Relation of Body Mass Index and Insulin Resistance to Cardiovascular Risk Factors, Inflammatory Factors, and Oxidative Stress During Adolescence

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Background—This study assessed the relation of fatness and insulin resistance and their interaction with cardiovascular risk factors, inflammatory factors, and oxidative stress in thin and heavy adolescents.

Methods and Results—Euglycemic insulin clamp studies were performed on 295 (169 male, 126 female) adolescents (mean±SE age, 15±0.1 years). Comparisons were made between (1) heavy and thin adolescents; (2) insulin-sensitive and insulin-resistant adolescents; and (3) thin insulin-sensitive (T-IS), thin insulin-resistant (T-IR), heavy insulin-sensitive (H-IS), and heavy insulin-resistant (H-IR) adolescents. Summed z scores were used to determine clustering of risk factors (fasting insulin, triglycerides, HDL-C, and systolic blood pressure [SBP]) among the groups. SBP, triglycerides, and fasting insulin were significantly higher and HDL-C significantly lower in the heavy adolescents. Fasting insulin and triglycerides were significantly higher and HDL-C significantly lower in the insulin-resistant adolescents. Among the 4 groups, the risk factors and cluster score followed a pattern of risk as follows: T-IS<T-IR<H-IS<H-IR, with H-IR significantly greater than the other groups and showing an interaction between fatness and insulin resistance.

Conclusions—These results show the significant association of both fatness and insulin resistance and their significant interaction with cardiovascular risk factors in adolescence. The finding that insulin resistance may be acting interactively with fatness suggests that interventions directed at insulin resistance in addition to weight loss may be required to alter early development of cardiovascular risk. (Circulation. 2005;111:1985-1991.)

Key Words: insulin ■ obesity ■ pediatrics ■ risk factors

Measures of obesity, in association with hypertension, dyslipidemia, and hyperglycemia, form the metabolic syndrome (MS), a well-recognized constellation of risk factors for type 2 diabetes and cardiovascular disease (CVD) in adults. The MS is not as well categorized before adulthood, however. Evidence from childhood studies confirms that the prevalences of type 2 diabetes and obesity are increasing, and recently published data from the Third National Health and Nutrition Examination Survey show that approximately 30% of overweight adolescents have criteria consistent with the MS. Moreover, as the degree of obesity increases in children, there is a corresponding increase in the levels of cardiovascular risk and inflammatory factors. Therefore, understanding the sequence of events that begins in childhood and leads to the onset of the MS has become increasingly important.

Insulin resistance and obesity are important components of the MS, but the joint effects of insulin resistance and obesity on the constituent elements of the syndrome are not well defined. The direct relation between insulin resistance and fatness is well known, and overweight is the single most important predictor of type 2 diabetes. Obesity is associated with impaired insulin signaling, and certain patterns of fat deposition (eg, central or intra-abdominal fat) are more highly related to insulin resistance. Although skeletal muscle is generally considered to be the primary tissue site for glucose utilization, glucose uptake also is affected by adipose tissue.

Despite these associations, it is clear that obesity per se cannot fully explain the development of insulin resistance. The relation between obesity and insulin resistance is not present in all obese individuals. Nonobese, nondiabetic, healthy individuals can be insulin resistant, and type 2 diabetes occurs in nonobese individuals.

Study of obesity and insulin resistance in children offers the potential to identify factors influencing the early development of cardiovascular risk and type 2 diabetes, ie, before...
the onset of disease. Overt CVD is rare in children, but previous studies in this age group have shown significant relations between fasting insulin and lipids, blood pressure, and weight, and more recent studies have confirmed the associations among obesity, insulin resistance, and the other risk factors. Although a high prevalence of impaired glucose tolerance is found among obese children, factors other than fatness must also be operative in children, as in adults, because the correlation between obesity and insulin resistance is only 0.26 in early adolescence.

Data in the present report were obtained as part of a longitudinal study designed to determine the relations between fatness and insulin resistance during the transition from childhood to adulthood. In the present report, their independent associations and interaction with individual cardiovascular risk factors, inflammatory factors, and oxidative stress were assessed in a cohort of thin and heavy 13- to 17-year-old adolescents undergoing insulin clamp studies.

Methods

The Human Subjects Committee of the University of Minnesota approved this study. Consent was obtained from all children and their parents/guardians.

Children were recruited after blood pressure, height, and weight screening of 12,043 fifth to eighth grade Minneapolis Public School students (3819 black, 4216 white, 4008 other, 6035 male, 6008 female), representing 93% of all students in those grades. Black and non-Hispanic white children were recruited with stratification according to sex, race, and systolic blood pressure (SBP) percentile (half from the upper 25th percentiles and half from the lower 75th percentiles to enrich the study population with potentially higher-risk children). At their second study visit, 310 completed a euglycemic insulin clamp at a mean age of 15 years. Complete data sets were available for 295 who form the cohort for this report. The screening BP, height, and weight of participants did not differ from the children who chose not to participate.

The children underwent a complete physical examination including Tanner staging and anthropometric measurements. Height was measured by a wall-mounted stadiometer. Weight was measured by a balance scale. BP was measured twice on the right arm with a random-zero sphygmomanometer with subjects in the seated position; the averages of the 2 measurements (systolic and fifth phase Korotkoff diastolic) were used in the analyses.

Euglycemic insulin clamp studies were conducted in the University of Minnesota Clinical Research Center as previously described. Blood samples for serum insulin and plasma glucose levels were obtained before starting the insulin infusion, and plasma glucose was measured every 5 minutes during the clamp. The insulin infusion was started at time 0 and continued at 1 mU · kg⁻¹ · min⁻¹ for 3 hours. An infusion of 20% glucose was started at time 0 and adjusted, based on plasma glucose levels, to maintain plasma glucose at 5.6 mmol/L (100 mg/dL). Insulin sensitivity was determined from the amount of glucose administered during the final 40 minutes of the euglycemic clamp and was expressed as M̄BM (ie, glucose utilization/kg lean body mass [LBM] per minute). LBM, or fat-free mass, was calculated by the skinfold formula method of Slaughter et al. Our studies (unpublished) in 140 11- to 17-year-old siblings of the cohort have shown high correlations for percent body fat (%BF) and fat body mass (FBM) between the Slaughter estimates and dual-energy x-ray absorptiometry, as follows: boys (n=72), %BF r=0.93, FBM r=0.96; girls (n=68), %BF r=0.92, FBM r=0.98; all P=0.0001.

Blood samples were analyzed for glucose immediately at the bedside with a Beckman Glucose Analyzer II (Beckman Instruments, Inc). Insulin levels (radioimmunoassay; 20% cross-reactivity with proinsulin) and serum lipids were determined in the laboratory of the Fairview University Medical Center, as previously reported.
The cohort consisted of 295 children (169 male, 126 female; 57 black, 238 white; Table 1). All were Tanner stage 4 or 5 with a mean Tanner score of 4.5±0.1. The mean age was 15±0.1 years. Males were significantly taller and had significantly greater weight, LBM, waist circumference, and waist-hip ratio, but females had significantly greater %BF and triceps and subscapular skinfolds. BMI was not significantly different between males and females. Males had significantly greater weight, LBM, waist circumference, and waist-hip ratio compared with all boys. P<0.0001 compared with all boys. P=0.0008 compared with all boys. *P<0.001 compared with all boys.

Comparison of body size and MLBM among the T-IS, T-IR, H-IS, and H-IR groups is shown in Figure 3. Virtually the differences in any of the anthropometric measurements or SBP between the 2 MLBM groups. However, fasting insulin and triglycerides were significantly higher and HDL-C was significantly lower in the insulin-resistant group (Figure 2).

Results

The cohort consisted of 295 children (169 male, 126 female; 57 black, 238 white; Table 1). All were Tanner stage 4 or 5 with a mean Tanner score of 4.5±0.1. The mean age was 15±0.1 years. Males were significantly taller and had significantly greater weight, LBM, waist circumference, and waist-hip ratio, but females had significantly greater %BF and triceps and subscapular skinfolds. BMI was not significantly different between males and females. Males had significantly greater systolic and females, greater diastolic blood pressure. Females had significantly lower fasting glucose and MLBM. Fasting insulin was similar in males and females. Triglycerides and HDL-C were not significantly different between males and females. There were few very significant differences between blacks and whites, in part reflecting the small number of black participants. Of note, HDL-C was significantly higher and triglycerides significantly lower in blacks.

The median MLBM was 12.45 for males and 11.52 for females. Age and Tanner stage were similar for the groups with MLBM above (insulin sensitive) and below (insulin resistant) the median MLBM. There were no significant differences in any of the anthropometric measurements or SBP between the 2 MLBM groups. However, fasting insulin and triglycerides were significantly higher and HDL-C was significantly lower in the insulin-resistant group (Figure 2).

Comparison of body size and MLBM among the T-IS, T-IR, H-IS, and H-IR groups is shown in Figure 3. Virtually

![Figure 1. Comparison of SBP, fasting insulin, triglycerides (Trig.), HDL-C, and MLBM between adolescents with BMI above median BMI and BMI below median BMI.](image-url)
identical values for BMI, waist (data not shown), BF, and LBM were found when T-IS was compared with T-IR or H-IS with H-IR. Figure 4 shows the differences among risk factors for the 4 groups. Examination of the differences in mean SBP across the 4 BMI-MLBM groups showed that BMI, not MLBM, was the important factor affecting SBP. Differences in mean SBP were significant between T-IS and H-IS and between T-IR and H-IR but not between T-IS and T-IR or H-IS and H-IR. Both BMI and MLBM affected the levels of other factors, with an increase in fasting insulin and triglycerides seen in the following pattern: T-IS > H-IS > T-IR > H-IR and an inverse of this pattern for HDL-C. The association of BMI with fasting insulin and blood lipids could be seen in both the IS and IR groups, with fasting insulin and triglycerides significantly higher and HDL-C significantly lower in the H-IS versus T-IS and in H-IR versus T-IR groups. In contrast, MLBM had an association only in the heavy subjects, with triglycerides and fasting insulin significantly higher and HDL-C significantly lower in the H-IR versus H-IS group, but only small differences were noted between T-IR and T-IS groups.

To explore this further, the clustering effect of triglycerides, HDL-C, SBP, and fasting insulin was analyzed by combining these factors as the average of their z scores. According to the standard normal distribution, z score values above 0.5 are the highest 30.8th percentiles and those below 0.5 are the lowest 30.8th percentiles. The percentage of participants with ≥2 risk factor z scores ≥0.5 increased sharply as the average z score increased; therefore, it was rare that a high z score reflected an isolated high risk-factor value. For the divisions of the cohort described in this study, the highest-risk group (H-IR) had 62% with 2 or more individual risk factor z scores ≥0.5, and the lowest-risk group (T-IS) had only 8%. The 2 middle groups had intermediate values of 14% (T-IR) and 23% (H-IS). As noted in Figure 5, BMI had a significant association in both the IR and IS groups; the average z score of the H-IR group was significantly greater than that in the T-IR group, and the average z score of H-IS was significantly greater than T-IS. In contrast, MLBM had a significant effect on the heavy but not the thin subjects. Thus, the average z score of H-IR was significantly greater than that of H-IS, but the average z scores of the T-IR and T-IS groups were not significantly different, despite a somewhat higher value in the T-IR group. These findings constituted a significant statistical interaction between BMI and MLBM; ie, the average z score of H-IR increased to a greater degree than would be expected by adding together the separate effects of high BMI and low MLBM. The results among groups, as shown in Figure 5, were the same when males, females, blacks, or whites were analyzed separately.

In a separate analysis, the composition of T-IS, T-IR, H-IS, and H-IR groups was changed by including only individuals at the greater extremes of the BMI and MLBM distribution, as follows: T-IS=lowest 25th percentile BMI and highest 25th percentile MLBM; T-IR=lowest 25th percentile BMI and lowest 25th percentile MLBM; H-IS=highest 25% BMI and highest 25% MLBM, and H-IR= highest 25th percentile BMI and lowest 25th percentile MLBM. Differences for the risk factors and cluster z scores among these more restricted groups were in the same direction but stronger, and the significance among the groups was the same as described earlier, when the cohort was divided according to median distribution of BMI and MLBM.
Data for CRP, IL-6, TNF-α, adiponectin, and 8-iso-PGF₂α are presented in Table 2. Levels for CRP increased steadily from the T-IS group to the H-IR group, with significantly greater CRP in the 2 groups (H-IS and H-IR) with above-median BMI. Adiponectin was significantly lower in the 2 above-median BMI groups, with the lowest level found in H-IR. Levels of 8-iso-PGF₂α were significantly higher in the 2 above-median BMI groups, and a significant interaction between BMI and MLBM was noted. There were no significant differences among the groups for IL-6 and TNF-α, although the highest level of IL-6 was found in the H-IR group.

**Discussion**

Previous reports for children have described a direct relation between obesity and insulin resistance and the role of visceral fat. The clinical importance of this relation has been confirmed in studies showing the high incidence of abnormal glucose tolerance in obese adolescents. Comparisons among white, black, and Hispanic children have shown that the obesity–insulin resistance relation is present in all 3 groups, but black children are more insulin resistant and hyperinsulinemic, and Hispanic children have higher degrees of insulin secretion than do whites.

The present study extends these findings to show that insulin resistance, particularly jointly with fatness, has a significant role in adolescence in the development of cardiovascular risk factors associated with the MS. By recruiting a cohort of both thin and heavy subjects with a broad range of insulin resistance, it has been possible not only to confirm the primary risk associated with fatness but also to identify an interaction between fatness and insulin resistance, such that the level of risk in heavy, insulin-resistance adolescents is greater than that of heavy, non–insulin-resistant adolescents and greater than that expected from the sum of the individual effects of fatness and insulin resistance.

Higher levels of fasting insulin, lipids, and blood pressure were found in the heavier adolescents, ie, those with BMI above the median BMI. The relation of fatness to insulin resistance is well established. Although the mediators of fatness-related cardiovascular risk have not been precisely identified, a common pathway via hyperinsulinemia has been suggested. Alterations in insulin signaling and glucose metabolism have been reported in obese individuals, and it has been suggested that the subsequent adverse effect of insulin resistance is mediated via a hyperglycemia-stimulated increase in insulin secretion. However, attention also has focused on the secretory function of adipose tissue and, in particular, the influence of visceral adiposity in insulin resistance. Visceral adipose tissue releases cytokines (eg, IL-6, TNF-α, and adiponectin). The proinflammatory cytokines (IL-6 and TNF-α) decrease insulin receptor signaling and increase insulin resistance. In contrast, adiponectin has an inverse relation to obesity, inflammation, and insulin resistance. Fatty acids released from visceral adipose tissue appear to stimulate the production of reactive oxygen species (oxidative stress), which are associated with a reduction in insulin-stimulated glucose transport and target-organ damage, such as that related to diabetes and atherosclerotic cardiovascular disease.

**TABLE 2. CRP, Cytokines, and Oxidative Stress According to BMI/MLBM Medians**

<table>
<thead>
<tr>
<th></th>
<th>T-IS (n=79)</th>
<th>T-IR (n=75)</th>
<th>H-IS (n=76)</th>
<th>H-IR (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, ng/mL*</td>
<td>0.78±0.16</td>
<td>1.00±0.17</td>
<td>1.21±0.16</td>
<td>1.34±0.16</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>2.73±0.26</td>
<td>2.86±0.27</td>
<td>2.69±0.27</td>
<td>3.16±0.26</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>5.90±0.83</td>
<td>4.58±0.86</td>
<td>5.84±0.85</td>
<td>6.56±0.83</td>
</tr>
<tr>
<td>Adiponectin, μg/mL*</td>
<td>15.2±6.2</td>
<td>15.0±6.3</td>
<td>13.4±6.3</td>
<td>11.7±6.1</td>
</tr>
<tr>
<td>8-iso-PGF₂α, nmol/mmol creatinine†‡</td>
<td>0.34±0.02</td>
<td>0.34±0.02</td>
<td>0.36±0.02</td>
<td>0.44±0.02</td>
</tr>
</tbody>
</table>

*Significant difference based on BMI.
†Significant difference based on M_LBM.
‡Significant interaction between BMI and M_LBM.
increase in CRP in association with both atherosclerotic cardiovascular disease and insulin resistance.\textsuperscript{41,42} Fewer data are available from studies of children. Positive correlations for CRP with blood pressure and lipids have been shown in univariate analyses,\textsuperscript{43} but in multivariate analyses, BMI is the only factor that remains statistically significant.\textsuperscript{44} In a large study focused on obesity and the MS in children and adolescents, CRP and IL-6 were significantly related to the degree of obesity but not to insulin resistance (as estimated by the homeostasis model assessment index method), and adiponectin was significantly related to both obesity and the homeostasis model assessment index.\textsuperscript{5} The present study also shows significant relations between CRP and adiponectin (reverse) with BMI but in addition shows a trend toward values associated with higher risk with increasing insulin resistance. IL-6 was not significantly related to either BMI or insulin resistance, but there also appeared to be a trend toward higher risk for the H-IR group. We are not aware of previously published data on oxidative stress. In the present study, oxidative stress was significantly related to BMI, and there was a significant interaction between BMI and insulin resistance.

Considerable attention has been given to the “epidemic of obesity” in childhood and adolescence. The incidence of type 2 diabetes is rising steadily in childhood and adolescence,\textsuperscript{3} and autopsy studies of individuals dying during their first 2 decades show a correlation between coronary artery lesions and risk factors (overweight, lipids, blood pressure).\textsuperscript{45} Obesity and its associated risk factors track from childhood into adulthood,\textsuperscript{46} and overweight in adolescence is related to coronary artery disease in adults.\textsuperscript{47} Therefore, understanding the early relations among overweight, insulin resistance, and cardiovascular risk would appear to be an important first step in developing intervention/prevention strategies.

In contrast, the role of insulin resistance in the development of cardiovascular risk is less well defined. In the present study, fasting insulin and triglycerides were significantly greater and HDL-C significantly lower in association with insulin resistance (ie, children with M\textsubscript{ABG} below the median M\textsubscript{ABG}), despite a BMI similar to that of insulin-sensitive children. Moreover, this same pattern of increased risk was seen in the T-IR group compared with the T-IS group, although the differences were not statistically significant. The initiating role of insulin resistance in type 2 diabetes has been reviewed in adults,\textsuperscript{8} but we are not aware of similar reports in nonobese subjects before adulthood.

The prognosis for the T-IR group is also of great interest. It seems reasonable that there would be a range of normal insulin sensitivity in humans, corresponding to a range of biological variability in cellular insulin action and glucose metabolism,\textsuperscript{48} and that insulin resistance in some individuals is not necessarily the hallmark of cardiovascular risk. However, type 2 diabetes develops in nonobese subjects.\textsuperscript{14} Therefore, it will be important to follow their developmental history.

The data from this study do not provide a specific mechanism for the interaction of insulin resistance with increased fat mass. The actions of each on the development of cardiovascular risk are complex, but there are a number of areas (eg, visceral adipose release of cytokines and fatty acids and their effect on insulin release and insulin resistance; insulin resistance effects on insulin release and adipose cells; relations of both to oxidative stress) that demonstrate an overlap and suggest the probability of an interaction. However, it seems unlikely that there would be overlap between all the actions associated with fatness or insulin resistance, so that the adverse processes could be synergistically enhanced in individuals unfortunate to have both conditions.

In summary, this study found a significant association for insulin resistance and a significant interaction between fatness and insulin resistance on the development of cardiovascular risk during adolescence. These data suggest that, in addition to current strategies directed at obesity, prevention of CVD may ultimately include strategies directed at a reduction in insulin resistance.

Acknowledgments

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