Relation of C-Reactive Protein to Insulin Resistance and Cardiovascular Risk Factors in Youth

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OBJECTIVE — Insulin resistance and C-reactive protein (CRP) levels are strongly correlated in adults. This study explored the relationship in youth.

RESEARCH DESIGN AND METHODS — Associations between CRP levels, cardiovascular risk, and insulin resistance measured by the euglycemic clamp were investigated in 342 healthy Minneapolis youth.

RESULTS — There was no difference in mean CRP levels among boys (n = 189, CRP 1.10 ± 0.46 mg/l) and girls (n = 153, CRP 1.16 ± 0.63 mg/l, P = 0.32). There was also no difference between CRP and Tanner stage. CRP, adjusted for BMI, was significantly greater in black subjects compared with white subjects (P = 0.03). CRP was strongly related to adiposity in both girls and boys. CRP levels were related to fasting insulin levels (r = 0.16, P = 0.003) but this association was not significant after adjustment for BMI (r = 0.07, P = 0.21). Similarly, M, the euglycemic clamp measure of insulin sensitivity, was significantly related to CRP levels (r = -0.13, P = 0.02) but not when M was normalized to lean body mass (M_bbm) (r = -0.10, P = 0.09). There was a significant inverse correlation between M_bbm and CRP quartiles, which disappeared after adjustment for BMI. There was no significant association between CRP levels and lipids, blood pressure, physical activity, or left ventricular mass.

CONCLUSIONS — In contrast to adult subjects, after adjustment for adiposity, CRP levels in children age 10–16 years were not significantly associated with insulin resistance or with other factors comprising the metabolic syndrome. This is consistent with the concept that insulin resistance may precede the development of CRP elevation in the evolution of the metabolic syndrome.

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C-reactive protein (CRP) is an acute-phase reactant usually associated with serious infection and inflammation. More recently, it has become clear that subtle elevation of CRP levels within the “normal” adult range is an indicator of subclinical disease, such as that related to 1) the risk of cardiovascular disease in healthy men and women and in individuals with type 1 diabetes (1), 2) the risk factors associated with the metabolic syndrome (1–3, and 3) the onset of type 2 diabetes (4). The mechanisms accounting for these relations are not well understood.

Insulin resistance and CRP levels are strongly correlated in adults (2,3,5,6). Although CRP is known to be associated with adiposity in youth (7–12), there are no data relating it to insulin resistance in children or adolescents. These data may be important because the insulin resistance syndrome has its origins in youth (13–15) and the independent roles of insulin resistance and adiposity in establishment of the syndrome (and in ongoing development from childhood into adulthood) have not been defined. If inflammation is a precursor to cardiovascular disease, determining these early relations would be highly relevant to the design of further investigation and prevention strategies. As recently suggested (16), this developmental period might be particularly conducive to studying the early relationships between these factors to better understand the pathogenesis.

In the current study, associations between CRP levels and insulin resistance, as measured by the euglycemic insulin clamp, were investigated in 342 healthy Minneapolis youth. These individuals, who were participating in a longitudinal cardiovascular risk factor study, offered a unique opportunity to explore the relation between this measure of chronic inflammation and obesity, insulin resistance, and cardiovascular risk.

RESEARCH DESIGN AND METHODS — This study was approved by the University of Minnesota Committee for the Use of Human Subjects in Research. Informed consent was obtained from parents and informed assent from the children. Participant recruitment and methods have been previously described in detail (13,17). The subjects were randomly selected after blood pressure screening of 12,043 fifth through eighth grade children in the Minneapolis public school system in 1996 and stratified according to sex, ethnicity (black and non-Hispanic white), and blood pressure

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percentile (50% from the upper 15 percentiles and 50% from the lower 75 percentiles for white children and black girls; 50% from the upper 25 percentiles and 50% from the lower 75 percentiles for black boys, to increase the percentage of children at potential cardiovascular risk).

Insulin clamps were completed in 357 participants, aged 10–16 years (mean age 13 years). Frozen fasting serum samples were available from 348 of the participants. Six subjects with CRP levels >5.05 mg/l were excluded from the analysis based on the expected normal range values of CRP (0.18–5.05 mg/l). The remaining 342 subjects form the study population for the present report.

Body composition measurement and physical activity
All participants attended a clinic dedicated to this study in which history was taken and physical examination was performed by board-certified pediatricians. Children were divided into Tanner stages according to pubic hair development in boys and breast and pubic hair development in girls. The greater of the two values in girls was used for statistical analysis so that pubertal maturation was not underestimated. Triceps and subscapular skinfold thickness were measured in duplicate with Lange calipers and were used to predict percent body fat by the method of Slaughter et al. (18). Blood pressure was measured twice on the right arm using a random-zero sphygmomanometer with subjects in the seated position; the averages of the two measurements (systolic and fifth-phase Korotkoff diastolic) were used in the analyses. Physical activity was measured using the Paffenbarger Physical Activity Survey (19).

Euglycemic clamps and echocardiograms
Participants were admitted to the University of Minnesota Clinical Research Center after a 10- to 12-h overnight fast. A medical history was obtained to exclude subjects who were acutely ill. An arm vein was cannulated for infusion of potassium phosphate, insulin, and dextrose. A contralateral vein was cannulated for blood sampling and the hand was placed in a heated box (65°C) to arterialize venous blood. Insulin was infused at a rate of 1 mU·kg⁻¹·min⁻¹ for 3 h and plasma glucose was measured every 5 min. A variable infusion of 20% dextrose was used to maintain the serum glucose level at 100 mg/dl. Echocardiographic examination for calculation of left ventricular mass was performed utilizing Philips equipment and included complete two-dimensional, spectral, and color Doppler examination and complete M-mode study.

Analytical methods and calculations
Plasma glucose was measured immediately at the bedside with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Blood samples were collected on ice, centrifuged within 20 min, and frozen and stored at −70°C. Insulin levels were measured within 1 week of the clamp studies by radioimmunoassay using a double-antibody method. The intra-assay coefficient of variation (CV) was 2–9%, the interassay CV was 5–7%, and cross-reactivity to proinsulin was 20%. Insulin sensitivity (M) was calculated as the amount of glucose (milligrams per kilogram per minute) required to maintain euglycemia during the last 40 min of the clamp and was expressed as M Archived [milligrams of glucose infused per kilogram lean body mass per minute]. A lower M Archived value represents a greater degree of insulin resistance.

C-reactive protein was measured by an ultrasensitive colorimetric competitive enzyme-linked immunosorbent assay (ELISA) (20). Biotinylated CRP competes with CRP in the sample for the coated antibody. Detection is via the enzyme horseradish peroxidase conjugated in an avidin-biotin complex, followed by the color reagent substrate orthophenylene diamine. Standardization is done using the World Health Organization CRP reference standard. Samples were measured in a single large batch at the end of the study. The laboratory reference range is 0.18–5.05 mg/l. Blood samples for serum lipids were analyzed utilizing a Cobas FARA. Cholesterol was determined by a standard enzymatic-cholesterol oxidase-based method; HDL cholesterol was determined after precipitation of non-HDL lipoproteins with magnesium/dextran precipitating reagent; and triglycerides were determined using a standard glycerol blanked, enzymatic, triglyceride method. LDL cholesterol was calculated by the Friedewald equation. Plasma leptin levels were measured by the DSL (Webster, TX) 10–23100 ACTIVE Human Leptin ELISA, an enzymatically amplified “two-step” immunosassay. The sensitivity (minimum detection limit) of the assay was 0.05 ng/ml. The intra-assay CV was 1.5–6.2% and the interassay CV was 3.3–5.3%. IGF-I and IGF-binding protein-3 assays were performed by ELISA (DSL) according to the manufacturer’s instructions.

Data analysis
Data are expressed as means ± SD. Pearson correlation analyses and multiple linear regression were used to assess the relationships between CRP and physical and clinical characteristics, with adjustments for sex, race, and Tanner stage. To normalize for skewed distribution, log (CRP + 1) levels were also used in these analyses. The results were similar and are not reported. We had 85% power to detect a correlation of at least ±0.16. All statistical analyses were conducted using the SAS statistical package, version 8.2 (SAS Institute, Cary, NC).

The clustering effect of the primary components of the insulin resistance syndrome (fasting insulin, BMI, systolic blood pressure [SBP], triglycerides, and HDL cholesterol) was assessed by comparing the average of the Z scores for the five variables (with reversed sign for HDL cholesterol) for participants in each of the four CRP quartiles. The Z score for each variable was computed as (value for the individual − the sex-specific mean)/sex-specific standard deviation.

RESULTS
CRP levels and sex, Tanner stage, race, and adiposity
Subject baseline characteristics are presented in Table 1. The six subjects excluded on the basis of CRP levels greater than the upper limit of normal for the assay (>5.05 mg/l) were all male: two were obese and insulin resistant, three were thin and insulin sensitive, and one was thin and insulin resistant. One of the excluded subjects was black (thin and insulin sensitive). The assumption was made that CRP elevation in these boys was the result of subclinical illness because none of the six had CRP levels outside the normal range when they were retested 2 years later.

There was no significant difference in mean CRP levels between boys (n = 189, CRP 1.10 ± 0.46 mg/l) and girls (n =
153, 1.16 ± 0.63 mg/l; \( P = 0.32 \) (Table 1). There were no significant differences in CRP levels between Tanner stages for either girls or boys (Fig. 1).

CRP levels adjusted for sex and Tanner stage were somewhat lower in white \( n = 271 \), CRP 1.09 ± 0.04 mg/l compared with black \( n = 71 \), 1.24 ± 0.07 mg/l subjects, but this difference did not quite achieve statistical significance (\( P = 0.052 \)). After additional adjustment for BMI, CRP was significantly higher in the black participants (\( P = 0.03 \)).

CRP, adjusted for race and Tanner stage, was strongly related to adiposity in both girls and boys. CRP in girls was related to BMI \( ( r = 0.27, P = 0.001 ) \), subscapular skinfold thickness \( ( r = 0.39, P = 0.0001 ) \), waist circumference \( ( r = 0.25, P = 0.002 ) \), triceps skinfold thickness \( ( r = 0.18, P = 0.03 ) \), hip circumference \( ( r = 0.17, P = 0.04 ) \), percent body fat \( ( r = 0.29, P = 0.0004 ) \), and serum leptin levels \( ( r = 0.27, P = 0.007 ) \). There were positive relationships of BMI, waist circumference, and serum leptin levels across increasing quartiles of CRP (Table 2).

**CRP levels and insulin levels, insulin resistance, and components of the insulin resistance syndrome**

CRP levels were significantly related to fasting insulin levels \( ( r = 0.16, P = 0.003 ) \), but this correlation was no longer significant after adjustment for BMI \( ( r = 0.07, P = 0.21 ) \). The euglycemic clamp measurement of insulin sensitivity, was significantly related to CRP levels \( ( r = -0.13, P = 0.02 ) \). However, the significance of this association disappeared when \( M \) was normalized to lean body mass \( ( M_{\text{LBM}} ) \); \( r = -0.10, P = 0.09 \). There was a significant inverse relationship between \( M_{\text{LBM}} \) levels and quartiles of CRP,

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**Figure 1** — CRP levels (mg/l) by sex and Tanner stage. Data are means ± SD.
CRP levels across quartiles adjusted for sex, race, and Tanner stage by linear regression analysis

<table>
<thead>
<tr>
<th>Quartiles of CRP Levels</th>
<th>CRP (ng/ml)</th>
<th>BMI (kg/m²)</th>
<th>Waist (cm)</th>
<th>Body fat</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>Total cholesterol (mmol/l)</th>
<th>LDL cholesterol (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
<th>Triglycerides (mmol/l)</th>
<th>Insulin (pmol/l)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (0.46–0.85)</td>
<td>0.76 ± 0.05</td>
<td>20.9 ± 0.5</td>
<td>73.1 ± 1.2</td>
<td>27.2 ± 1.2</td>
<td>106 ± 1</td>
<td>56 ± 2</td>
<td>4.14 ± 0.09</td>
<td>2.42 ± 0.08</td>
<td>1.24 ± 0.03</td>
<td>1.03 ± 0.07</td>
<td>69 ± 0.00</td>
<td>19 ± 0.00</td>
</tr>
<tr>
<td>Q2 (0.86–0.99)</td>
<td>0.95 ± 0.05</td>
<td>20.6 ± 0.5</td>
<td>73.5 ± 1.3</td>
<td>26.3 ± 1.3</td>
<td>106 ± 1</td>
<td>57 ± 2</td>
<td>3.90 ± 0.09</td>
<td>2.27 ± 0.08</td>
<td>1.20 ± 0.03</td>
<td>0.93 ± 0.07</td>
<td>73 ± 0.00</td>
<td>68 ± 0.00</td>
</tr>
<tr>
<td>Q3 (1.00–1.16)</td>
<td>1.09 ± 0.05</td>
<td>21.1 ± 0.5</td>
<td>74.7 ± 1.3</td>
<td>27.6 ± 1.3</td>
<td>106 ± 1</td>
<td>56 ± 2</td>
<td>3.89 ± 0.09</td>
<td>2.30 ± 0.08</td>
<td>1.20 ± 0.03</td>
<td>0.86 ± 0.07</td>
<td>80 ± 0.00</td>
<td>69 ± 0.00</td>
</tr>
<tr>
<td>Q4 (1.17–1.72)</td>
<td>1.77 ± 0.05</td>
<td>22.6 ± 0.5</td>
<td>76.9 ± 1.2</td>
<td>29.3 ± 1.2</td>
<td>108 ± 1</td>
<td>58 ± 2</td>
<td>4.07 ± 0.09</td>
<td>2.38 ± 0.07</td>
<td>1.22 ± 0.03</td>
<td>1.01 ± 0.07</td>
<td>86 ± 0.00</td>
<td>72 ± 0.00</td>
</tr>
<tr>
<td>p</td>
<td>0.0001</td>
<td>0.01</td>
<td>0.02</td>
<td>0.19</td>
<td>0.20</td>
<td>0.50</td>
<td>0.53</td>
<td>0.69</td>
<td>0.63</td>
<td>0.83</td>
<td>0.08</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 342). *P = 0.17 when adjusted for BMI. M_	ext{fem}, mg glucose infused · kg lean body mass$^{-1}$ · min$^{-1}$ during the euglycemic clamp. DBP, diastolic blood pressure.

but the significance disappeared after adjustment for BMI (Table 2). Similar BMI-adjusted correlations of fasting insulin and M_	ext{fem} with CRP were found in separate analyses of boys (insulin $r = 0.13$, $P = 0.10$; M_	ext{fem} $r = -0.004$, $P = 0.96$) and girls (insulin $r = 0.03$, $P = 0.70$; M_	ext{fem} $r = -0.08$, $P = 0.31$).

There were no associations between CRP and lipid levels (total cholesterol, HDL, calculated LDL, and triglycerides; $P > 0.15$ for all), SBP ($r = 0.08$, $P = 0.14$), diastolic blood pressure ($r = 0.02$, $P = 0.77$), physical activity score ($r = -0.06$, $P = 0.29$), and left ventricular mass ($r = 0.02$, $P = 0.73$). There was no relationship between CRP and clustering of cardiovascular risk factors. The cluster Z scores for the CRP quartiles were all about zero (range $-0.05$ to 0.11), and there was no significant difference in cluster Z scores among the CRP quartiles ($P > 0.1$).

CRP levels were higher in subjects in the highest quartile versus those in the lowest quartile for measures of adiposity including waist ($P < 0.001$), BMI ($P < 0.001$), percent body fat ($P = 0.01$), and leptin levels ($P < 0.001$) (Table 3). CRP levels were higher in subjects in the lowest quartile for M_	ext{fem} (the most insulin resistant) than in subjects in the other three quartiles for M_	ext{fem} ($P = 0.02$), which were equivalent to each other, but the significance of this difference disappeared after adjustment for BMI.

**CONCLUSIONS** — This is the first study to explore the relationship in youth between CRP levels and insulin resistance as measured by the hyperinsulinemic-euglycemic clamp. In contrast to adult subjects (3,21), after adjustment for adiposity, healthy children age 10–16 years did not show a significant linear association between continuous values of CRP and insulin resistance or Z scores representing the clustering of cardiovascular risk factors. Although M_	ext{fem} was lower among those in the highest quartile for CRP and CRP was greater in the lowest quartile for M_	ext{fem}, the significance of these associations disappeared after adjustment for BMI. The finding of a significant relationship between CRP and obesity in youth who are just beginning to show evidence of the insulin resistance syndrome (13) raises the possibility that with additional maturation, the CRP/insulin resistance association will become evident in these at-risk individuals.

Significant correlations have been found in adults between CRP levels and hypertension, dyslipidemia, cardiovascular disease, and diabetes (2,3,22). It is unclear whether CRP elevation or insulin resistance develops first and different theories have emerged to explain this association (3,5). CRP may contribute to cardiovascular disease by binding to the membranes of damaged cells, activating complement, or enhancing production of thrombogenic agents (23–25), and the resultant vascular inflammation may contribute to the development of insulin

Table 3—CRP levels across quartiles adjusted for sex, race, and Tanner stage by linear regression analysis

<table>
<thead>
<tr>
<th>Quartiles of CRP Levels</th>
<th>M_	ext{fem}</th>
<th>Waist</th>
<th>BMI</th>
<th>Body fat (%)</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>1.31 ± 0.06</td>
<td>1.06 ± 0.06</td>
<td>1.08 ± 0.06</td>
<td>1.10 ± 0.06</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>Q2</td>
<td>1.11 ± 0.06</td>
<td>1.17 ± 0.06</td>
<td>1.10 ± 0.06</td>
<td>1.10 ± 0.06</td>
<td>1.13 ± 0.06</td>
</tr>
<tr>
<td>Q3</td>
<td>1.12 ± 0.06</td>
<td>1.15 ± 0.07</td>
<td>1.19 ± 0.07</td>
<td>1.12 ± 0.06</td>
<td>1.13 ± 0.06</td>
</tr>
<tr>
<td>Q4</td>
<td>1.12 ± 0.06</td>
<td>1.42 ± 0.07</td>
<td>1.40 ± 0.07</td>
<td>1.31 ± 0.06</td>
<td>1.41 ± 0.06</td>
</tr>
<tr>
<td>p value (Q1-Q4)</td>
<td>0.02*</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.01</td>
<td>&lt; 0.001†</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 342). *P = 0.24 when adjusted for BMI; †P = 0.98 when adjusted for BMI. M_	ext{fem}, mg glucose infused · kg lean body mass$^{-1}$ · min$^{-1}$ during the euglycemic clamp.
resistance. Alternatively, insulin resistance may initiate or contribute to CRP elevation by reducing insulin-induced suppression of hepatic acute-phase reactants (26). Although causality cannot be inferred from a cross-sectional study, the current data are consistent with the theory that insulin resistance precedes the development of CRP elevation in the evolution of the insulin resistance syndrome.

As in adults, features of the insulin resistance syndrome are apparent in youth. We previously demonstrated significant relationships between insulin resistance and cardiovascular risk factors in this pediatric cohort (13,27). It may be too early in development, however, to see a direct independent relationship with CRP. These data suggest that a longer period of exposure to either excessive obesity or insulin resistance may be required before the association between CRP and the risk factors becomes apparent or that a critical degree of vascular inflammation must be reached before these associations are clinically evident. Alternatively, there may be protective factors related to rapid growth and development during puberty which prevent or retard the progression of this association during the adolescent years. Hormonal changes are probably not a factor because the physiological insulin resistance of puberty is not correlated with the increase in testosterone and estrogen (17) and because CRP levels in this study were not associated with Tanner stage.

Prior results in the literature regarding the relationship between CRP and features of the metabolic syndrome in youth are conflicting. In a cohort of 470 obese and overweight youth, CRP levels were not related to fasting insulin levels or to the product or fasting insulin and glucose (homeostatic model of insulin resistance). Although ~40–50% of subjects were characterized as having the metabolic syndrome, CRP levels were not significantly related to components of the syndrome (28). In 2,846 boys and girls 3–17 years of age participating in the National Health and Nutrition Examination Survey in 1999–2000, significant associations were observed between CRP levels and SBP and triglyceride concentrations. However, these associations (except for the association with SBP in 12- to 17-year-old girls) disappeared after adjustment for BMI (10). A study of English schoolchildren aged 10–11 years found an association between CRP levels and both HDL cholesterol and SBP (7), but those data may have been influenced by a large cohort of Southeast Asian children with high CRP levels and insulin resistance. A relationship was found between CRP and HDL cholesterol levels in 835 children from Taipei aged 12–16 years (11). A study of 79 Finnish children showed no significant relationship between CRP levels and blood pressure or lipid levels (29).

Several studies have related CRP levels to the degree of adiposity in adults (3,5) and children (7–12,28). CRP was related to BMI and adiposity in Hispanic children as young as 2 years of age (12). In the large cohort of children aged 6–18 years in the Third National Health and Nutrition Examination Survey (8,9), CRP concentrations were highest among children with a BMI >85th percentile, and this association did not differ by age, sex, race, or ethnicity. The present study also showed a significant relationship between CRP and measures of adiposity.

Few data are available regarding the role of race in the CRP/insulin resistance/cardiovascular risk relationship. CRP levels have been reported to be elevated in children of color. Hispanic children (10,30) and South Asian children (7) have greater CRP levels than white children of similar BMI or adiposity. Although 50 black youth had higher mean levels of CRP than 24 white youth of similar percent body fat, the difference was not statistically significant (31). In the current cohort after adjustment for body size, black youth were shown to have higher CRP levels than white youth. This is of interest because this particular cohort of black subjects tends to be more physically active, slender, and insulin sensitive (19). How this affects future cardiovascular risk remains to be seen. Race did not affect the relationship between CRP and stroke in the Third National Health and Nutrition Examination Survey (32).

In summary, study of a healthy pediatric cohort shows that CRP levels are related to adiposity but are not yet independently related to insulin resistance, as measured by the euglycemic clamp. These results add to the growing evidence of the early establishment of cardiovascular risk factors before adulthood and are consistent with the hypothesis that insulin resistance may precede CRP elevation. To explain the evolution from childhood to the adult relationships between insulin resistance, CRP elevation, and cardiovascular risk, it will be important to build on these current results by following the natural history of a cohort of adolescents as they progress into adulthood.

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References
CRP and insulin resistance in youth


