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Fitness Level and Body Composition are Associated with Inflammation in Non-obese Children

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ABSTRACT

Childhood obesity and poor fitness are associated with insulin resistance (IR), risk for coronary heart disease (CHD), and type 2 diabetes mellitus. Elevated markers of inflammation (e.g. C-reactive protein [CRP]) are independent predictors of CHD. Whether higher percent body fat and poor fitness in *non-obese* children are associated with evidence of inflammation and IR is unclear. We evaluated 75 children with non-obese body mass index (BMI) for age (<95th percentile), ages 11-14 years for fasting insulin, glucose, adiponectin, CRP, body composition, and maximum oxygen consumption (VO_{2max}). CRP correlated positively with body composition (BMI z-score, p = 0.00062; percent body fat, p = 0.00007; and total body fat in grams, p = 0.00006) and negatively with VO_{2max}, p = 0.036. Using multivariate analysis, VO_{2max} and percent body fat were both independent predictors of CRP. Fasting insulin and insulin resistance as assessed by QUICKI did not correlate with CRP, fitness, or fatness in these non-obese children. Adiponectin showed no significant correlations, and gender did not influence correlation analyses. We conclude that in non-obese children, low fitness and higher body fat are both associated with inflammation (i.e. higher levels of CRP). This observation strengthens the importance of promoting both fitness and healthy body composition in all children.

KEY WORDS

fitness, inflammation, non-obese children

INTRODUCTION

Obese children demonstrate evidence for inflammation linked to future development of cardiovascular disease. Obese adolescents have higher levels of C-reactive protein (CRP) and lower levels of anti-inflammatory adiponectin compared to lean adolescents[1]. Children with higher CRP values have evidence of early atherosclerosis with lower brachial artery flow-mediated dilatation and increased carotid intima-media thickness[2]. It has also been demonstrated that childhood CRP values predict adult CRP[3].

In addition to obesity, poor physical fitness is a risk factor for cardiovascular disease and the development of insulin resistance (IR)[4,5]. In adults, poor cardiovascular fitness (as measured by maximum oxygen consumption [VO_{2max}] is a risk factor for IR and inflammation, independent of obesity[6]. Recently, associations between blood markers of inflammation and poor fitness have been reported in obese children[7-10]. In obese adolescents, levels of fitness correlate more closely with measures of insulin resistance than percent body fat[11-13], suggesting that poor physical fitness may be an *independent* contributor to the development of IR in children, as well as in adults. It is therefore of concern that one-third of children, both obese and non-obese, aged 12 to 19 years fail to meet current standards for acceptable minimum cardiorespiratory fitness[14]. Whether markers of inflammation are independently correlated with body composition and fitness levels in non-obese children is unknown. In this study, we analyzed the relationship between CRP, adiponectin, body composition, and fitness levels in *non-obese* children.

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CHILDREN AND METHODS

Study population

Seventy-five children, 11-14 years of age, who were identified as being non-obese (body mass index [BMI] <95th percentile for age; median 20 kg/m^2 [range 14-24]) were included in this study[15]. All testing was completed by the same investigators during a single early morning visit after an overnight fast. The procedures were approved by the Human Subjects Committee, and informed consent was obtained from children and a parent before initiating the testing protocol. Testing included a history and physical examination, laboratory evaluation to determine fasting glucose and insulin levels, adiponectin, CRP, body composition, and cardiovascular fitness assessment. Height was measured using a wall-mounted stadiometer to the nearest 0.5 cm. Weight was measured on a calibrated beam balance platform scale to the nearest 0.25 kg.

Measurements

Percent body fat and fat free mass were measured by dual-energy X-ray absorptiometry (DXA). Whole body scans were performed using the Norland XR-36 whole body bone densitometer (Norland Corporation, Ft. Atkinson, WI) and tissue masses were analyzed using software version 3.7.4/2.1.0.

Children underwent measurement of VO_{2max} performed by open-circuit spirometry using a progressive treadmill walking protocol to volitional fatigue on a CPX-D treadmill (Medical Graphics Corp, St. Paul, MN). Requirements to ensure that children reached their VO_{2max} with this protocol included at least two of the following: (1) maximum heart rate of more than 200 beats/min; (2) respiratory exchange ratio (ratio of maximum carbon dioxide to VO_{2max}) of more than 1.0; and (3) a plateau in oxygen consumption. All participants reached their VO_{2max} according to these criteria.

A 10-ml fasting blood sample for insulin and glucose levels, adiponectin, and CRP measurement was obtained from an antecubital vein. Fasting insulin concentration was determined using a chemiluminescent immunoassay (ARUP Laboratories, Salt Lake City, UT), and glucose concentration was determined by the hexokinase method (University of Wisconsin Hospital and Clinics Laboratory, Madison, WI). The Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated using the formula[16]: 1/[log fasting insulin + log fasting glucose]. Adiponectin was measured by radioimmunoassay (Northwest Lipid Research Laboratories, Seattle, WA). High sensitivity C-reactive protein was measured immunochemically using the Dade-Behring reagent on a nephelometer autoanalyzer (Northwest Lipid Research Laboratories, Seattle, WA).

Statistical analysis

Inflammatory markers, body composition, and cardiovascular fitness parameters were summarized by standard descriptive statistics in terms of means and standard deviations. The bivariate associations between inflammatory marker levels and body composition/cardiovascular fitness parameters were examined using non-parametric Spearman's rank correlation analysis. Multiple regression analyses were used to evaluate the independent effects of cardiovascular fitness and body composition parameters on CRP and adiponectin. Gender, BMI z-score, percentage body fat, total fat and VO_{2max} were included as possible covariate variables in the multiple regression analysis models. Predictive variables were selected by applying a backward regression selection procedure with a p-value cut-off of <0.05. Multicollinearity was assessed by examining the variance inflation factor associated with each variable included in the multiple regression model. In these analyses, inflammatory markers were log-transformed to satisfy the necessary statistical assumptions of linear regression. Model assumptions were checked graphically using residual plots. All statistical analyses were performed with SAS software (version 9.1, SAS Institute Inc, Cary, NC). All p values were two sided and p values less than or equal to 0.05 were considered statistically significant.

RESULTS

The participants' characteristics are presented in Table 1. Study results are presented in Table 2.

Association of CRP with body composition

There was a significant positive correlation between CRP and BMI z-score (rs = 0.39, p <0.001, 95% CI: 0.18-0.57), percent body fat (rs = 0.46, p <0.001, 95% CI: 0.25-0.64) (Fig. 1), and total body fat in grams (rs = 0.46, p <0.001, 95% CI: 0.25-0.63). Of the body composition variables tested, CRP had a stronger correlation with body fat and total fat in grams than BMI z-score. Percent body fat remained a significant predictor of CRP in the multiple regression analysis (p <0.001), adjusted for age, gender and cardiovascular fitness (VO_{2max}). There was no correlation between CRP and fat free mass (p = 0.38).

Association of CRP with cardiovascular fitness

There was a significant negative correlation between CRP and fitness level as measured by VO_{2max} (rs = -0.25, p = 0.04, 95% CI: -0.45 to -0.01) in the univariate analysis (Fig. 2). There was no significant gender effect demonstrated by a two-way factorial ANOVA model with VO_{2max} (≤44 ml/kg.min vs >44 ml/kg.min) and gender as factors. In the multiple regression analysis with VO_{2max}, age, gender and percent body fat as independent variables, VO_{2max} remained a significant predictor (p = 0.019) of CRP.

Association of CRP with insulin resistance

There was no significant correlation between CRP and fasting insulin or IR as measured by QUICKI in this group of non-obese children.

Adiponectin

We found no significant correlation between adiponectin and IR, body composition or fitness level.

DISCUSSION

Increasingly sedentary lifestyles are fostering increased body fat and poor fitness in both children and adults. It is well established that CRP levels are increased[10,17,18] and related to fitness[19,20] in obese children and adolescents. However, reduced fitness is pervasive today among both obese and non-obese children, and the relationship between maximal cardiovascular fitness (the 'gold standard' in assessing cardiovascular fitness), body composition and CRP in healthy, non-obese children, has yet to be delineated. We measured markers of inflammation, body composition, and cardiovascular fitness by testing VO_{2max} in exclusively non-obese children.

Our data show that inflammation (measured by CRP) is associated with both body composition and fitness level in *non-obese* children. In this cohort of children with BMI <95th percentile, children with greater percent body fat, greater total body fat mass in grams, and higher BMI z-scores demonstrated higher CRP levels. With respect to fitness, children with lower VO_{2max} measurements had higher CRP levels. It is important to note that the overlap of cardiovascular fitness and fatness often makes it difficult to separate these important, yet distinct influences. However, after adjusting for body composition in the multivariate analysis of these data, VO_{2max} remained a significant predictor of CRP. These findings suggest that low fitness and poor body composition are independently associated with inflammation in non-obese children.

Previous studies in obese children have shown a strong positive relationship between adiponectin and IR and an inverse relationship between adiponectin and fat mass[21,22]. Unexpectedly, we found no correlations between adiponectin and IR, body composition or fitness level in this group of non-obese children. This suggests that the relationships between adiponectin and IR, body composition and fitness level do not become measurable until some greater threshold of obesity is met.

The limitations of this study include the relatively small sample size (n = 75) and the inclusion of fewer boys. While the majority (59%) of the participants was female, we did not find any significant differences when the data were analyzed by gender. Finally, since pubertal staging was not

TABLE 1

Participants' characteristics

	n	%	
Gender			
Female	44	59	
Male	31	41	
	Mean ± SD	**Median**	**Range**
Age (years)	12.5 ± 0.6	13	11.0 – 14.0
BMI	19.7 ± 2.7	19.9	14.5 – 24.0
BMI z-score	0.27 ± 0.94	0.56	-1.9 – 1.63
BMI percentile	59.2 ± 29.0	71.2	2.8 – 94.8
VO_{2max} (ml/kg per minute)	44.0 ± 7.8	43.9	30.7 – 65.5
Percent body fat	24.5 ± 6.7	24.1	9.2 – 38.9
Total body fat (kg)	16.6 ± 5.9	15.6	5.4 – 29.4
Fasting glucose (mg/dl)	94.6 ± 5.6	94.5	78.0 – 111.0
Fasting insulin (μU/ml)	11.5 ± 5.0	11.0	4.0 – 33.0
hsCRP (mg/dl)	0.032 ± 0.04	0.014	0.002 – 0.22
Adiponectin (ng/ml)	16,604 ± 6,502	16,650	5,350 – 41,700

TABLE 2

Spearman's rank correlation coefficients (95% confidence intervals) between markers of inflammation and insulin resistance, body composition, and fitness

	Fasting insulin	**QUICKI**	**BMI z-score**	**Percent body fat**	**Total fat** (g)	**VO_2max**
C-reactive protein	r_s = 0.14 (-0.10 to 0.36) p = NS	r_s = -0.15 (-0.37 to 0.08) p = NS	**r_s = 0.39** **(0.18 to 0.57)** **p <0.001**	**r_s = 0.46** **(0.25 to 0.64)** **p <0.001**	**r_s = 0.46** **(0.25 to 0.63)** **p <0.001**	**r_s = -0.25** **(-0.45 to 0.01)** **p = 0.04**
Adiponectin	r_s = -0.02 (-0.25 to 0.22) p = NS	r_s = 0.02 (-0.22 to 0.25) p = NS	r_s = -0.18 (-0.39 to 0.05) p = NS	r_s = -0.04 (-0.27 to 0.20) p = NS	r_s = -0.09 (-0.32 to 0.14) p = NS	r_s = 0.09 (-0.15 to 0.31) p = NS

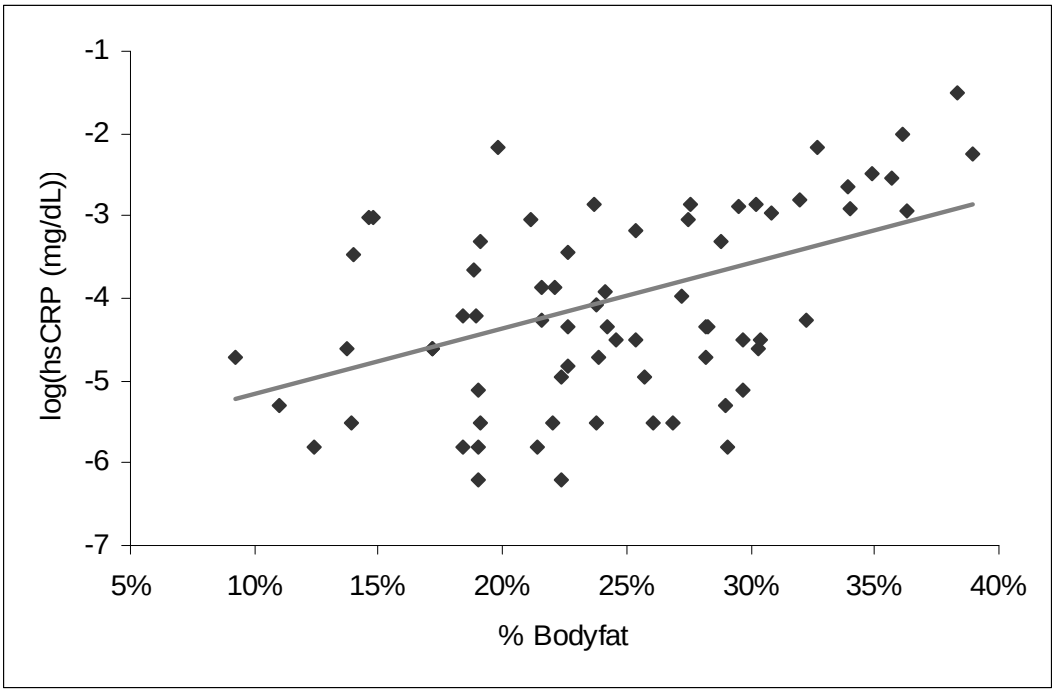

Fig. 1: Correlation between hsCRP and % body fat measured by DEXA. $r_s = 0.46$, $p < 0.001$.

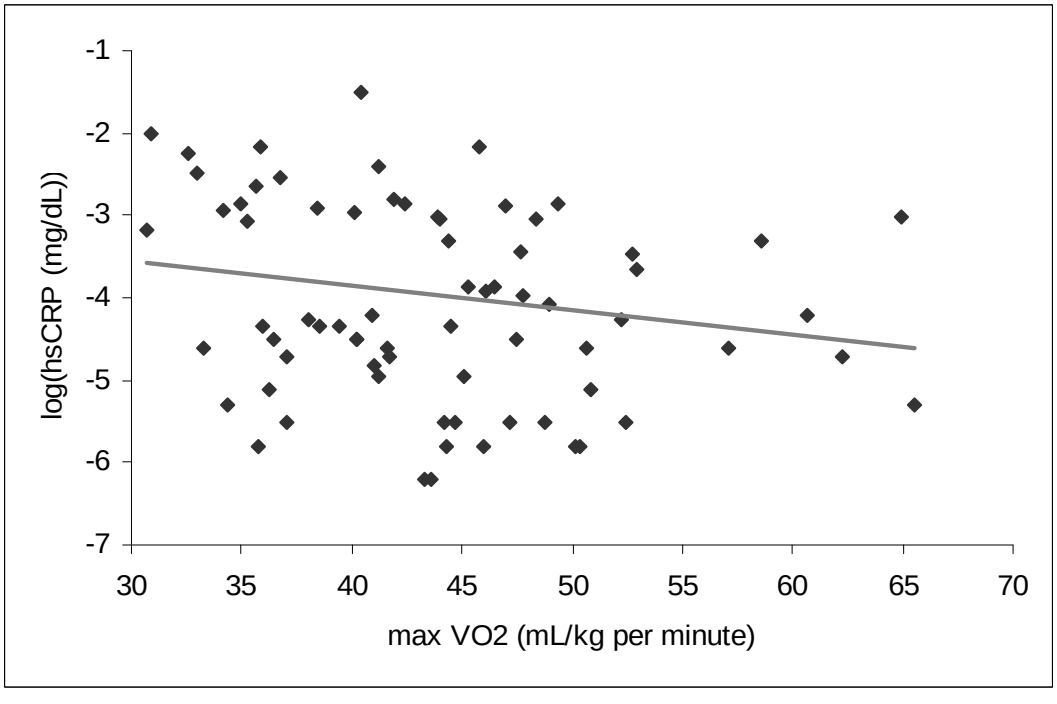

Fig. 2: Correlation between hsCRP and fitness measured by VO_2max. $r_s = -0.25$, $p = 0.04$.

performed, we cannot report the effect of pubertal status on these relationships.

In addition, although non-obese children with relatively increased body fat and poor fitness show evidence of inflammation, the question of clinical relevance remains. While CRP varied significantly with increasing body fat and reduced fitness, the absolute increases were small and the values remained within the normal adult range for cardiovascular risk assessment. However, while children with increased body fat and low fitness levels are asymptomatic, increased markers of inflammation suggest that initiation of a cascade of inflammation, increasing risk for type 2 diabetes mellitus (DM2) and coronary heart disease (CHD), may have already begun. Thus, being sedentary is not only a risk factor for obesity, but also the inflammatory process that accompanies low fitness.

This study suggests that risk factors for the development of CHD and DM2 are associated with fitness as well as adiposity in non-obese children. While all children with a BMI less than the 95th percentile for age are considered 'non-obese', there is likely a continuum of risk for future adverse cardiovascular outcomes which ranges from higher to lower BMI. Thus, BMI determination may still be useful in large scale settings, such as schools, to identify and target children at increased risk for inflammation who do not meet criteria for 'obesity'.

In conclusion, in non-obese children, reduced fitness and increased adiposity are associated with elevated CRP, a surrogate marker of inflammation. This strengthens the emphasis on promoting fitness and healthy body composition in all children, obese and non-obese alike.

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Disclosure statement

None of the authors report any potential conflicts of interest with entities directly related to the material being published.

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