Changes in Insulin Resistance and Cardiovascular Risk During Adolescence

Establishment of Differential Risk in Males and Females

Antoinette Moran, MD; David R. Jacobs, Jr, PhD; Julia Steinberger, MD, MS; Lyn M. Steffen, PhD; James S. Pankow, PhD; Ching-Ping Hong, MS; Alan R. Sinaiko, MD

Background—Developmental changes in insulin resistance and cardiovascular risk were studied in youths 11 to 19 years of age.

Methods and Results—A cohort was randomly selected after blood pressure screening of Minneapolis, Minn, school children. Studies were done 3 times on this cohort and once on their siblings (996 observations on 507 individuals from 363 families). Insulin sensitivity was determined by euglycemic clamp. Body mass index and waist circumference increased similarly in both sexes from ages 11 to 19 years. Body fat decreased in males and increased in females ($P<0.001$). Lean body mass increased at a steeper rate in males ($P<0.0001$). Insulin resistance was lower in males at 11 years but increased steadily to 19 years ($P=0.003$); in contrast, it did not increase in females. Thus, despite being less insulin resistant at 11 years and decreasing in fatness during puberty, males became more insulin resistant than females by 19 years of age. Triglycerides increased in males and high-density lipoprotein cholesterol decreased, whereas the opposite pattern was seen in females, which resulted in higher triglycerides and lower high-density lipoprotein cholesterol in males at 19 years. No gender difference in low-density lipoprotein or total cholesterol was seen. Systolic blood pressure increased in both sexes but at a greater rate in boys ($P=0.03$).

Conclusions—During the transition from late childhood through adolescence, insulin resistance in males increased in association with increased triglycerides and decreased high-density lipoprotein cholesterol, despite a concurrent reduction in body fatness, whereas the opposite occurred in females. These gender-related developmental changes in insulin resistance, which were independent from changes in fatness, total cholesterol, and low-density lipoprotein cholesterol, are consistent with an early role for insulin resistance in the increased cardiovascular risk found in males.

Key Words: insulin resistance ■ risk factors ■ youth ■ sex ■ metabolic syndrome

I
nterest in the metabolic syndrome and long-term cardiovascular risk in children and adolescents has increased over the past decade because of recognition of increasing childhood prevalence of the full syndrome and increasing levels in children of the individual risk factors that make up the syndrome.1–3 Adverse childhood levels of risk factors have been related to development of target-organ disease in adults.4 During the first 2 decades of life, insulin resistance and metabolic syndrome factors are influenced by significant developmental changes mediated by puberty and growth. Thus, studying changes in insulin resistance and its relation to measures of body composition and other metabolic syndrome factors during adolescence may help clarify the causative relations among insulin resistance, body fatness, and the development of cardiovascular risk.
The initial cohort was selected randomly after blood pressure screening of 12,043 fifth- to eighth-grade public school children in Minneapolis, Minn (93% of eligible children in those grades), with stratification according to sex, race (black and non-Hispanic white), 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) as described previously. Of 2915 children receiving a recruitment letter, 537 attended an information meeting (groups of 20 to 30) with their parents, 401 provided informed consent, and 357 completed an initial clinic visit. The screening blood pressure, height, and weight of these participants did not differ significantly from the children who chose not to participate. The original cohort of 401 participants was reexamined twice, with a target reexamination interval of 2 years between visits 1 and 2 and at age 18 to 19 years for visit 3. After enrollment of the initial cohort, siblings were recruited. Of 266 siblings who were 11 to 19 years old, clinic visits and insulin clamps were completed on 150. There were 13 pregnancies; no data were obtained during pregnancy or in the postpartum period.

Participants were seen in a dedicated clinic. Blood pressure was measured with a random-zero sphygmomanometer on the right arm of seated subjects, and the average of 2 SBP and fifth-phase Korotkoff diastolic blood pressure (DBP) measurements were analyzed. The euglycemic insulin clamp and other laboratory studies were conducted 3 times on the initial cohort and once on their siblings in the University of Minnesota Clinical Research Center, as described previously. Insulin was infused at a rate of 1 U·kg⁻¹·min⁻¹ for 3 hours. A variable amount of glucose was added to maintain plasma glucose at 100 mg/dL (5.6 mmol/L). Insulin sensitivity, M, was determined from the amount of glucose required to maintain euglycemia over the final 40 minutes of the clamp, corrected for lean body mass (LBM) and expressed as MLBM (milligrams of glucose per kilogram of LBM per minute). A lower MLBM value represents greater insulin resistance. LBM was calculated by the method of Slaughter et al for data collected at the first and second cohort clamps and by dual-photon x-ray absorptiometry for the third cohort clamp and the sibling clamps. The Slaughter estimates were adjusted to the dual-photon x-ray absorptiometry estimates on the basis of formulas from our previously published studies in 140 children 11 to 17 years of age who showed high correlations for percent body fat (%BF) and fat body mass between the Slaughter estimates and dual-photon x-ray absorptiometry, as follows: boys (n=72), %BF r=0.93, fat body mass r=0.96; girls (n=68), %BF r=0.92, fat body mass r=0.98 (all P<0.0001).

Plasma glucose was measured immediately at the bedside with a Beckman glucose analyzer II (Beckman Instruments Inc, Fullerton, Calif). Fasting plasma insulin levels were obtained at baseline (15, 10, and 5 minutes before the insulin infusion was begun) and averaged. Analyses were conducted in the University of Minnesota Fairview laboratory by radioimmunoassay (Diagnostic Products Corp, Los Angeles, Calif) for the first and second cohort clamps. The laboratory then changed the method for insulin measurements to a chemiluminescence solid-phase immunometric assay (Immulite, Diagnostic Products Corp), and this method was used for the third cohort clamp and the sibling clamp studies. After analysis of 26 samples with radioimmunoassay insulin <80 mU/L measured both ways, insulin values were recalibrated from the radioimmunoassay to the Immulite method, as follows: Immulite insulin value=(−2.195+0.9349)×radioimmunoassay insulin value (r=0.98, P<0.0001). Serum lipids were analyzed as described previously; low-density lipoprotein cholesterol was estimated by the Friedewald equation.

Data Analysis
We analyzed linear and year-by-year age trends in each dependent variable separately for boys and girls using SAS PROC MIXED to account for the correlated observations. The observations were correlated in 2 ways. There were up to 3 repeated measures in original study participants (tracking correlations), and family membership induced lesser correlations among siblings (excluding 4 original participants who were siblings of other original participants). The model included terms for sex, race, sibship (an indicator for sib or original proband), visit number, current age at observation, and the products of each of sex, race, and sibship with current age. Further adjustment for whether or not the original participant was selected for high SBP did not alter the findings (data not shown). The linear slopes with increasing age were derived from this model with current age as a continuous variable. Because none of the sibship-by-age interaction terms were significant, it was appropriate to pool the age slopes across original participants and their siblings. Findings were more variable, but patterns were consistent with those reported here when analyses were restricted to the 193 original participants who underwent all 3 clamp studies. Adjusted mean levels of the dependent variable at each examination were derived from a similar model in which year of age was treated as a categorical variable and the race-by-age and sibship-by-age terms were dropped. The age-year–by-age-year means serve as an indication of goodness of fit of the linear slopes. The term for visit number in these models was categorical and removed systematic measurement differences (matching on age) between examinations.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
Subjects
After missing data were removed in 10 clamp studies, the ages and number of clamps performed on the initial cohort were as follows: age 11 to 15 years (visit 1) n=350; age 13 to 17 years (visit 2) n=303; and age 18 to 19 years (visit 3) n=193. Clamps were completed once on each of 150 siblings, 11 to 19 years of age, for a total of 996 data measurements from 507 individuals in 363 families that form the cohort for the present study (Table 1). A total of 57% of observations were in males, whereas 43% were in females, and racial composition was 80% white and 20% black. Girls entered puberty earlier than boys, but by age 18 years, all subjects were sexually mature at Tanner stage 5 (Table 1). Changes over age in body composition, insulin resistance, lipid levels, and blood pressure are shown in Table 2 and described below.

Body Composition
Between ages 11 and 19 years, body mass index (BMI) increased in both sexes (each P<0.0001), with no significant difference in BMI or in the rate of increase in BMI over time between sexes (P=0.44). Waist circumference increased in both sexes (P<0.0001), and the rate of increase did not differ between males and females (P=0.16). Despite similar changes in BMI, very different sex-associated changes in body composition occurred during the adolescent years (Figure 1). Both %BF and LBDM increased at a modest but significant rate in females (P<0.0001). LBDM increased at a steeper rate in males (P<0.0001), and, in contrast to females, %BF decreased (P<0.0001). Height reached a plateau at approximately age 13 years in females and age 16 to 17 years in males.

Insulin Resistance and Fasting Insulin Levels
Mean MLBM was significantly greater in males than in females at age 11 years (Figure 2); however, MLBM began to decrease in early adolescence, stabilized in mid adolescence, and then continued to significantly decrease into young adulthood (P=0.003). In contrast, after an early decrease in females,
M_{LBM} recovered to early adolescent levels, where it remained, and it was significantly greater than in males by late adolescence. Thus, the sex differences in insulin sensitivity were reversed during adolescence, and by age 19 years, females had greater insulin sensitivity than males. Comparable results were also seen when data were analyzed only for the initial participants without inclusion of siblings.

M_{BW} (BW indicates body weight, not adjusted for LBM) showed a similar pattern as M_{LBM}. M_{BW} decreased significantly between age 11 and 19 years in males (P=0.01) and did not change between age 11 and 19 in females (P=0.47); these patterns were significantly different between the sexes (P=0.002). However, at age 19 years, M_{BW} was not significantly different between the sexes.

Fasting insulin showed little change in males from 11 to 19 years (P=0.93). Although fasting insulin was significantly higher in females than males at age 11, it decreased from 11 to 19 years (P=0.06), which resulted in a mean level that was not significantly different from males at age 19. The homeostasis model assessment (HOMA; fasting insulin × fasting glucose/22.5) followed the same pattern as fasting insulin (data not shown).

Table 2. Rates of Change per Year of Age (Linear Slope) in Study Variables Between Age 11 and 19 Years Adjusted for Age, Race, and Sex

<table>
<thead>
<tr>
<th>Variable</th>
<th>Female P</th>
<th>Male P</th>
<th>P (Between Sexes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>0.68±0.11 (&lt;0.0001)</td>
<td>0.72±0.10 (&lt;0.0001)</td>
<td>0.44</td>
</tr>
<tr>
<td>%BF</td>
<td>0.69±0.26 (&lt;0.0001)</td>
<td>-0.42±0.25 (&lt;0.0001)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>1.23±0.18 (&lt;0.0001)</td>
<td>3.64±0.17 (&lt;0.0001)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>1.03±0.24 (&lt;0.0001)</td>
<td>1.29±0.23 (&lt;0.0001)</td>
<td>0.16</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>-0.23±0.18 (0.18)</td>
<td>-0.11±0.16 (0.46)</td>
<td>0.52</td>
</tr>
<tr>
<td>M_{LBM}, mg · kg LBM⁻¹ · min⁻¹</td>
<td>0.12±0.11 (0.29)</td>
<td>-0.31±0.10 (0.003)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>-0.46±0.24 (0.06)</td>
<td>-0.02±0.23 (0.93)</td>
<td>0.05</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>-4.09±1.38 (0.003)</td>
<td>1.45±1.31 (0.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>0.51±0.23 (0.02)</td>
<td>-0.43±0.22 (0.05)</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>0.51±0.18 (0.01)</td>
<td>0.41±0.18 (0.02)</td>
<td>0.80</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL (.linear coefficient)</td>
<td>0.01±0.54 (0.99)</td>
<td>-0.63±0.50 (0.21)</td>
<td>0.19</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL (quadratic coefficient)</td>
<td>-0.02±0.62 (0.98)</td>
<td>-0.36±0.58 (0.53)</td>
<td>0.54</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL (linear coefficient)</td>
<td>0.69±0.21 (0.001)</td>
<td>0.50±0.20 (0.01)</td>
<td>0.48</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>1.20±0.36 (0.001)</td>
<td>1.66±0.34 (&lt;0.0001)</td>
<td>0.17</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>0.53±0.23 (0.02)</td>
<td>0.94±0.22 (&lt;0.0001)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein. The P value within each sex is noted parenthetically after the mean±SEM. The P value for the difference in slopes between genders is noted in the last column. Findings were similar when only the probands (no siblings) were evaluated. For LDL and total cholesterol, the linear coefficient and P value are from regression analysis that ignores the teenage dip and considers only general tendency to change upward or downward. The quadratic coefficient and P value are from regression analysis that also includes a linear term (not shown) and shows that the amount of curvature is not significantly different between boys and girls.
Blood Pressure and Lipid Levels and Smoking

Lipid levels and blood pressure were not significantly different between the sexes at age 11 years (Figure 3). Low-density lipoprotein and total cholesterol followed similar U-shaped patterns in both sexes (Table 2). High-density lipoprotein cholesterol decreased in males ($P=0.05$) and increased in females ($P=0.02$). In contrast, triglycerides increased in males ($P=0.03$) but decreased in females ($P=0.003$). SBP increased significantly in both sexes but at a greater rate in boys (sex interaction $P=0.03$). DBP increased similarly in both sexes (sex interaction $P=0.17$). Smoking data were only available for the present cohort at age 17 to 19 years, at which time 27% of youths and their siblings smoked regularly (averaging $≈10$ cigarettes per day), with no sex difference ($P=0.72$).

Results by Median Baseline BMI and Racial Differences

The male and female cohorts were divided by median baseline BMI and the analyses repeated. Comparable patterns were seen in heavy and thin youths. The trends in the variables between 11 and 19 years for both groups were similar to those seen in the respective sexes for the entire cohort, and there were no significant differences in the slopes between the groups above or below median BMI.

There were few significant differences between black and white participants. $M_{LBM}$ decreased significantly in blacks but not whites ($−0.40$ versus $−0.02$ mg per kg $\text{lbm} \cdot \text{min}^{-1} \cdot \text{y}^{-1}$, $P=0.003$), and high-density lipoprotein cholesterol decreased at a significantly steeper rate in blacks ($−0.32$ versus $0.19$ mg $\cdot \text{dL}^{-1} \cdot \text{y}^{-1}$, $P=0.01$). There was no significant racial difference in BMI, $\%\text{BF}$, LBM, fasting insulin, triglycerides, or SBP. No significant differences were noted in sex-by-race comparisons, but the number of blacks in each sex limited the statistical power for meaningful conclusions.

Discussion

Previous studies in children and adolescents have tended to focus on the cross-sectional relation of insulin resistance to obesity and other cardiovascular risk factors. The present report provides information on the change in insulin resistance during the transition from late childhood through adolescence, presenting it in the context of normal developmental changes in body size and fatness and in relation to changes in other risk factors. The results show 2 new findings that are highly relevant to the development of cardiovascular
risk. First, a relatively rapid increase in insulin resistance was seen in males during adolescence despite a decrease in %BF, with an opposite pattern occurring in females. Although girls and boys had similar body composition at age 11 years and similar BMI throughout the teenage years, by age 19, males were more insulin resistant, even though they had greater LBM and less adiposity than females. Second, an overall adverse change in lipid levels and blood pressure was found in males, which, in association with the changes in insulin resistance, established significantly greater cardiovascular risk by age 19 than for females. No sex difference in cardiovascular risk was present at the onset of adolescence, but significantly higher SBP and triglycerides and lower high-density lipoprotein cholesterol were present in males by age 19. In contrast, low-density lipoprotein and total cholesterol, traditional measures of cardiovascular risk in adults, had not yet emerged as risk factors in males in the present youth cohort. Thus, a focus on traditional measures to the exclusion of factors more typically associated with the metabolic syndrome may overlook at-risk children and adolescents. Smoking, another important risk factor, was not studied in the present cohort until age 17 to 19 years, at which time no sex difference was apparent.

Obesity is well known to be associated with insulin resistance in both adults and children. Several mechanisms have been proposed to explain this, including impaired insulin signaling, interference with glucose transport, decreased insulin clearance related to elevated intraportal free fatty acids, and systemic effects of adipocyte cytokines. Although these factors may be operative in the development of insulin resistance, the present study shows that they or other mechanisms act, in part, independent of obesity, because insulin resistance during adolescence did not follow the expected course in relation to developmental changes in adiposity.

The changes in insulin resistance during adolescence also appear to be independent of puberty. Early puberty in both sexes is associated with insulin resistance. This occurs earlier in girls than boys because girls enter puberty earlier. Our previous study in the present cohort at age 11 to 14 years showed that insulin sensitivity recovers in girls and boys by Tanner stage 5. However, as noted in the present study, which extends the cohort evaluation to age 19 years, after a period of stabilization in mid adolescence, insulin resistance increases in males. Although this may be related to some ongoing pubertal changes in males, the fact that MLBM continues to fall despite virtual completion of puberty by age 18 years suggests that other mechanisms are involved.

During puberty, boys gain more LBM than girls, whereas fat mass increases in girls and decreases in boys. The result is a change from a childhood body composition that is similar between the sexes to an adult-type pattern in which, for a given BMI, there is increased lean mass in males and greater adiposity in females. This normal physiological differentiation in body habitus is largely due to sex hormones, which also influence fat distribution such that young women primarily accumulate peripheral (gluteal and subcutaneous) rather than central (intra-abdominal) fat. Despite the decrease in %BF in males in the present cohort, it could be suggested that the increase in insulin resistance was related to greater accumulation of abdominal fat in males than in females; however, waist circumference increased at a similar rate in both sexes. Thus, developmental changes in insulin resistance during adolescence appear to be independent of normal maturational changes in adiposity, although when obesity occurs, it may overwhelm these natural relations, because excessive accumulation of adipose tissue in either sex is not physiological and is associated with insulin resistance. There was no sex difference in insulin sensitivity at age 19 years when total body glucose uptake was not

Figure 3. Changes in lipid levels and blood pressure in boys and girls from age 11 to 19 years. Plotted in each figure are means and SEs estimated in the repeated-measures model described in Methods. HDL-C indicates high-density lipoprotein cholesterol; Total C, total cholesterol; and LDL-C, low-density lipoprotein cholesterol.
normalized for LBM; this adjustment was performed because skeletal muscle is the primary site of the metabolic activity of insulin.

Other cross-sectional and longitudinal studies in the present cohort and cross-sectional studies in other youth cohorts have suggested an independent effect for insulin resistance on development of cardiovascular risk. The sex-associated differences in insulin resistance shown in the present study to emerge during the adolescent years parallel changes in cardiovascular risk and suggest an association in the early establishment of increased risk in males. Before puberty, boys and girls have similar lipid profiles and blood pressure, whereas in adulthood, men have higher SBP, total cholesterol, and low-density lipoprotein cholesterol than women. The incidence of coronary artery disease is 3 to 4 times higher in adult men, and there is a male predominance in the incidence of myocardial infarction. The degree of coronary atherosclerosis in women at autopsy lags behind men by several years: A slight increase in advanced lesions is already seen in young men compared with women by late adolescence (2% versus none), whereas by 30 to 34 years of age, 20% of men compared with only 8% of women have advanced lesions. Similar to the present findings in youth at the end of adolescence, the few clamp studies available in adults have tended to show greater insulin resistance in white and black men than in women. The exception is a second study in blacks from investigators who showed greater insulin resistance in black females, which disappeared when glucose uptake was corrected for body fat.

No single risk factor or combination of factors fully explains reduced cardiovascular risk in women. Although the present study does not have data on hormonal changes, the finding that female sex exerts a positive influence on insulin sensitivity, lipids, and blood pressure despite a seemingly paradoxical increase in adiposity, combined with the fact that risk protection in women is known to disappear after menopause, strongly suggests that it is mediated directly or indirectly by female sex hormones.

Estrogen exerts many physiological effects that might influence cardiovascular risk and insulin resistance. It has both antiinflammatory and antioxidant properties, and it directly upregulates expression of antioxidant genes. Estrogen protects the pancreatic β-cell from glucolipotoxicity, oxidative stress, and apoptosis. It enhances insulin sensitivity by decreasing hepatic glucose production and increasing muscle glucose transporter content. It stimulates lipolysis in the abdominal fat depots and promotes use of lipid as a fuel by muscle, the net effect of which is a reduction in central adiposity.

There are situations, however, when estrogen has been associated with insulin resistance in women. The effect of hormone replacement therapy in postmenopausal women is controversial, with some investigators reporting improved insulin sensitivity and others reporting insulin resistance. Pregnancy, a high estrogen state, is characterized by insulin resistance. Interpretation of these data may be complicated by the fact that hormone replacement therapy studies have generally involved nonhuman estrogen preparations, and pregnancy is influenced by other complex hormonal interac-

Sources of Funding

This project was supported by National Institutes of Health grants HL-52851 and M01-RR-00400 (General Clinic Research Centers).

Disclosures

None.
References


CLINICAL PERSPECTIVE

The present study assessed changes in insulin resistance and overall cardiovascular risk during the transition from late childhood through adolescence (ie, age 11 to 19 years). During these years, boys decreased their percentage of body fat and become more lean and muscular, whereas girls gained body fat. Insulin resistance at age 11 years was significantly lower in boys but increased during adolescence, so that by age 19, boys were more insulin resistant than girls. Over the same period, triglycerides increased and high-density lipoprotein cholesterol decreased in males, whereas the opposite pattern was seen in females, and systolic blood pressure increased at a greater rate in males. Total cholesterol and low-density lipoprotein cholesterol changes did not differ between the sexes. Thus, adolescence in males was associated with increased insulin resistance despite decreased adiposity, as well as an increase in cardiovascular risk. Because this was independent of adverse changes in low-density lipoprotein and total cholesterol, it suggests that early development of risk is related to insulin resistance. Although the cause of these sex-related changes is not clear, it is possible that the increased cardiovascular risk that emerges in males during normal adolescence is related to hormonal differences between the sexes.
Changes in Insulin Resistance and Cardiovascular Risk During Adolescence: Establishment of Differential Risk in Males and Females
Antoinette Moran, David R. Jacobs, Jr, Julia Steinberger, Lyn M. Steffen, James S. Pankow, Ching-Ping Hong and Alan R. Sinaiko

*Circulation.* 2008;117:2361-2368; originally published online April 21, 2008; doi: 10.1161/CIRCULATIONAHA.107.704569

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/18/2361

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/