We found similar results for abnormal ANC with those in the 70-89 percentile had two times the odds of having elevated ANC and those with ≥90th percentile had three times the odds of having an elevated ANC. We did not find any statistically significant interactions for age, sex, or race with body fat percentiles.

In additional models, for each one percentile increase in body fat, there was a 13% higher odds of having abnormal CRP ($P < 0.001$) and for each 1% increase in body fat percentile there was a 7% odds of having elevated ANC ($P < 0.001$). In linear regression analyses, each 1 percentile increase in body fat was associated with a 0.01 increase in CRP ($P < 0.001$) and each 1 percentile increase in body fat was associated with a 0.05 increase in ANC ($P < 0.001$).

We found a consistent association of body fat with inflammatory markers, even when we used raw body fat mass, as the independent variable (see Tables 3 and 4).

Discussion

To our knowledge this is the first study to report a link between body fat percentile, a standard measure of adiposity, and inflammation, in a large representative population-based sample of US children. We found that a higher level of body fat was associated with higher odds of having abnormal CRP and ANC, which highlights the fact that the association between adiposity and inflammation occurs during childhood as well as in adulthood.

Our study provides evidence of a robust link between obesity and inflammation in children (9,11), which builds upon prior pediatric studies that have used proxy measures of body fat. Smaller pediatric studies have found a significant association between adiposity and CRP in even nonobese children as defined by BMI. McVean et al. investigated 75 children with BMI <95th percentile and found that percent body fat was a predictor of CRP (24).

Other pediatric studies have used the NHANES data to evaluate the link between adiposity and inflammation using proxy measures for

TABLE 2 Unadjusted and Adjusted analyses showing the odds ratio (OR) for elevated inflammatory parameters (C-Reactive Protein or Absolute Neutrophil Count) when compared to referent group of 5-69 percentile body fat for age and gender

Categories of percentile body fat	Abnormal C-reactive Protein (>1.0 mg/dl) OR (95% CI)	
	Unadjusted	Adjusted for age, race, gender
5-69%	Referent	Referent
70-84%	2.88** (1.35-6.12)	2.85** (1.32-6.26)
85-89%	3.67*** (1.69-7.96)	3.61*** (1.66-7.86)
≥90%	10.69**** (5.69-20.09)	10.52**** (5.66-20.25)

Categories of percentile body fat	Absolute Neutrophil Count (>6,600) OR (95% CI)	
	Unadjusted	Adjusted for age, race, gender
5-69%	Referent	Referent
70-84%	2.14**** (1.44-3.16)	2.14*** (1.42-3.21)
85-89%	2.03* (1.11-3.73)	2.06* (1.07-3.95)
≥90%	3.24**** (2.17-4.85)	3.38**** (2.22-5.08)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, ****$P < 0.001$

TABLE 3 Linear regression of CRP or ANC as outcome with total fat as predictor adjusted for total body mass, sex, race/ethnicity, and age

	CRP			ANC		
	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value
Total Body Fat Mass	0.000020	0.000002	<0.001	0.00007	0.00001	<0.001

TABLE 4 Logistic regression of CRP or ANC as dichotomous outcomes with total fat as predictor adjusted for total body mass, sex, race/ethnicity, and age

	CRP			ANC		
	OR	SE	*P*-value	OR	SE	*P*-value
Total Body Fat Mass	1.00015	0.00003	<0.001	1.00007	0.00002	0.001

body fat. Skinner et al. found an increased hazard ratio for elevated CRP in very obese children (>99th percentile by BMI) starting at 3-5 years of age (HR 2.29; $P < 0.01$) up to children 15-17 years of age (HR 4.73; $P < 0.01$). Similarly, they also found an increased HR for elevated ANC of 2.0 in very obese children starting as young as 6-8 years of age (9). More recently, Going et al. assessed NHANES data for the relationship between skin fold measures of body fat and elevated CRP (25). They reported that those with higher percent body fat by skin fold (>20-30% in boys and > 25-35% in girls) had 6-10 folds higher odds of elevated CRP. These data are consistent with our results but are proxy measures for the percent body fat assessments used in this study.

Our findings are consistent with adult studies that have used population based data. For example, using NHANES data, Visser et al. found that obese males were 2.13 times more likely and obese females were 6.21 times more likely compared to normal-weight counterparts to have an increased CRP/cardiovascular disease risk with adiposity estimates by BMI with (8). Menke et al. used a variety of adiposity measures (waist circumference, BMI, % body fat, and skin fold thickness) and also found that adiposity was linked with elevated CRP in males and females (8,26).

Strengths of our study include the representative nature of the NHANES database, the racial/ethnic diversity of the population, and the relatively large number of children with DEXA scans performed. We used measures of body fat from DEXA scans, which represent a current standardized measure for body fat, and we used recently published percentiles for percentage body fat which account for age and gender differences (22). Finally, we found an association between percentile body fat and inflammation using two different inflammatory markers, although we acknowledge that further studies are necessary to determine the clinical relevance of these markers.

We also acknowledge limitations of this study. Because DEXA was only performed in older children, we were unable to examine the association among younger children (27). We used a cross-sectional study design and therefore cannot prove causality. We acknowledge that multiple measurements are needed to categorize an individual as having failure to thrive, so we may have missed children in this category by relying on just one measurement. Finally, we acknowledge that there is not a clear consensus what constitutes a "normal" percent body fat in children, but we did find similar results in our analyses which evaluated body fat percentile as a continuous variable and which evaluated raw total body fat as well.

Obesity is a significant problem starting in young children, yet we do not clearly understand the long-term sequelae of obese children and this early inflammation. In adult studies, obesity and its correlated inflammation place individuals at risk for early metabolic and cardiovascular disease. Early inflammatory activation may be associated with other disease processes such as cancer (28), autoimmune conditions (29), and asthma (30). Our findings emphasize that young children with increased body fat for age and gender also have increased systemic inflammation and emphasizes the need to further investigate the relationship of inflammation and body fat accumulation in longitudinal studies. Such studies can clarify the temporal relationship between inflammation and adiposity along with the time course for development of subsequent metabolic and cardiovascular disease. O

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