Effect of insulin on glycerol production in obese adolescents

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Departments of Pediatrics and Internal Medicine, and the Yale Children's General Clinical Research Center, Yale University School of Medicine, New Haven, Connecticut 06520; and the Department of Vascular Surgery, Huddinge University Hospital, Karolinska Institute, S-105 21 Stockholm, Sweden

Robinson, Childsy, William V. Tamborlane, David G. Maggs, Staffan Enoksson, Robert S. Sherwin, David Silver, Gerald I. Shulman, and Sonia Caprio. Effect of insulin on glycerol production in obese adolescents. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E737–E743, 1998.—Impaired stimulation of glucose metabolism and reduced suppression of lipolytic activity have both been suggested as important defects related to the insulin resistance of adolescent obesity. To further explore the relationship between these abnormalities, we studied seven obese [body mass index (BMI) 35 ± 2 kg/m²] and seven lean [BMI 21 ± 1 kg/m²] adolescents aged 13–15 yr and compared them with nine lean adults (aged 21–27 yr, BMI 23 ± 1 kg/m²) during a two-step euglycemic-hyperinsulinemic clamp in combination with 1) a constant [2H5]glycerol (1.2 mg·m⁻²·min⁻¹) infusion to quantify glycerol turnover and 2) indirect calorimetry to estimate glucose and net lipid oxidation rates. In absolute terms, basal glycerol turnover was increased and suppression by insulin was impaired in obese adolescents compared with both groups of lean subjects (P < 0.01). However, when the rates of glycerol turnover were adjusted for differences in body fat mass, the rates were similar in all three groups. Basal plasma free fatty acid (FFA) concentrations were significantly elevated, and the suppression by physiological increments in plasma insulin was impaired in obese adolescents compared with lean adults (P < 0.05). In parallel with the high circulating FFA levels, net lipid oxidation in the basal state and during the clamp was also elevated in the obese group compared with lean adults. Net lipid oxidation was inversely correlated with glucose oxidation (r = −0.50, P < 0.01). In conclusion, these data suggest that lipolysis is increased in obese adolescents (vs. lean adolescents and adults) as a consequence of an enlarged adipose mass rather than altered sensitivity of adipocytes to the suppressing action of insulin.

glycerol turnover; lipid oxidation; glucose metabolism

The prevalence of obesity is steadily increasing in adolescents (15) and, according to the National Health and Nutrition Examination Survey (NHANES III), ~20% of adolescent males and females are obese (32). Most obese adolescents will remain obese adults with an increased risk of metabolic and cardiovascular complications, which may be related to the central deposition of fat that occurs in adolescence (25). In view of these facts, understanding the early metabolic defects and the antecedents of obesity-related complications in obese adolescents is of paramount interest. Most studies on the interactions between glucose and lipid metabolism have been performed in obese adults, most of whom have had long-lasting obesity (8, 16, 19); therefore, any metabolic defect detected in adult obesity may represent an adaptation to the long-term obesity rather than being causally related. On the other hand, childhood obesity and adolescent obesity represent ideal models that offer the unique opportunity to elucidate early metabolic defects occurring at the time of excessive fat accumulation. Recent studies from our laboratory (11) that have examined in vivo insulin action in adolescent girls suggest that increased visceral fat, hyperinsulinemia, and insulin resistance are closely linked abnormalities that are expressed early in the natural history of obesity. Similar defects were found also in preadolescent obese children (9). Such insulin resistance does not appear to be confined to the glucose regulatory actions of insulin, because elevated fasting plasma free fatty acid (FFA) levels have also been reported in obese and nonobese adolescents despite hyperinsulinemia (9, 11). Indeed, the ability of insulin to stimulate glucose oxidation is, in part, indirectly mediated by its capacity to suppress FFA derived from adipose and possibly intramuscular triglyceride stores (1, 2, 4, 5). More recently, Boden and colleagues (5, 6) and Kelley et al. (21) have shown that fat infusion abolished the ability of insulin to stimulate glycogen synthase activity in muscle and thus could explain the impairment in nonoxidative glucose metabolism seen in obesity. A further study, by Roden et al. (31), suggests that increased concentrations of plasma FFA induce insulin resistance through inhibition of glucose transport/phosphorylation activity.

Despite the important interactions between glucose and FFA metabolism, it is still unclear how lipolysis is altered in obesity during adolescence, a developmental stage that appears to be of crucial importance in adipose tissue cellular proliferation (22). In vivo studies that have examined the effect of insulin on FFA levels and turnover in obese adults with long-lasting obesity concluded that the increased plasma FFA concentration and FFA turnover were the consequence of the enlarged fat mass rather than impaired insulin sensitivity of the adipocyte (8, 16). FFA turnover, however, may not necessarily be a reliable indicator of lipolysis, because FFA can be reesterified within adipose tissue after triglyceride hydrolysis (27). On the other hand, glycerol liberated as a result of lipolysis cannot be reincorporated into triglyceride, because adipose tissue lacks glycerol kinase (2, 16). Release of glycerol into
plasma should therefore be a more reliable index of lipolysis than plasma FFA. In this study, we used \[^{[2H_5]}\text{glycerol}\] kinetics to determine whether there is increased lipolysis in obese adolescents compared with lean adolescents and adults. Differences in rates of lipolysis were analyzed with and without adjustments for variations in total body fat mass.

**METHODS**

Subjects. We studied three groups of subjects: seven obese adolescents [mean age 13.6 ± 0.6 yr, mean body mass index (BMI) 35 ± 2 kg/m²], seven lean adolescents (age 13.1 ± 0.4 yr, BMI 20.5 ± 1.1 kg/m²), and nine lean adults (age 23.8 ± 1.2 yr, BMI 23 ± 1 kg/m²) (Table 1). All obese adolescents had a BMI >95th percentile specific for age and sex (based on percentile curves for girls and boys computed from the first NHANES, 1971 to 1974) (17). All seven obese adolescents had at least one obese parent (BMI > 30), and one subject had a parent with type II diabetes and morbid obesity. Tanner stage of pubertal development was assessed by physical exam in the adolescent subjects; the stages ranged between II and IV for pubic hair, breast, or genital development. Plasma estradiol, testosterone, and insulin-like growth factor I levels were obtained and used as additional markers of puberty. All subjects were healthy and taking no medications. The protocol was approved by the Human Investigation Committee of the Yale School of Medicine, and informed written consent was obtained from all subjects and the parents of the adolescent subjects. Subjects were seen on two separate visits for measurement of fat mass and 2) euglycemic-hyperinsulinemic clamp.

Measurement of fat mass. Bioelectrical impedance analysis was used to estimate fat-free mass. At the time of measurement, subjects were instructed to fast for 3 h and to abstain from exercise for 12 h before measurement. The total body resistance and reactance were measured using a bioelectric impedance analyzer (RJ L 101A, Detroit, MI). Four surface self-adhesive electrodes were placed according to the procedures recommended by the manufacturer. A standard conduction current of 800 µA and 50 kH was used. The equations used for calculating the amount of fat-free mass were the Kushner equation for the adults (23) and the Hautkooper equation for the adolescents (20).

Euglycemic-hyperinsulinemic clamp. Subjects were admitted at 0800 to the Yale General Clinical Research Center for metabolic study. All studies were performed in a postabsorptive state after an overnight fast of 10–12 h with the subject lying supine in a quiet room, as described previously (9, 11). A retrograde cannula was inserted into a vein in the dorsum of the hand, which was positioned in a heated box for sampling of arterialized venous blood. A small volume of normal saline (0.9%) was infused through the intravenous cannula to maintain patency. A second intravenous catheter was inserted in a contralateral antecubital vein for infusion of insulin, glucose, and stable isotopes. At the start of a 2-h baseline period, \[^{[2H_5]}\text{glycerol}\] was infused at a constant rate of 1.2 mg·m⁻²·min⁻¹; this was continued for the duration of the study (6 h). After the baseline period, a two-step hyperinsulinemic clamp was initiated. Insulin was administered intravenously as a primed-continuous infusion at 8 and 40 mU·m⁻²·min⁻¹. Each step lasted for 2 h. Plasma glucose levels were kept constant (90 mg/dl) using a variable rate infusion of a 20% dextrose solution. Samples for the measurement of \[^{[2H_5]}\text{glycerol}\] enrichment, plasma glycerol, FFA, insulin, and C-peptide were collected at 10-min intervals for the last 30 min of the basal period and each step of the clamp. Indirect calorimetry using the ventilated hood technique (10) was also employed during the last 30 min of the basal period and of each step of the clamp to estimate net rates of carbohydrate and lipid oxidation.

Determinations. Plasma glucose levels were measured by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Brea, CA). Plasma insulin and C-peptide were measured by a double-antibody radioimmunoassay. Plasma nonesterified fatty acids (NEFA) were assayed by a colorimetric method (18) and urinary nitrogen by the Kjeldahl procedure (26). Plasma glycerol was measured by an automatic ultrasensitive bioluminescence kinetic assay (19).

Blood for analysis of \[^{[2H_5]}\text{glycerol}\] enrichment was collected in heparinized tubes and immediately placed in a 4°C ice bath. The plasma was then separated by centrifugation and stored at −70°C until time of analysis. Plasma proteins were precipitated with Ba(OH)₂ and ZnSO₄, and the resultant supernatant was passed through anion (Dowex AG1-X8) and cation (Dowex AG-50-X8) exchange columns. The trimethylsilyl derivative is formed and enrichment determined by a gas chromatography-mass spectrometry system (Hewlett-Packard 5985, Palo Alto, CA) described by Wolfe (33).

Calculations: glycerol kinetics. Glycerol rate of appearance (Ra) was calculated according to the steady-state equation (infusion rate divided by enrichment)

\[
Ra = \frac{IE_{inf}}{IE_{pla}} \times f
\]

where \(Ra\) is the rate of appearance of glycerol (µmol/kg), \(IE_{inf}\) is the isotopic enrichment of the infusion [in atoms % excess (APE)], \(IE_{pla}\) is the isotopic enrichment of plasma (APE) at isotopic equilibrium, and \(f\) is the isotope rate of infusion (µmol·kg⁻¹·min⁻¹). This calculation was made with the knowledge that glycerol enrichments reach a steady state in a relatively short time (34), and we observed stable plasma glycerol enrichments during the last 30 min of the baseline period and both insulin clamp steps; therefore, all calculated rates of glycerol turnover pertain to steady-state conditions. Whole body glycerol fluxes were adjusted for adipose tissue mass.

During the insulin clamp study, the amount of glucose required to maintain euglycemia provides an index of insulin-stimulated glucose metabolism. The glucose infusion rate (mg·m⁻²·min⁻¹) was calculated at 20-min intervals and corrected for deviations from the target plasma glucose level, as previously described (12). The average rate of glucose infusion during the last 30 min of each step of the clamp was used for comparison. Respiratory gas exchange rates were measured by a computerized open-circuit indirect calorimetry (DeltaTrac; Sensor Medics, Helsinki, Finland) with a ventilated hood system. Oxidation rates for carbohydrate, fat, and
protein before and during the clamp procedure were calculated from the measured \( O_2 \) consumption, \( CO_2 \) production, and urinary nitrogen excretion, as previously described (10).

Statistical analysis. All values are presented as means ± SE. Repeated-measures analysis of variance (ANOVA) was performed with a single factor to compare the responses of the different groups over time (Systat v.5.0; SPSS, Chicago, IL). One-sample ANOVA and two-group repeated-measures ANOVA tests and Tukey’s procedure for multiple comparisons were used to localize effects found in the initial set of repeated-measures analyses.

RESULTS

Plasma insulin, C-peptide, FFA, and glycerol concentrations. Fasting plasma insulin concentrations were higher in the obese adolescents than in either of the lean groups (\( P < 0.0001 \), Table 2). During the clamp, although absolute insulin levels were higher in the obese group at each time point, there was no significant difference in the relative change from baseline between any of the groups (Table 2). Basal C-peptide concentrations were also significantly higher in the obese adolescents compared with lean groups, and they did not change significantly during the low-dose insulin infusion in the obese group. In contrast, a substantial suppression of C-peptide was observed in both lean groups during the low-dose clamp. During the high-dose clamp, the change in C-peptide from baseline was significant in all three groups. Despite elevated plasma insulin levels, basal plasma FFA concentrations were significantly higher in obese adolescents than in lean adults (\( P < 0.005 \); Table 2). As shown in Table 3, during both the low- and high-dose exogenous insulin infusions, circulating plasma FFA concentrations were significantly elevated in obese adolescents compared with lean adults (\( P < 0.005 \)). Although obese adolescents tended to have higher plasma FFA than lean adolescents throughout the study, the difference was not statistically significant (\( F = 2.25, P = 0.159 \)). However, these seemingly similar levels occurred in the presence of much higher insulin levels in the obese group, indicating a shift to the right in the dose-response curve for insulin’s suppression of lipolysis in obese adolescents. FFA levels at baseline and during the clamp studies tended to be higher in lean adolescents than in lean adults; however, the difference was statistically significant only during the 40 mU insulin clamp (\( P < 0.01 \)). Basal and insulin-damped plasma glycerol levels were not statistically different among the three groups. The fall in plasma glycerol concentrations was significant during the low and high insulin infusions in all three groups (\( P < 0.02 \) vs. basal, Table 3).

Glyceral kinetics. The values for plasma \([\text{H}_5]\)glycerol enrichment in each group are reported in Table 3. As can be seen, a steady state of plasma enrichment was achieved in all three groups before and during each step of the insulin clamp, and there was no difference among the groups at any time during the study (\( F = 0.9, P = 0.424 \)).

Table 2. Plasma insulin and C-peptide levels during low- and high-insulin dose clamp studies

<table>
<thead>
<tr>
<th>Plasma insulin, pmol/l</th>
<th>Obese Adolescents</th>
<th>Lean Adolescents</th>
<th>Lean Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>117 ± 15*</td>
<td>61 ± 8†</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>8 mU·m⁻²·min⁻¹</td>
<td>192 ± 24 (78 ± 18)</td>
<td>108 ± 6 (48 ± 12)</td>
<td>96 ± 6 (60 ± 12)</td>
</tr>
<tr>
<td>40 mU·m⁻²·min⁻¹</td>
<td>552 ± 72 (438 ± 60)</td>
<td>378 ± 18 (318 ± 30)</td>
<td>372 ± 24 (336 ± 30)</td>
</tr>
<tr>
<td>Plasma C-peptide, pmol/l</td>
<td>879 ± 111*</td>
<td>466 ± 71†</td>
<td>361 ± 23</td>
</tr>
<tr>
<td>Basal</td>
<td>836 ± 115 (42 ± 75†)</td>
<td>285 ± 68 (180 ± 20)</td>
<td>255 ± 35 (106 ± 31)</td>
</tr>
<tr>
<td>40 mU·m⁻²·min⁻¹</td>
<td>691 ± 130 (187 ± 115)</td>
<td>205 ± 85 (261 ± 58)</td>
<td>185 ± 39 (175 ± 33)</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>88 ± 2</td>
<td>92 ± 2</td>
<td>88 ± 1</td>
</tr>
<tr>
<td>Basal</td>
<td>90 ± 5</td>
<td>91 ± 3</td>
<td></td>
</tr>
<tr>
<td>40 mU·m⁻²·min⁻¹</td>
<td>92 ± 3</td>
<td>92 ± 5</td>
<td></td>
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</tbody>
</table>

Values are means ± SE, with changes from baseline in parentheses. * \( P < 0.01 \) vs. lean adolescents and adults; † \( P < 0.05 \) vs. lean adults.
Glucose metabolism. Glucose infusion rates changed minimally in all three groups during the low-dose infusion. In contrast, glucose infusion rates increased in all three groups during the high-dose insulin infusion, but to a lesser degree in the obese adolescents (140 + 10 mg·m⁻²·min⁻¹) compared with lean adolescents (220 + 20 mg·m⁻²·min⁻¹) and lean adults (305 + 12 mg·m⁻²·min⁻¹) (P < 0.01). As shown in Table 4, glucose oxidation rates at baseline and during the lower insulin dose infusion were similar in all three groups. However, during the high-dose insulin infusion, glucose oxidation rates were 25% greater in the lean adults than in both adolescent groups (P < 0.05) (Table 4). Of note, as shown in Table 4, basal fat oxidation rates were greater in both obese and lean adolescents than in lean adults (P < 0.01). Insulin infusion suppressed fat oxidation rates by 80 and 40% in the lean adults and lean adolescents, respectively, during the higher dose (P < 0.05). In marked contrast, in the obese adolescents, no change from baseline in fat oxidation rates occurred at any step of the clamp study. Fat oxidation correlated inversely with glucose oxidation (r = −0.50, r² = 0.25, P < 0.001) in the three groups.

**DISCUSSION**

In the present study, we have evaluated insulin action on lipolysis in obese adolescents and compared their response with responses observed in groups of lean adolescents and lean adults. To assess lipolysis we

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**Table 3. Plasma glycerol enrichment, plasma glycerol and free fatty acid levels during insulin infusions**

<table>
<thead>
<tr>
<th>Insulin Infusion Time, min</th>
<th>8 mL·m⁻²·min⁻¹</th>
<th>40 mL·m⁻²·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>240</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[³H₂]glycerol, APE</th>
<th>Obese adolescents</th>
<th>Lean adolescents</th>
<th>Lean adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese adolescents</td>
<td>7.73 ± 0.81</td>
<td>8.11 ± 0.74</td>
<td>8.08 ± 0.68</td>
</tr>
<tr>
<td>Lean adolescents</td>
<td>8.63 ± 0.75</td>
<td>8.98 ± 1.00</td>
<td>8.89 ± 0.58</td>
</tr>
<tr>
<td>Lean adults</td>
<td>10.59 ± 1.45</td>
<td>11.49 ± 0.74</td>
<td>9.83 ± 1.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma glycerol, µmol/l</th>
<th>Obese adolescents</th>
<th>Lean adolescents</th>
<th>Lean adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese adolescents</td>
<td>107 ± 29</td>
<td>116 ± 19</td>
<td>93 ± 23</td>
</tr>
<tr>
<td>Lean adolescents</td>
<td>66 ± 11*</td>
<td>72 ± 14*</td>
<td>52 ± 15*</td>
</tr>
<tr>
<td>Lean adults</td>
<td>67 ± 14*</td>
<td>74 ± 14*</td>
<td>50 ± 15*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma free fatty acid, µM</th>
<th>Obese adolescents</th>
<th>Lean adolescents</th>
<th>Lean adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese adolescents</td>
<td>860 ± 83†</td>
<td>871 ± 91†</td>
<td>533 ± 62†</td>
</tr>
<tr>
<td>Lean adolescents</td>
<td>809 ± 83</td>
<td>781 ± 92</td>
<td>373 ± 63</td>
</tr>
<tr>
<td>Lean adults</td>
<td>567 ± 73</td>
<td>577 ± 80</td>
<td>272 ± 55</td>
</tr>
</tbody>
</table>

Values are means ± SE. **APE**, atoms % excess. *P < 0.02 vs. basal values and significantly higher than lean adults; †P < 0.02 vs. lean adults; ‡P < 0.05 vs. lean adults.

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**Table 4. Glucose and fat oxidation rates in basal state and during 8 and 40 mL·m⁻²·min⁻¹ clamp**

<table>
<thead>
<tr>
<th>Glucose oxidation, µmol·kg LBM⁻¹·min⁻¹</th>
<th>Obese Adolescents</th>
<th>Lean Adolescents</th>
<th>Lean Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>10 ± 2</td>
<td>11 ± 2</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>8 mL·m⁻²·min⁻¹</td>
<td>10 ± 2</td>
<td>14 ± 3</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>40 mL·m⁻²·min⁻¹</td>
<td>17 ± 3</td>
<td>19 ± 4*</td>
<td>24 ± 3*</td>
</tr>
<tr>
<td>Fat oxidation, µmol·kg LBM⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>5 ± 2†</td>
<td>7 ± 2†</td>
<td>3 ± 0.8</td>
</tr>
<tr>
<td>8 mL·m⁻²·min⁻¹</td>
<td>5 ± 1†</td>
<td>6 ± 2†</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>40 mL·m⁻²·min⁻¹</td>
<td>4 ± 0.8†</td>
<td>4 ± 1†</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Glucose infusion rates, mg·m⁻²·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>50 ± 7</td>
<td>37 ± 12</td>
<td>56 ± 7</td>
</tr>
<tr>
<td>8 mL·m⁻²·min⁻¹</td>
<td>140 ± 10†</td>
<td>220 ± 20†</td>
<td>305 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE. LBM, lean body mass. *P < 0.05 vs. baseline; †P < 0.01 vs. lean adolescents; §P < 0.01 vs. lean adolescents and adults; $P < 0.05 vs. lean adults.
used the appearance rate of glycerol in plasma, because it is thought to be a more reliable index of lipolysis than that of FFA turnover (5, 27). In the postabsorptive state, glycerol turnover in absolute terms was enhanced and less suppressed by insulin in obese adolescents compared with either lean group. To correct for differences in body composition, we also analyzed glycerol flux per kilogram of adipose tissue. With this analysis, basal glycerol flux adjusted per fat mass tended to be lower in obese adolescents than in either lean adolescents or lean adults, but the difference was not significant and was appropriate for the level of insulin present. Thus, when the enlarged fat mass and increased insulin levels of the obese adolescents are taken into account, the elevated fasting glycerol turnover rates are normalized.

Although absolute basal glycerol turnover rates were elevated in obese adolescents, their basal plasma glycerol levels were not significantly different from those of lean adolescents. The reason for this is not clear, but it may be due to a poor relationship between plasma levels and turnover rates or to a different glycerol clearance. Previous studies in obese adults found increased basal lipolytic rate expressed per lean body mass, whereas these rates appeared to be decreased when adjusted for differences in fat mass. Campbell et al. (8) showed that, despite impaired insulin sensitivity, the release of FFA from adipose tissues, expressed per kilogram fat mass, was less in obese vs. lean subjects after an overnight fast, as the result of a reduction in lipolysis and an increase in FFA reesterification. Using a stepwise euglycemic insulin clamp technique in combination with an infusion of \(^{13}C\)palmitate and indirect calorimetry, Groop et al. (16) reported that the absolute rate of basal FFA turnover was greater and less suppressed by insulin in obese compared with lean subjects. To our knowledge none of these studies was performed in subjects still accumulating fat. Our study, on the other hand, provides a unique documentation of fatty acid metabolism and lipolysis during the active phase of excessive fat accretion.

Our results are also somewhat similar to those of LeStunff and Bougnères (24), who reported that obese prepubertal children have greater absolute rates of glycerol turnover than lean prepubertal children. However, even in these younger subjects, the rates of lipolysis were significantly lower than those of controls when corrected for adipose tissue mass. They concluded that, during the initial dynamic phase of human obesity, the sensitivity of lipolysis to insulin may actually be increased. However, the greater suppression in glycerol turnover rates per kilogram of fat mass reported by those investigators is likely to be due to the higher ambient basal plasma insulin levels compared with levels observed in the nonobese children. As shown in Fig. 1B, the lower basal glycerol turnover rates per kilogram of adipose tissue in obese subjects can be accounted for by basal hyperinsulinemia. When data relating glycerol turnover to plasma insulin under basal and insulin-stimulated conditions are interpolated (Fig. 1B), it is notable that the curve for obese adolescents is superimposable on curves of the two lean groups, indicating a normal rather than an increased ratio of lipolysis to insulin. Although our study suggests that the antilipolytic effects of insulin are normal in juvenile obesity, it is important to acknowledge that mobilization of lipid stores in response to epinephrine has recently been found to be reduced during the dynamic phase of fat accumulation (7). Further studies are, however, needed to clearly establish whether alterations in the regulation of lipolysis contribute to the development of obesity.

Even though individual adipocytes may be normally sensitive to insulin in obese adolescents, the enlarged fat mass resulted in a net increase in the rates of lipolysis before and during the insulin clamp procedure. In parallel with the increased circulating plasma FFA concentrations, we have found increased net lipid oxidation rates in the basal state and during the clamp in the obese adolescents compared with lean adults. The coexistence of increased fat oxidation and decreased glucose metabolism suggests that the defects in glucose metabolism in obese adolescents at least in part result from substrate competition between glucose and FFA, as proposed by the Randle cycle (30).

Another interesting finding of the current study is the lack of feedback inhibition of endogenous insulin secretion in the obese group. This is indicated by the significantly greater changes in and percent suppression of plasma C-peptide levels in both lean groups compared with the obese group. Thus, in contrast to nonobese individuals, at a very early stage of obesity the \(\beta\)-cell appears to be blind to the circulating levels of insulin and does not promptly decrease its secretion as occurs in lean individuals, even during a very low insulin infusion. Insulin and C-peptide are secreted in an equimolar fashion and, unlike insulin, C-peptide is not extracted by the liver and therefore is a better reflection of “true” insulin secretion (28, 29). It is conceivable that these higher plasma C-peptide levels are due to an altered metabolic clearance rate in obese compared with lean children. However, studies in obese adults have indicated that the metabolic clearance of C-peptide is unaltered by the induction of hyperinsulinemia. Thus inadequate feedback suppression may account for the prevailing hyperinsulinemia of juvenile obesity, as also described in studies by Elahi et al. (13) in obese Pima Indians. This chronic elevation in insulin levels may favor accumulation of triglycerides in muscle, which in turn will interfere with glucose storage and oxidation, leading to insulin resistance. It is conceivable that alterations in skeletal muscle metabolism might play a role in the etiology of obesity. This is, however, an area that clearly needs further study.

Our present study also provides information about insulin’s effects on lipolysis and fat oxidation rates in the insulin resistance of puberty. We found no differences between the lean adolescents and adults with regard to the ability of insulin to suppress glycerol turnover, an index of lipolysis. In contrast, suppression of lipid oxidation was impaired in lean pubertal subjects vs. lean adults despite equivalent insulin concen-
trations during both steps of the clamp (Tables 2 and 4). These findings are in agreement with those of Arslanian and Kalhan (3), who reported that increased lipid oxidation may contribute to the insulin resistance normally observed during puberty. Of particular interest, in their study Arslanian and Kalhan found a relationship between plasma insulin-like growth factor I levels and total body lipid oxidation rates, suggesting that increased growth hormone secretion during puberty might be responsible for the augmented rates of lipid oxidation. Attractive as it is, however, this hypothesis needs further evaluation.

In summary, in contrast to the marked resistance to insulin’s stimulatory effect on glucose metabolism, the lipolytic rate in adipose tissue of obese adolescents is normally sensitive to the insulin’s suppressive effects. Lipolysis, as assessed by the glycerol appearance rate in plasma in obese adolescents, is increased as a consequence of the expanded adipose mass, which in turn results in increased plasma FFA. Inhibition of net lipid oxidation rates is markedly impaired in early onset obesity. The augmented supply of fatty acid substrates and their ability to interfere with glucose oxidation and storage may be an important component of insulin resistance in adolescent obesity.

We are grateful to all the children for participating in the study. We thank the nursing staff of the Pediatric General Clinical Research Center for the excellent care given to our subjects during these studies. We are indebted to the staff of the Core Laboratory of the Center for the excellent care given to our subjects during these studies. We are particularly thank Dr. G. Cline for measurements of [2H5]glycerol. We are grateful to all the children for participating in the study.

REFERENCES


