In Vivo Resistance of Lipolysis to Epinephrine

A New Feature of Childhood Onset Obesity

Pierre Bougnères,* Catherine Le Stunff,* Claire Pecqueur,*‡ Evelyne Pinglier,* Pierre Adnot,§ and Daniel Ricquier‡

**Institut National de le Santé et de la Recherche Médicale U342, Hôpital Saint-Vincent-de-Paul, Institut Cochin Génétique Moléculaire, Université René Descartes, 75014 Paris; ‡Centre National de la Recherche-Scientifique Unité de Recherche Propre 1511, 92190 Meudon-Bellevue, France; and §Centre de Recherche, Groupe Lyonnaise Industrielle Pharmaceutique, 91380 Chilly-Mazarin, France*

Abstract

A decreased mobilization of triglycerides may contribute to fat accumulation in adipocytes, leading to obesity. However, this hypothesis remains to be proven. In this study, epinephrine-induced lipid mobilization was investigated in vivo in nine markedly obese children (160±5% ideal body weight) aged 12.1±0.1 yr during the dynamic phase of fat deposition, compared with six age-matched nonobese children. As an in vivo index of lipolysis, we measured glycerol flux using a nonradioactive tracer dilution approach, and plasma free fatty acid concentrations. In the basal state, the obese children had a 30% lower rate of glycerol release per unit fat mass than the lean children. To study the regulation of lipolysis, epinephrine was infused stepwise at fixed doses of 0.75 and then 1.50 μg/min in both groups. In lean children, glycerol flux and plasma free fatty acid increased to an average of 249–246% of basal values, respectively, in response to a mean plasma epinephrine of 396±41 pg/ml. The corresponding increase was only 55–72% in the obese children, although their mean plasma epinephrine reached 606±68 pg/ml. All obese and nonobese children, except an Arg64Trp heterozygote, were homozygotes for Trp at position 64 of their beta$_3$-adrenoreceptor. The resistance of lipolysis to epinephrine showed no relationship with the Arg64 polymorphism of the beta$_3$-adrenoreceptor gene.

In summary, in vivo lipolysis, which mostly reflects the mobilization of lipid stores from subcutaneous adipose tissue, shows a decreased sensitivity to epinephrine in childhood onset obesity. Since our study was carried out in obese children during the dynamic phase of fat accumulation, the observed resistance to catecholamines might possibly be causative rather than the result of obesity. (*J. Clin. Invest.* 1997. 99:2568–2573.) Key words: childhood onset obesity • epinephrine resistance • lipolysis

Address correspondance to Pierre Bougnères, INSERM U342, ICGM Hôpital Saint-Vincent-de-Paul 82, Avenue Denfert Rochereau 75014 Paris, France. Phone: 33-1-40-48-82-49; FAX: 33-1-40-48-83-52.

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Introduction

Most metabolic and endocrine studies in the literature have dealt with adult obesity. However, childhood obesity has nowadays become a major health problem in developed countries. Not only is it frequent and of growing prevalence (1), but most obese adolescents will remain obese adults, known to be exposed to an increased risk of metabolic and vascular complications (2, 3). Some of these complications, like atherosclerosis, are known to be more strongly associated with juvenile than with maturity onset obesity (3), suggesting that specific morbid effects could be related to early fat deposition.

Juvenile obesity is usually characterized by a continuous increase of body weight spanning the whole childhood and adolescence. Unlike stable long-established adult obesity, childhood onset obesity allows for the study of the mechanisms associated with adipose tissue overgrowth. Two concurrent processes are certainly of major importance to the constitution of fat depots in childhood obesity: one is the proliferation of numerous relatively small fat cells (hyperplastic obesity) (4), and the other is an increased storage of energy as triglycerides. Net lipid deposition in adipocytes results from a chronic imbalance between triglyceride synthesis and mobilization, which itself reflects the imbalance between energy intake and expenditure. Mobilization of fat stores, the subject of the present study, occurs predominantly (> 95%) in adipose tissue through the hydrolysis of triglycerides into glycerol and fatty acids (lipolysis). In human physiology, adipose-tissue lipolysis is by far the major provider of endogenous energy supply during periods of deficient nutritional availability.

It is tempting to speculate that primary or acquired alterations in the regulation of lipolysis contribute to the rapid increase in fat mass observed during the dynamic phase of juvenile obesity. While insulin has a powerful antilipolytic effect, catecholamines are the major hormones stimulating triglyceride hydrolysis in humans (5). Catecholamine effects are initiated by binding to lipolytic beta-adrenoreceptors and antilipolytic alpha$_2$-adrenoreceptors of white adipose cells, as recently reviewed by Lafontan and Berlan (5). Activated adrenoreceptors regulate adenylate cyclase activity, cyclic AMP production, and protein kinase A, resulting in phosphorylation and activation of hormone-sensitive lipase, which breaks down triglycerides to glycerol and free fatty acids, via di- and monoacylglycerol intermediates. The rate-limiting step of adipose tissue lipolysis is this hydrolysis of triglycerides by hormone-sensitive lipase, which in the postabsorptive state accounts for most of the detectable lipolysis (6).

Several in vivo studies (7–9) suggested that the lipolytic effect of catecholamines is altered in adults with long standing

stable obesity. However, it is not known if this alteration is primary or secondary to other obesity-induced dysfunctions of white adipose cell metabolism. Decreased beta$_2$-adrenoreceptor function, increased alpha$_2$-receptor function, and decreased activation of hormone sensitive lipase have been reported in the subcutaneous adipocytes of obese adults (10–12). More recently, Hellström et al. demonstrated a resistance of lipolysis to catecholamines in abdominal subcutaneous fat cells of normal weight first degree relatives of obese adults (13), suggesting that this alteration could predispose to obesity.

To test if resistance to catecholamines is associated with excess fat deposition and could thus play a role in the pathogenesis of morbid obesity, we studied in vivo the response of lipid mobilization to epinephrine during the dynamic phase of childhood onset obesity.

Methods

Subjects. The obese group consisted of nine children (four boys, five girls), aged 12.1±0.1 yr, who were in good health. Obesity was defined by a weight exceeding ideal body weight by 20% or more (14). To be sure to study the early dynamic phase of obesity, we selected patients with obesity of < 10 yr duration, recruited as reported (15, 16) and who had continuously increased their body weight since the onset of obesity.

The control group consisted of six children (four boys, two girls), aged 12.9±0.7 yr, recruited according to the approval of the institutional ethical committee.

Patients and controls were normoglycemic and had no familial history of diabetes mellitus or endocrine diseases. They were in good health, had normal clinical, EKG, and routine biology exams. All were exercising within normal limits. We enrolled only prepubertal children to avoid the interference of puberty with insulin action and other hormonal pathways. Boys had testes volume ranging 2–3 ml, penis length 45–55 mm, and pubic hair stage P1. All had plasma testosterone < 0.50 ng/ml, except one who had 0.70 ng/ml. Girls had no breast or pubic hair development, and their plasma estradiol was < 15 pg/ml. Bone age averaged 12±0.2 yr in boys, and 11.1±0.2 yr in girls, with no significant difference between obese and normal lean children.

Adipose tissue mass was determined as reported in previous studies (11, 12), using multiple skinfold measurements (triceps, biceps, subscapular, supra-iliac) with a Harpenden caliper by the same investigator to reduce the relative inaccuracy of the method (17). Lean body mass was approximated by subtracting adipose tissue mass from total body weight (15, 16).

The main clinical characteristics of the two groups of children are presented in Table I.

Materials. The [1,1,2,3,3-$^2H_5$] glycerol tracer material, composed of 85% pentadeuterated molecules, was purchased from ISOTEC Inc. (Miamisburg, OH). The tracer material was prepared for sterile intravenous administration as a 0.9% saline solution. A 5 μg/ml epinephrine solution was prepared by diluting Adrénaline Aguettant (Aguettant, Lyon, France) (0.25 mg/ml) in 0.9% saline. A 0.8 U/ml insulin solution was obtained with hemisynthetic insulin (Actrapid®; Novo Nordisk, Copenhagen, Denmark). Glucose, glycerol tracer, epinephrine, and insulin solutions were infused by separate pumps (IVAC Medical Systems, Los Angeles, CA).

Table I. Clinical Characteristics of the Studied Children

	Obese children	Lean controls
	(n = 9)	*(n = 6)*
Sex (M/F)	4/5	4/2
Age (yr)	12.1±0.1	12.4±0.4
Body weight (kg)	76±7*	44±4
% IBW (kg/m^2)	160±5*	99.5±4
BMI	29.7±1.1*	18.7±0.9
Fat mass (kg)	29±2*	16±1.5
Duration of obesity	5.4±0.4	—

*$P < 0.01$. IBW, ideal body weight.

Procedural methods. During the 72 h before study, all subjects consumed an isocaloric standard diet with 20, 40, and 40% of daily calories given at 0800 h, 1200 h, and 2000 h, respectively, and consisting of 45% carbohydrate, 35% fat, and 20% protein, a protocol used in our previous studies of juvenile obesity (15, 16, 18).

Studies were performed at 8.00 h after an overnight 12-h fast. An antecubital vein was cannulated to administer the infusates, and a dorsal vein of the opposite hand for sampling. Between sampling, a cannula was left in this vein, and the hand was placed in a heating box designed to warm the palm. After collection of a baseline sample, infusion of [1,1,2,3,3-$^2H_5$] glycerol began with a priming dose followed by the continuous infusion of the tracer at a constant rate of 0.047±0.002 μmol·kg^{-1}·min^{-1}.

Before epinephrine infusion, a 1-h period of isotope equilibration was allowed in obese children. In lean controls, insulin was infused for 3 h at a minimal rate of 0.20 mU·kg^{-1}·min^{-1}, starting simultaneously with glycerol infusion, in order to reach a plasma level of insulin comparable to obese children in the basal state. Plasma glucose was maintained nearly constant as reported (15, 18). Mean glucose concentration was 4.2±0.1 mM during insulin infusion in the normal children.

Epinephrine infusion started at $t = 60$ min at a rate of 0.75 μg/min for 60 min, then 1.5 μg/min for another 60-min period. Epinephrine doses were similar for obese and lean children, and were calculated to induce lipolysis while minimally stimulating the cardiovascular system, according to previous studies in adult men (19–21). According to the Micromedex Pharmacological Data Base, pediatric doses of epinephrine are 0.020–1.50 μg/kg/min. In our experiment, we used epinephrine at doses of 0.015 and 0.030 μg/kg/min. Blood samples for the measurement of plasma glycerol concentration and [$^2H_5$] enrichment, plasma FFA, epinephrine, norepinephrine, and insulin were taken at baseline, then at 10-min intervals during the last 30 min of each study period.

Analytical procedures. Blood samples for the subsequent determination of glycerol concentration and [$^2H_5$] enrichment, insulin, C-peptide and FFA concentrations were collected into iced tubes containing EDTA and rapidly centrifuged. The plasma was separated for glycerol, glucose, FFA, and insulin and stored at −20°C. For catecholamine determination, blood samples were collected into iced tubes containing glutathion and stored at −80°C.

FFA concentrations were measured by a standard enzymatic colorimetric method (22). Insulin concentrations were measured by radioimmunoassay (23).

Plasma epinephrine and norepinephrine content was determined using high performance liquid chromatography after alumina extraction. High performance liquid chromatography was performed on a system including autosampler, isocratic pump, and integrator (Shimadzu Medical Instruments, Columbia, MD); the electrochemical detector was a Kontron 402 (Velizy-Villacoublay, France). Separation was carried out using a 150 × 2 mm Beckmann column (Gagny, France) packed with Ultrasphere ODS C18 (5 μm average particle size). The mobile phase (pH = 3.76) contained 0.1 M potassium dihydrogen phosphate, 0.1 mM EDTA, and 5 mM heptane sulfonic acid. Flow rate was 0.15 ml/min, potential 0.7 V (oxidation), sensitivity 0.2 nA/V, and temperature 25°C. 500-μl plasma samples were added to a tube containing 10 mg of alumina and 0.5 ml of Tris-HCl buffer (1 M; pH = 8.7) with EDTA (0.05 M). After agitation for 8 min and centrifugation at 2,800 rpm for 15 min at 4°C, supernatants were discarded and alumina were washed twice with water. Catecholamines were eluted from alumina with 0.1 ml of a 0.1 M perchloric acid solution containing

0.6 mM sodium dithionite. After 4 min of agitation and centrifugation, 10 μl of supernatants was injected into the HPLC system for determination of epinephrine and norepinephrine. Extractions were carried out in an ice-water bath. Recoveries were calculated on the basis of peak heights measured by the integrator. Calibration curves were made with spiked plasma. The limits for precise quantification were < 30 pg/ml for both epinephrine and norepinephrine.

Glycerol was converted into a tri-acetyl derivative and its [$^2H_5$] enrichment was measured using gas chromatography-mass spectrometry (15). Ions at m/z 145 (M) and 148 (M + 3), issued, respectively, from the electron impact fragmentation of natural glycerol and its deuterated tracer, were selectively monitored using a mass spectrometer (model 5970 B; Finnigan Mat, Orsay, France). Plasma glycerol concentration was measured separately by the same method using a fixed amount of [$^2H_5$]glycerol as an internal standard added to plasma samples, with an appropriate correction for the small contribution of the tracer infused in vivo (15).

Leptin was measured in plasma or serum samples by radioimmunoassay (Linco Research, St. Louis, MO). The antileptin antiserum was raised in rabbit against highly purified recombinant human leptin. This antiserum did not cross-react with insulin, insulin-like growth factors, glucagon or interleukin 2 in doses of 10 mg/ml. The tracer was 125-iodinated human leptin. Recombinant leptin standard or serum sample in duplicate was incubated in phosphate-buffered saline, pH 7.4, containing 0.1% Triton X-100 and 1% bovine serum albumin with antileptin antiserum and 125-iodinated leptin (~ 15,000 cpm in 100 ml) for 21 h at 4°C. Then the bound fraction was precipitated with a conjugate of polyethylene-glycol and anti–rabbit antiserum and centrifuged. The supernatant was decanted and the pellet was counted in a gamma counter. The performance of the standard curve was as follows: ED_{80}: 1.48±0.09 ng, ED_{50}: 6.07±0.51 ng, ED_{20}: 31.7±3.49 ng. The detection limit was 0.1 ng/ml. The intraassay coefficient of variation was 3.6 and 3.3% at levels of 1.4 and 14.2 ng/ml, respectively. The interassay coefficient of variation was 4.6 and 3.7% at levels of 1.4 and 14.2 ng/ml, respectively. Recovery of human leptin added to a plasma pool was 105±4% (mean±SD). Serial dilutions of high level samples strictly parallel the standard curve.

Calculations. Ra, the rate of appearance of endogenous glycerol in plasma, and Rd, the rate of its disposal, were quantified in basal conditions and during the epinephrine infusion. Since individual glycerol concentrations and enrichments varied within < 10% of mean value during the last 20 min of each 60-min study period, we used a steady state dilution equation to calculate glycerol turnover (24). Ra and Rd were equal and expressed in $\mu mol \cdot m^2 \cdot min^{-1}$ to figure whole body glycerol fluxes. These fluxes representing lipid fuel production were normalized to fat mass in order to estimate adipose tissue lipolysis from body fat stores and its sensitivity to hormonal changes, as recommended (25, 26).

Data are presented as mean±SE. Statistical comparison between groups was performed with a two-tailed Student's *t* test for unpaired data. The relationship between variables was evaluated using simple linear regression analysis. We calculated the slopes of regression lines for each response variable (glycerol flux, plasma FFA, heart rate, or glucose) for each subject of the form log (response) = $m' \cdot [Epi] + b$. The slope of such a regression line represents the proportional increase in response to a unit change in [Epi]. The means±SE of these regression lines in obese versus nonobese children were calculated and compared by unpaired *t* test, according to the STS (NIH) method described by Feldman (27).

Polymorphism at position 64 of the β3 AR gene. Blood samples were drawn for the extraction of genomic DNA from leukocytes. Amplification of DNA by polymerase chain reaction (PCR) was carried out in a volume of 40 μl containing 200 ng of genomic DNA; 20 pmol each of the primers (forward 5′ CCAGTGGGCTGCCAGGGG 3′ and reverse 5′ GCCAGTGGCGCCCAACGG 3′); 200 mM each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythimidine triphosphate in 1.5 mM magnesium chloride; 10 mM Tris-hydrochloridic acid (pH 9.0); 50 mM potassium chloride; 0.1% Triton X-100; and 1 U of *Taq* polymerase (Appligène, Illkirch, France). The PCR reactions began with denaturation at 94°C for 10 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 30 s, with a final extension at 72°C for 10 min.

The amplified PCR products (248 bp) were digested with the addition of 10 mM Tris-hydrochloric acid, 10 mM magnesium chloride, 5 mM sodium chloride, dithiothreitol (pH 7.9), 100 mg/ml BSA, and 0.2 U of BstNI, a restriction enzyme specific for the sequence CC(A/T)GG (Biolabs, Montigny le Bretonneux, France); this mixture was incubated at 60°C for 2 h. The digested samples were separated by electrophoresis through a 3% agarose gel and visualized by staining with ethidium bromide. Digestion of the 248-bp product with BstNI produced fragments of the following sizes: 97, 64 and 61 bp in Trp64 homozygotes, 158, 97, 64, and 61 bp in Trp64/Arg64 heterozygotes, and 158 and 64 bp in Arg64 homozygotes.

Results

Plasma substrates and hormones

Basal state. Table II presents the basal plasma concentrations of substrates and hormones in the two studied groups. Basal glycerol and FFA concentrations were comparable in obese and nonobese children. All children, obese or nonobese, had normal fasting plasma glucose. Mean basal insulin was moderately elevated in the obese group. In obese children, as already observed in a previous study (15), plasma epinephrine was increased twofold ($P < 0.025$), compared with normal children. In contrast, norepinephrine was decreased by 35% ($P < 0.05$) in the obese children. Plasma leptin concentration was 24±2.5 ng/ml in the obese and 3±0.5 ng/ml in the nonobese children ($P < 0.01$). Leptin was positively correlated with fat mass ($r = 0.79$, $P = 0.0003$) and plasma epinephrine ($r = 0.56$, $P < 0.025$) and negatively with plasma norepinephrine ($r = 0.52$, $P < 0.05$).

In the nonobese children, low dose infusion of insulin increased plasma insulin level to a plateau averaging 94±8 pM, a value comparable to that observed in the obese patients in the basal state.

Epinephrine infusion. During the first step of epinephrine infusion, plasma epinephrine increased to 466±67 pg/ml in the obese and to 290±51 pg/ml in the nonobese children ($P < 0.02$), then to 606±68 pg/ml in the obese and 396±41 pg/ml in the nonobese children ($P < 0.02$) during the second step (Fig. 1). Norepinephrine concentrations remained unchanged. Plasma insulin increased slightly in obese (120±12 and 126±18 pM) and nonobese (106±10 and 121±8 pM) children.

Table II. Plasma Substrates and Hormones in the Children Studied in the Basal State after Overnight Fasting

	Obese children	Lean controls
	(n = 9)	*(n = 6)*
Plasma glucose (mmol/liter)	4.02±0.10	3.95±0.18
Plasma FFA (μmol/liter)	614±33	761±122
Plasma glycerol (μmol/liter)	83±13	91±14
Plasma insulin (pmol/liter)	102±4*	36±6
Serum epinephrine (pg/ml)	299±62*	150±22‡
Serum norepinephrine (pg/ml)	979±155‡	1507±234
Serum leptin (ng/ml)	24±2.5§	3±0.5

‡ $P < 0.05$; * $P < 0.025$; § $P < 0.01$.

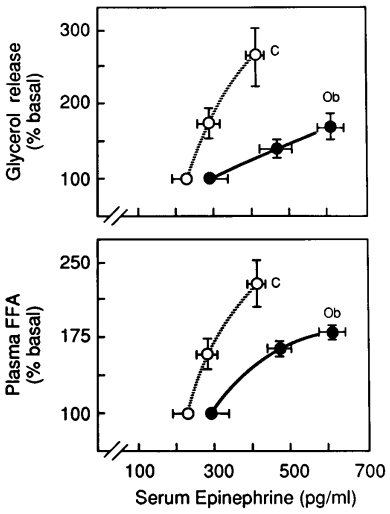

Figure 1. Dose–response curve of lipolytic rate (glycerol flux), and plasma FFA concentration, both expressed as the percentage of basal values, versus serum epinephrine levels in obese (●) and nonobese children (○). Data are means±SE. Results of statistical analyses are given in the text.

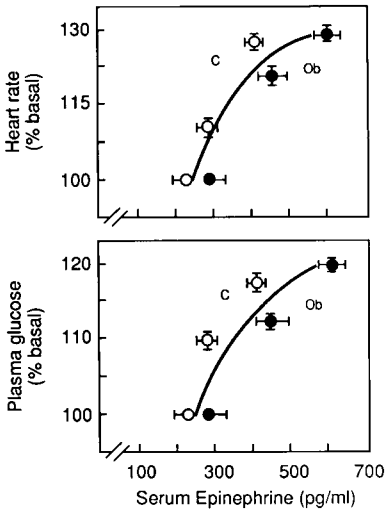

Figure 2. Dose–response curve of heart rate and plasma glucose concentration, expressed as the percentage of basal values, versus serum epinephrine levels in obese (●) and nonobese children (○). No significant differences in these measures were detected among the groups studied.

Plasma glycerol increased to 104±13 μM in obese subjects, and 140±12 μM in control subjects during step one, then to 137±12 μM in obese and 188±29 μM in control subjects during step two. Plasma FFA increased to 98±3 μM in obese and 135±19 μM in control children during step one, then to 105±5 μM in obese, 195±24 μM in control children during step two. The relative increase in plasma FFA was therefore only 149 and 172% of basal value in obese patients during epinephrine infusion, while it reached 170 and 246% in nonobese children ($P < 0.025$) (Fig. 1). From regression lines of the form log (FFA) = $m' \cdot [Epi] + b'$ (27), we calculated mean slopes to be $77 \pm 11 \cdot 10^{-5}$ in obese and $171 \pm 21 \cdot 10^{-5}$ in nonobese children ($P < 0.025$), indicating an important decrease of FFA release in response to epinephrine infusion.

Glycerol production

Basal. The total amount of glycerol produced in the basal state was higher in the obese (365±31 μmol·min^{-1}) than in the control children (289±22 μmol·min^{-1}, $P < 0.05$). But normalization to adipose tissue mass revealed that glycerol release per unit fat mass was in fact 32% lower in the obese (12.2±1.1 μmol·min^{-1}·kg fat mass^{-1}) than in the normal children (17.6±1.5 μmol·min^{-1}·kg fat mass^{-1}, $P < 0.05$).

Glycerol utilization expressed per unit of body surface or kilogram of lean body mass was comparable in obese and nonobese children, respectively 203±23 and 194±17 μmol·m^2·min, 8.3±0.9 and 8.6±0.7 μmol·kg lean body mass·min.

Epinephrine infusion. During the first step of epinephrine infusion, glycerol production increased to 480±40 μmol·min^{-1} (133% of basal value) in the obese and to 493±41 μmol·min^{-1} (170% of basal value) in the nonobese children, then to 557±52 μmol·min^{-1} (155% of basal value) in the obese and 721±64 μmol·min^{-1} (249% of basal value) in the nonobese during the second step. The relative increase of glycerol release during epinephrine infusion was therefore largely decreased in obese patients (Fig. 1). From regression lines of the form log (glycerol flux) = $m' \cdot [Epi] + b'$ (27), we calculated mean slopes to be $56 \pm 14 \cdot 10^{-5}$ in obese and $162 \pm 27 \cdot 10^{-5}$ in nonobese children ($P < 0.025$), indicating a decrease of glycerol release in response to epinephrine infusion. The magnitude of glycerol flux and FFA increases in response to epinephrine were closely correlated ($r = 0.84$, $P < 0.0001$).

Using the STS approach for comparison (27), we found that heart rate and plasma glucose showed comparable response to epinephrine infusion in the obese and nonobese groups, as presented in Fig. 2. Heart rate increased to 110±2 and 127±2% of basal value in the lean children, and to 117±2 and 129±2% in the obese children. Plasma glucose increased to 108±1 and 118±2% of basal values in the lean controls and 119±1% in the obese group.

Allelic variation at position 64 of the β3 AR gene. Among the obese and nonobese children, all except one child were found to be homozygotes for Trp64, the most frequent genotype. Only one obese child was an Arg64/Trp64 heterozygote; this child had a slightly subnormal response to epinephrine infusion. We therefore concluded that there was no relationship between the polymorphism at position 64 of the beta$_3$-adrenoreceptor and the diminished response of obese children to epinephrine infusion.

Discussion

During the dynamic phase of childhood onset obesity, this study indicates that the in vivo mobilization of triglyceride stores per unit of fat mass is decreased by ~ 30% in the basal state and is resistant to epinephrine action. While epinephrine infusion raising mean plasma level at 396±41 pg/ml resulted in a simultaneous 249–246% increase of the average glycerol release and FFA concentrations, respectively, in lean children, raising plasma epinephrine at 606±68 pg/ml in obese children resulted only in a 55–72% increase in glycerol release and plasma FFA. Note that such levels of epinephrine are near physiological. Both the maximal response and the ED_{50} characterizing the dose–response curve of glycerol release to serum epinephrine were diminished in the obese children.

Our measurement of the overall lipolytic rate in vivo reflects mainly the lipolytic effect of catecholamines on the subcutaneous fat depot which, due to its high proportion of the total fat mass, dominates the response quantitatively. In the obese children, CT scans show that > 85% of the adipose tissue is subcutaneous and evenly distributed on the trunk and on

gluteofemoral and proximal limb areas. Obese children, characterized by a massive overweight and a continuing rapid growth of adipose tissue, are known to have a hyperplastic or hypercellular form of obesity (4), i.e., an increased total number of fat cells. One unit of fat mass in obese children contains approximately the same number of adipocytes as in normal children (4). We previously confirmed these data in our patients by showing that their fat depots were made of numerous adipocytes of near-normal size and slightly increased lipid content (28). The fact that the heart rate and plasma glucose responded normally to epinephrine infusion indicates that obese children do not have a generalized resistance to epinephrine.

Basal plasma catecholamine values in our children deserve discussion. Using a sensitive HPLC technique similar to ours, Candito et al. (29) reported mean plasma concentrations of epinephrine of 135±36 pg/ml (SE) (range 18–568 pg/ml) in children studied in the same resting conditions as the present subjects. These values are comparable to the basal epinephrine values in our nonobese children (150±22 pg/ml). For additional comparison, other studies of basal plasma epinephrine concentrations in normal adolescents yielded mean values of 440 pg/ml using HPLC (30) and 30–41 pg/ml using a radioenzymatic method (31, 32). Mean resting norepinephrine values measured with HPLC were 442±91 pg/ml (SE) (range 85–1,923 pg/ml) (29) and 580 pg/ml (30) in normal children. Plasma concentrations of adolescents in the resting state measured with a radioenzymatic method were lower: 288±20 pg/ml (31) and 285±30 pg/ml (32). Our mean norepinephrine value in nonobese children (1,507±234 pg/ml) are at the upper limit of the normal range of HPLC measurements (29). These relatively high norepinephrine values in normal children were possibly due to a slight degree of unseen agitation in a subset of resting lean children, as discussed (29). In those normal children with the highest basal norepinephrine values, however, basal lipolysis and heart rate were not increased, nor were responses of these variables to epinephrine infusion decreased. Nevertheless it cannot be excluded that high norepinephrine concentrations at time 0 of experiment favored sympathetically mediated vasoconstriction, leading to modifications of tissue extraction of catecholamines. It is important in this respect that norepinephrine levels remained stable during the whole experimental period.

In obese children, basal plasma epinephrine concentrations were increased compared with lean controls, an observation consistent with epinephrine resistance. Obese children had decreased norepinephrine, indicating a lower degree of sympathetic activation than in normal children, which remained stable later during the study period.

The observed diminution of epinephrine-stimulated lipolysis could have several causes. An impaired ability of the hormone-sensitive lipase to be activated could be at the level of the enzyme molecule itself (33), or at any step in the regulation of lipolysis between the binding of catecholamines to their receptors and the hormone-sensitive lipase. In abdominal subcutaneous fat cells of normal weight first degree relatives of obese adults, Hellström et al. recently demonstrated a resistance of lipolysis to catecholamines, apparently associated with impaired hormone-sensitive lipase activation by cyclic AMP (13), suggesting that this alteration could predispose to obesity.

Deficient lipolysis could also be due to a supranormal activity of the $alpha_2$-adrenoreceptors in the adipocytes of our obese patients. Constitutively activated $alpha_2$-adrenoreceptors obtained by directed mutagenesis can inhibit lipolysis (34), and an enhanced $alpha_2$-adrenergic responsiveness, without changes in beta adrenergic responses, has been described in abdominal subcutaneous adipose tissue of obese men and women (35). Adipocytes obtained from normal chidren have a higher content of $alpha_2$-adrenoreceptors than adipocytes from adults (36). No data, however, are yet available to support a role of these receptors in the catecholamine resistance of early-childhood obesity.

Another hypothesis is that the lipolysis resistance to epinephrine observed in the obese children is due to a deficient signal transduction by beta-adrenoreceptors. A decreased function of $beta_2$-adrenoreceptors has been reported in subcutaneous fat cells of adult obese patients (10). Regarding the $beta_3$-adrenoreceptor, there is still controversy as to how much it contributes to lipolysis regulation in man (5, 37). In lean young women, $beta_3$-adrenoreceptors are expressed in subcutaneous white adipocytes, but represent only 20% of $beta_1$ and $beta_2$-adrenoreceptor transcripts and have little lipolytic effect (38). The association of a $beta_3$-adrenergic receptor variant with obesity in genetically distinct populations, including Finns (39), Pima Indians (40), and French Caucasians (41) might suggest a consistent functional defect. This variant, however, has been associated with body mass index in one study, degree of insulin resistance in another, and early age of onset in a third, suggesting differences in the ultimate phenotype depending on the population genetic background. In our childhood-onset obesity model, the polymorphism in position 64 of the $beta_3$-adrenoreceptor was clearly not associated with the observed defect of lipolysis.

Since obese children have increased epinephrine levels in the basal state, another mechanism for catecholamine resistance could be the desensitization of beta-adrenergic receptors (42). Elevated levels of circulating insulin could also play a role, since downregulation of beta-adrenoreceptors can be acutely induced in human subcutaneous cells by insulin (43). However, long-term experiments in human or rat adipose tissue kept in primary culture failed to show insulin effects upon hormone-sensitive lipase or catecholamine-induced lipolysis (44, 45). The fasting level of insulin was slightly increased in our obese children, as previously reported (15, 18), but we found no correlation between insulin levels and the decreased sensitivity to epinephrine. Note in addition that during the present study, low dose insulin was infused in the lean children to compensate for their lower insulin levels. In conclusion, although chronic moderate hyperinsulinemia does not appear as an important causative factor for the observed resistance of lipolysis to epinephrine, its possible participation cannot be excluded.

Previous in vivo studies reported decreased lipolytic responses to catecholamine infusions in obese adults (7–9). In upper-body massively obese adults, the lipolytic sensitivity to epinephrine and norepinephrine is impaired in subcutaneous adipocytes (10, 11). In contrast, the catecholamine-induced FFA mobilization from omental fat cells is accelerated, due to an increased $beta_3$-receptor function and partly to a decreased $alpha_2$-adrenoreceptor function (24). These studies also indicate regional differences in the alterations of lipolysis associated with massive long-term obesity: increased catecholamine action in the visceral depot but decreased action in the subcutaneous fat depot. However, to our knowledge none of these

studies were carried out in patients still accumulating fat, nor longitudinally during the evolution of obesity. It is therefore impossible to know at which point during the evolution of obesity the resistance to catecholamines was acquired; before the onset, as part of the evolution, or as an end result of obesity.

Our data are the first to support that epinephrine resistance may be a major pathogenic feature of morbid forms of childhood-onset obesity. By limiting lipid mobilization, it could directly favor the abnormal accumulation of fat depots which is associated with accelerated adipogenesis (4).

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