Impaired In Vivo Stimulation of Lipolysis in Adipose Tissue By Selective β_2-Adrenergic Agonist in Obese Adolescent Girls

Staffan Enoksson, Meredith Talbot, Frances Rife, William V. Tamborlane, Robert S. Sherwin, and Sonia Caprio

Studies performed in adults with long-standing obesity suggest a reduced lipolytic sensitivity to catecholamines in subcutaneous abdominal adipose tissue (AT). We used microdialysis to study the in situ lipolytic effects of dobutamine (selective β_1-agonist) and terbutaline (selective β_2-agonist) on glycerol release (lipolytic index) in abdominal subcutaneous AT in 10 obese girls aged 13–17 years, BMI 38 ± 2.1 kg/m^2, and in 7 lean girls aged 11–17 years, BMI 21 ± 1.1 kg/m^2, and compared them with 10 obese women aged 21–39 years, BMI 36 ± 1.6 kg/m^2, and 10 lean women aged 18–42 years, BMI 21 ± 0.4 kg/m^2. Terbutaline at 10^{-6} mol/l stimulated glycerol release more efficiently in lean girls than in obese girls (peak response ~350 vs. 150% of control, $P < 0.01$). At the lower concentration of agonist, no significant difference was seen. In women, terbutaline was more effective in lean than in obese women in stimulating glycerol release at both 10^{-8} mol/l (peak response lean ~175% vs. obese 125% of control) and 10^{-6} mol/l (~300 vs. 150% of control, $P < 0.05$). No significant difference in glycerol release between obese and lean girls or women was detected with selective β_1-stimulation. Our data demonstrate a specific impairment in the capacity of β_2-adrenergic agonists to promote lipolysis in subcutaneous abdominal adipose tissue of obese adolescent girls and women. Thus, decreased mobilization of fat during activation of the adrenergic system might be present early in the development of adolescent obesity. *Diabetes* 49:2149–2153, 2000

The prevalence of obesity in children as well as in adults is steadily increasing in the U.S. (1). The early-onset type of obesity deserves special attention because youth-onset obesity is often the precursor of the most intractable form of adult obesity (2). Furthermore, the comorbid conditions that accompany obesity in adults, such as type 2 diabetes, dyslipidemia, and hypertension, are seen with increasing frequency in overweight adolescents and even preadolescents (3,4).

Although the pathogenesis of obesity is poorly understood, it is believed to result from a complex interaction between genetic and environmental factors (5), leading to a more efficient accumulation of fat and to an impaired ability to mobilize fat. Because catecholamines are the only hormones with pronounced lipolytic action in humans (6), the effect of obesity in adults on the responsiveness to catecholamines has been extensively studied. Both in vitro and in vivo studies have demonstrated that subcutaneous abdominal adipose tissue in obese adults is resistant to the lipolytic effects of catecholamines (7–9). Moreover, even though subcutaneous adipose tissue contains β_1-, β_2-, and β_3-adrenoceptors, obesity-induced catecholamine resistance appears primarily because of defects in β_2-stimulation (7), with impaired β_1-stimulation having a secondary role. Whereas obese children have been shown to have a reduced lipolytic response to systemic epinephrine infusion (9), the effect of juvenile obesity on adrenoceptor function has not been established.

To investigate the possibility that reduced function of β-adrenoceptors is an early event in the development of juvenile obesity, we used the microdialysis technique to selectively target the abdominal subcutaneous adipose tissue in obese and lean adolescent girls and women and study the in vivo lipolytic response to locally delivered selective β-adrenergic agonists. Our findings indicate that defects in β_2-adrenergic stimulation of lipolysis are expressed early in the natural history of obesity—a factor that may contribute to the persistence of problems with excessive weight gain into adulthood.

RESEARCH DESIGN AND METHODS

Subjects. A total of 37 healthy volunteers participated in the study (10 lean women, 10 obese women, 7 lean adolescent girls, and 10 obese adolescent girls); subject characteristics are given in Table 1. Adiposity was determined by calculating BMI and measurements of body fat composition using the dual-energy X-ray absorption scan. Obesity in adults was defined as BMI ≥30 kg/m^2 and in adolescents a BMI >95th percentile specific for age and sex (10). None of the participants were taking any drugs. 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.

Microdialysis technique. The principles of the microdialysis technique for lipolysis studies in adipose tissue have been described in detail (11,12). Briefly, a tubular polyamide dialysis membrane (0.62 × 30 mm, molecular cutoff 20,000 Da) is glued to the end of the outer cylinder of concentric double-lumen polyurethane tubing. The perfusate is continuously propelled by a microinfusion pump (CMA/100; CMA/Microdialysis, Stockholm, Sweden) and

From the Departments of Internal Medicine (S.E., M.T., F.R., R.S.S.) and Pediatrics (W.V.T., S.C.), Yale University School of Medicine; and the General Clinical Research Center, New Haven, Connecticut.

Address correspondence and reprint requests Sonia Caprio, MD, Department of Pediatrics, 333 Cedar St., Yale University School of Medicine, New Haven, CT 06520. E-mail: sonia.caprio@yale.edu.

Received for publication 19 April 2000 and accepted in revised form 21 August 2000.

R.S.S. is the current president of the American Diabetes Association.

ANOVA, analysis of variance; ECF, extracellular fluid; NEFA, nonesterified fatty acid.

TABLE 1
Clinical characteristics of the study subjects

	Lean girls	Obese girls	Lean women	Obese women
Age (years)	14 ± 0.7	15 ± 0.3	27 ± 2.2	30 ± 1.7
BMI (kg/m^2)	21 ± 1.1	38 ± 2.1	21 ± 2.2	36 ± 1.6
Fat mass (%)	31 ± 1.8	44 ± 0.7	26 ± 1.0	44 ± 1.3

Data are means ± SE.

flows through the outer tubing into the space between the concentric cylinders to the distal end of the probe. The exchange of molecules between the extracellular fluid and the perfusate occurs across the semipermeable dialysis membrane, after which the perfusate enters the inner cannula in a retrograde direction and is collected in timed fractions for later analysis of glycerol. Glycerol is not reused to any large extent in adipose tissue in the fasting state. Thus, changes in extracellular glycerol concentrations reflect changes in lipolysis, as discussed in detail elsewhere (11).

Study protocol. All subjects were investigated in the supine position after an overnight fast. A retrograde cannula was inserted into a vein in the dorsum of the right hand, which was positioned in a heated box (60–65°C) for sampling of arterialized venous blood (13). Three to five microdialysis catheters (CMA/60; CMA/Microdialysis, Acton, MA) were inserted percutaneously in the subcutaneous adipose tissue of the anterior abdominal wall a distance of at least 30 mm apart. Before the insertion, the skin was superficially anestethized (EMLA; Astra, Sodertalje, Sweden). After allowing a 30-min rest to avoid a potential traumatic artifact caused by the insertion itself (14), the microdialysis catheters were continuously perfused with artificial extracellular fluid (ECF) (135 mmol/l NaCl, 3 mmol/l KCl, 1 mmol/l $MgCl_2$, 1.2 mmol/l $CaCl_2$, 300 µmol/l ascorbate, and 2 mmol/l Na phosphate buffer adjusted to pH 7.4) with the addition of either the selective β_2-agonist terbutaline or the selective β_1-agonist dobutamine at a concentration of 10^{-8} mol/l. Another catheter was perfused only with artificial ECF and served as the control. In addition, six subjects from each group received two additional catheters (a total of five) perfused with terbutaline and dobutamine at a concentration of 10^{-6} mol/l. The perfusion rate in all catheters was 0.3 µl/min. The dialysate was collected in 30-min fractions and analyzed for glycerol. Plasma samples were drawn every 30 min for determination of glycerol, nonesterified fatty acids (NEFAs), insulin, glucose, epinephrine, and norepinephrine.

Analytical procedures. Plasma glucose levels were measured by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Brea, CA). Plasma insulin was measured by a double-antibody radioimmunoassay (Linco Research, St. Louis, MO). Catecholamines were collected in iced tubes containing glutathione and assayed by high-performance liquid chromatography using an electrochemical detector. Plasma NEFAs were assayed by a colorimetric method (15). Glycerol in plasma and dialysates was measured by an enzymatic fluorometric method using an automated multianalyzer (CMA/600; CMA/Microdialysis, Stockholm, Sweden).

Statistical analysis. All data are presented as means ± SE. Area under curve (0–120 min) for plasma concentrations of glycerol and for the increase in interstitial glycerol concentration induced by the β-adrenergic agonists and in the control catheters was calculated using trapezoidal integration. Student's *t* test, using the paired *t* test when applicable, was performed in cases for which two groups of values were compared. One-factor analysis of variance (ANOVA) was used to compare the lipolytic response to various concentrations of agonist and control within a group. Differences in age, BMI, fat mass, and concentrations of substrates or hormones at a particular time point were tested using one-factor ANOVA. The level of significance was set at $P < 0.05$.

Drugs and chemicals. Artificial ECF and drugs were prepared by the Investigational Drug Service at Yale-New Haven Hospital. Terbutaline was obtained from Geneva Pharmaceuticals (Basel), and dobutamine hydrochloride was obtained from Baxter Pharmaceutical Products (Raritan, NJ).

RESULTS

Basal values. As shown in Table 2, plasma glucose in obese women was significantly higher than in each of the other study subjects ($P < 0.05$), although it was within the normal fasting range. Basal circulating concentrations of NEFAs in obese girls tended to be higher than those in lean girls and women. The NEFA concentrations in the lean or obese adolescent girls were not significantly different from the concentrations in the other groups ($F = 1.69$, $P = 0.08$). The plasma insulin concentrations were significantly increased in both obese adolescent girls and obese women compared with their lean counterparts ($P < 0.05$ and $P < 0.001$, respectively). Notably, although the insulin concentration in the lean adolescents was lower than that in the obese adolescents, it was increased to the same level as that in the obese women. Epinephrine and norepinephrine concentrations in serum were not significantly different between the obese and lean adolescent girls or women.

Glycerol concentration in plasma and control dialysate. Figure 1 depicts the glycerol concentration in plasma and dialysate for the full duration of the study. In both obese and lean adolescents and women, the dialysate concentration of glycerol was approximately three times as high as the glycerol concentration in plasma. The mean dialysate glycerol concentration in lean and obese girls was 184 ± 7 and 198 ± 6 µmol/l, and in lean and obese women, 216 ± 6 and 212 ± 5 µmol/l, respectively. The corresponding circulating levels were 55 ± 1.5, 61 ± 2.9, 58 ± 1.4, and 57 ± 1.0 µmol/l, respectively. There was no significant difference in the mean plasma or dialysate glycerol concentration between lean and obese individuals in either the adolescent or the adult groups. Also, in lean and obese adolescent girls and women, plasma concentrations of glycerol were unchanged over time, whereas dialysate glycerol concentration increased initially and subsequently reached a plateau after ~60 min.

Effect of the selective β_2-adrenergic agonist terbutaline on lipolysis. Lipolysis was stimulated locally in abdominal subcutaneous adipose tissue by the addition of increasing concentrations of the selective β_2-adrenergic agonist terbutaline to the microdialysis perfusion medium, and the resulting changes in dialysate glycerol concentration

TABLE 2
Basal circulating concentrations of substrates and hormones

	Lean girls	Obese girls	Lean women	Obese women
Glucose (mmol/l)	5.1 ± 0.11	4.9 ± 0.09	4.7 ± 0.17	5.3 ± 0.08†
FFA (µmol/l)	430 ± 40	636 ± 88	585 ± 44	577 ± 48
Insulin (pmol/l)	87 ± 8	127 ± 17*	39 ± 6	89 ± 7‡
Epinephrine (pg/ml)	28 ± 6	48 ± 19	25 ± 7	36 ± 8
Norepinephrine (pg/ml)	160 ± 11	208 ± 81	270 ± 44	216 ± 36

Data are means ± SE. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

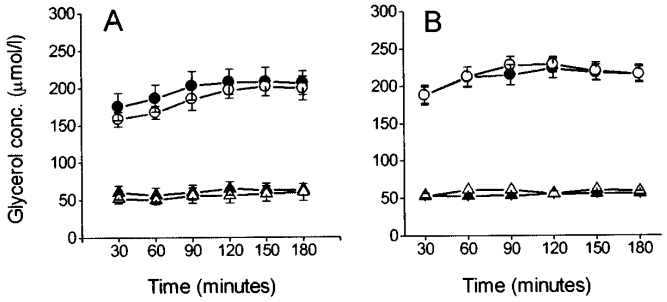

FIG. 1. Plasma and control dialysate glycerol concentration during the study. Microdialysis was performed during 180 min in the subcutaneous abdominal adipose tissue of lean and obese adolescent girls (*A*) and women (*B*). A control catheter was perfused only with artificial extracellular fluid. The concentrations of glycerol were determined in plasma (△, lean subjects; ▲, obese subjects) and 30-min collections of dialysate (○, lean subjects; ●, obese subjects). Values are means ± SE. conc., concentration.

(lipolysis index) over time are presented as the percent of control dialysate glycerol concentration in Fig. 2*A* and *B* (girls) and Fig. 3*A* and *B* (women). A dose-dependent increase in dialysate glycerol concentration was seen in both lean adolescent girls ($P < 0.001$) and lean women ($P < 0.01$). In both lean groups, the onset in glycerol release was rapid and followed by a gradual decline over time toward the level in the control catheter. The maximal response to the low concentration of β_2-agonist in lean adolescents was 156% of the control and almost 350% of the control at the high concentration of agonist. In lean women, the maximum increase in glycerol concentration was 167% of the control at the low dose of β_2-agonist and nearly 300% of the control at the high concentration of agonist.

In contrast, the lipolytic response in the obese subjects was very different. Although the dialysate glycerol concentration increased during stimulation with terbutaline at a concentration of 10^{-8} and 10^{-6} mol/l in both obese adolescents and women ($P < 0.05$), there was no significant difference in stimulatory effect with increased dose of the agonist in either group. More important, however, the glycerol release in response to the selective β_2-adrenergic agonist at 10^{-6} mol/l was markedly blunted in the obese girls ($P < 0.01$) as well as in the obese women ($P < 0.05$) compared with their lean control subjects. With the lower dose of terbutaline, the diminished response in obese women was less striking but still significant ($P < 0.05$). In the adolescent girls, no significant difference in response between lean and obese subjects was apparent at the low dose of β_2-agonist. Thus, the dose-response curve in the obese subjects seems to be shifted to the right and flattened, indicating both an altered sensitivity and responsiveness to β_2-adrenergic stimulation.

Effect of the selective β_1-adrenergic agonist dobutamine on lipolysis. Lipolysis was also stimulated in subcutaneous abdominal adipose tissue by the addition of increasing concentrations of the selective β_1-adrenergic agonist

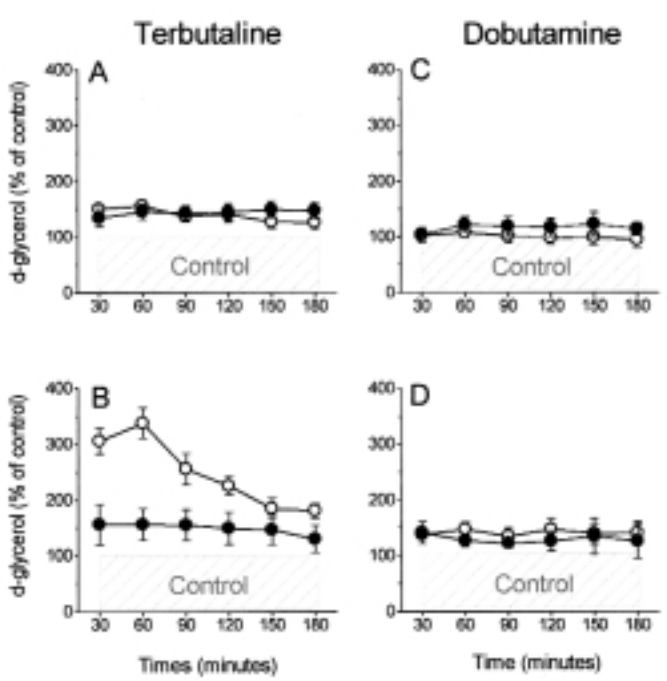

FIG. 2. Effect of the β_2-adrenergic agonist terbutaline (*A* and *B*) and the β_1-adrenergic agonist dobutamine (*C* and *D*) on the glycerol level in adipose tissue of lean and obese adolescent girls. Subcutaneous abdominal adipose tissue was microdialyzed for 180 min with artificial ECF alone (control) or with the addition of terbutaline or dobutamine at a concentration of 10^{-8} mol/l (upper console) or 10^{-6} mol/l (lower console). Glycerol was determined in the dialysate, which was collected at 30-min intervals, and all individual fractions were expressed as the percent of the control value (○, lean subjects; ●, obese subjects). Values are means ± SE.

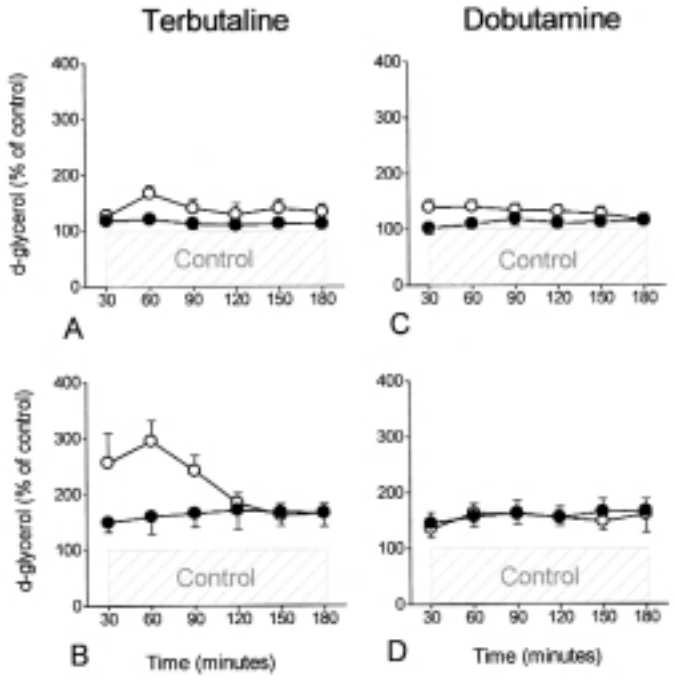

FIG. 3. Effect of the β_2-adrenergic agonist terbutaline (*A* and *B*) and the β_1-adrenergic agonist dobutamine (*C* and *D*) on the glycerol level in adipose tissue of lean and obese women. Subcutaneous abdominal adipose tissue was microdialyzed for 180 min with artificial ECF alone (control) or with the addition of terbutaline or dobutamine at a concentration of 10^{-8} mol/l (upper console) or 10^{-6} mol/l (lower console). Glycerol was determined in the dialysate, which was collected at 30-min intervals, and all individual fractions were expressed as the percent of the control value (○, lean subjects; ●, obese subjects). Values are means ± SE.

dobutamine to the microdialysis perfusion medium in Fig. 2*C* and *D* (girls) and Fig. 3*C* and *D* (women). The increase in dialysate glycerol concentration induced by dobutamine at a concentration of 10^{-6} mol/l was significantly higher than the control in lean adolescent girls but not in obese adolescent girls ($P < 0.01$). The low concentration of dobutamine did not significantly stimulate glycerol release in lean or obese adolescents. In lean women, a significant increase in dialysate glycerol concentration was seen in response to the selective β_1-adrenergic agonist dobutamine at 10^{-8} and 10^{-6} mol/l ($P < 0.001$). In obese women, only the high concentration of agonist resulted in a significant increase in extracellular glycerol concentration. The maximal response to the low concentration of dobutamine was 108 and 122% of the control in lean and obese adolescents and 140 and 118% of the control in lean and obese women, respectively. At the high concentration of β_1-agonist, the dialysate glycerol was 147 and 141% of the control in lean and obese adolescents and 164 and 167% of the control in lean and obese women, respectively. However, the increase in glycerol release with increased doses of agonist did not reach significance in any group when dobutamine was administered locally at the current concentrations, and there was no significant difference in maximal glycerol release discernible between lean and obese subjects.

DISCUSSION

Juvenile-onset obesity is a common disorder with serious medical consequences. Nevertheless, the mechanisms involved in its development are largely unknown. Fat accumulation results from an imbalance between lipid synthesis and lipid mobilization. The present study demonstrates a striking defect in the capacity of β_2-adrenergic activation to stimulate lipid mobilization in obese adolescents, which may have implications for the development and/or maintenance of the obese state.

We used the microdialysis technique to examine the effect of age and obesity on the in vivo lipolytic response of subcutaneous abdominal fat tissue to selective β_1- and β_2-adrenergic stimulation. We do not know the final effective extracellular concentration of dobutamine or terbutaline. Because of diffusion characteristics and dilution by local blood flow, the concentration of the agonist may be significantly less in the tissue than in the perfusate. Nevertheless, in previous microdialysis and in vitro studies on human adipose tissue, these concentrations of agonists have been shown to submaximally stimulate in a selective fashion the designated receptors (16). The perfusion rate in all catheters was 0.3 µl/min. At this flow rate and with the presently used dialysis membrane length, recovery of glycerol has recently been shown to exceed 90%, i.e., near absolute interstitial levels are measured (17). The bidirectional nature of the microdialysis system allowed us to selectively stimulate only a small depot of fat tissue in the absence of systemic effects, and the minimally invasive character of this approach allowed us to study teenagers as well as adults. By limiting the study to females, the confounding effects of sex were avoided. Several studies have shown a greater lipolytic response to adrenergic stimulation in females than in males (18).

Our results demonstrate that rates of lipolysis in subcutaneous fat are increased by both β_1- and β_2-adrenergic stimulation in lean and obese adolescents and adults. However, in both age-groups, the response to β_2-adrenergic stimulation was blunted in obese subjects compared with lean subjects. In contrast, there was no effect of age or adiposity on the lipolytic response to β_1-adrenergic stimulation. Our findings of a blunted lipolytic response to β_2-adrenergic stimulation in obese women compared with lean women is consistent with in vitro and in vivo data from other investigators (6–8). Little data are available, however, that address this question in juvenile obesity. Bougnères et al. (9) recently reported diminished lipolytic response to intravenous infusion of epinephrine in obese versus lean prepubertal children. In this study, we examined the lipolytic response to selective β-agonists in the tissue bed of interest (i.e., subcutaneous abdominal adipose tissue) rather than the response to systemic catecholamine stimulation. By studying lean and obese adolescent girls, we were also able to examine the interaction between puberty, aging, and obesity on responses to selective β-adrenergic stimulation. Our results indicate that normal puberty or aging did not adversely affect the lipolytic response to either β_1- or β_2-adrenergic stimulation. Moreover, obesity caused the same impairment of responses to β_2-adrenergic stimulation in adolescents as it did in adults.

It is not possible from our experiment to determine whether the observed defect is located at the receptor level, located further downstream intracellularly, or if it involves the hormone-sensitive lipase directly. Irregularities have been demonstrated at all these levels in adult fat cells (19,20). However, the β_1-adrenoceptor signal transduction in the current experiment appeared to be unaffected in the obese subjects because their response was not significantly different from the response in the lean control subjects. Knowing that β_1- and β_2-adrenoceptors share an intracellular final common pathway leading to increased cAMP and subsequent activation of hormone-sensitive lipase (20), it is likely that a defect at the receptor level engaging the β_2-adrenoceptors is a dominant feature. The notion of downregulation of β_2-adrenoceptors as an important mechanism behind catecholamine resistance is supported by in vitro data from Reynisdottir et al. (7), who reported decreased expression of β_2-adrenoceptors in fat cells of obese women with catecholamine resistance.

In addition to β_1- and β_2-adrenoceptors, white adipocytes derived from subcutaneous abdominal depots also express β_3-adrenoceptors (21). Although these receptors play a functional role in lipolysis regulation, the β_2-adrenoceptors seem to be of a greater importance for catecholamine stimulation of mobilization of lipids, particularly from abdominal subcutaneous adipose tissue (22). Studies by Lonquist et al. (23) provide evidence that visceral adipocytes obtained from individuals with upper obesity have increased lipolysis in response to catecholamines and that this is mediated, in large part, by an increase in β_3-adrenoceptor function. Whether the β_3-adrenoceptor function in the subcutaneous tissue is also altered in obesity is not known at the present time.

Whereas catecholamines are the major hormones stimulating lipolysis in humans, insulin is the dominant anti-lipolytic agent. Insulin has been shown to acutely downregulate β-adrenoceptors in vitro (24), but the physiological relevance of this propensity for insulin remains to be established in vivo. In a report from Hagstrom-Toft et al. (25), in which adipose tissue was stimulated in situ with the β-adrenergic agonist isoproterenol before and during a 2-h hyperinsulinemic-euglycemic clamp, a decrease in glycerol release in response to the

agonist during insulin infusion was not observed (25). It may be that a longer period of hyperinsulinemia is needed to unveil this mechanism in vivo. In the present study, circulating levels of insulin were increased in the obese adolescents and may therefore have contributed to the observed depressed lipolytic response. However, the observed resistance of lipolysis to β_2-agonist in the present study cannot be fully accounted for by insulin. Physiological hyperinsulinemia and insulin resistance are normally associated with adolescence. As expected, plasma levels of insulin in our lean adolescent girls were elevated to the same degree as in the obese women, yet obese women were significantly resistant to β_2-adrenergic stimulation compared with the lean adolescents. Similarly, even if insulin is infused in lean children to match hyperinsulinemia in the obese, the lean children are more responsive to catecholamine stimulation than obese children (9). It is conceivable that the downregulation of β_2-adrenoceptors is secondary to a chronic stimulation of sympathetic activity by the hyperinsulinemia seen in obesity. Another possible factor that might be involved in the reduced β_2-adrenergic effect on lipolysis is the presence of locally increased levels of leptin. How might high leptin levels influence β-adrenergic response is totally unknown. Our study suggests that in addition to the enlargement of the adipose mass, there are clearly qualitative alterations in this tissue occurring early in the development of obesity. Studies by Caro et al. (26) suggested that in obesity, compared with muscle tissue, the adipose tissue remains relatively insulin sensitive and that differences in the degree of insulin resistance in these critical tissues could be a mechanism contributing to the initiation and perpetuation of the obese state.

In summary, we investigated the hypothesis that decreased mobilization of fat during activation of the adrenergic system might be present early in the evolution of juvenile obesity, and we studied specifically the relative importance of β_1- and β_2-adrenergic receptors to locate the defect. A markedly blunted lipolytic response to selective β_2-adrenergic stimulation in subcutaneous abdominal adipose tissue of obese adolescent girls and women was observed. Hence, our data identify for the first time the β-adrenoceptor signaling pathway involved in lipolytic catecholamine resistance in children and support the idea that inability of the fat cell to respond to catecholamines is an early defect that may contribute to the development and/or maintenance of the obese state.

ACKNOWLEDGMENTS

The work was supported by grants from the National Institutes of Health (RO1 HD 28016 [to S.C.], MO1 RR 00125, MO1 RR 06022, and HD30671), the American Diabetes Association, the foundation of Henning and Johan Throne-Holst, and the Swedish Institute.

We are grateful for the invaluable assistance of the staff of the General Clinical Research Center and the Core Laboratory. We are also thankful to Kathy Catalano for excellent preparation of the manuscript.

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