Original Article

Adipocyte Lipases and Defect of Lipolysis in Human Obesity

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The mobilization of fat stored in adipose tissue is mediated by hormone-sensitive lipase (HSL) and the recently characterized adipose triglyceride lipase (ATGL), yet their relative importance in lipolysis is unknown. We show that a novel potent inhibitor of HSL does not inhibit other lipases. The compound counteracted catecholamine-stimulated lipolysis in mouse adipocytes and had no effect on residual triglyceride hydrolysis and lipolysis in HSL-null mice. In human adipocytes, catecholamine- and natriuretic peptide–induced lipolysis were completely blunted by the HSL inhibitor. When fat cells were not stimulated, glycerol but not fatty acid release was inhibited. HSL and ATGL mRNA levels increased concomitantly during adipocyte differentiation. Abundance of the two transcripts in human adipose tissue was highly correlated in habitual dietary conditions and during a hypocaloric diet, suggesting common regulatory mechanisms for the two genes. Comparison of obese and nonobese subjects showed that obesity was associated with a decrease in catecholamine-induced lipolysis and HSL expression in mature fat cells and in differentiated preadipocytes. In conclusion, HSL is the major lipase for catecholamine- and natriuretic peptide–stimulated lipolysis, whereas ATGL mediates the hydrolysis of triglycerides during basal lipolysis. Decreased catecholamine-induced lipolysis and low HSL expression constitute a possibly primary defect in obesity. *Diabetes* 54:3190–3197, 2005

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ATGL, adipose triglyceride lipase; BAY, 4-isopropyl-3-methyl-2-[1-(3-(S)-methyl-piperidin-1-yl)-methanoyl]-2*H*-isoxazol-5–1; FFA, free fatty acid; HSL, hormone-sensitive lipase; KRBA, Krebs-Ringer bicarbonate buffer containing albumin; MOME, 1(3)-monooleoyl-2-0-monooleyl glycerol.

Obesity, which is characterized by an excess of fat stores, is the most important risk factor for type 2 diabetes. Adipose tissue lipolysis leads to the hydrolysis of triglycerides and release of free fatty acids (FFAs). Because of the link between elevated circulating FFA levels and the development of insulin resistance and the metabolic syndrome (1,2), adipose tissue lipolysis constitutes a target for the drug industry. Nicotinic acid, which acts by inhibiting adipose tissue lipolysis, was the first extensively used lipid-lowering agent (3). Catecholamines and natriuretic peptides are the major hormones stimulating this catabolic pathway in humans (4). Resistance to catecholamine-induced lipolysis in subcutaneous adipose tissue has been demonstrated in obese adults and children (5,6) and is attributed to decreased expression of lipolytic β_2-adrenoceptors (7), increased antilipolytic properties of α_2-adrenoceptors (8), and decreased expression of hormone-sensitive lipase (HSL) (9). It is possible that the HSL defect is the most important factor because it is also observed in nonobese first-degree relatives to obese subjects (10) and because there is a positive relationship between lipolytic capacity and HSL expression in human subcutaneous fat cells (11).

The rate-limiting role of HSL in adipose tissue lipolysis has been challenged by the data from HSL knockout mice (12–15). Catecholamine-induced lipolysis is abrogated, but residual basal lipolysis is observed in adipocytes from HSL-null mice. These data suggest the existence of non-HSL lipases in adipose tissue. Recently, a novel triglyceride lipase termed adipose triglyceride lipase (ATGL), desnutrin, or iPLA2ζ has been identified (16–18). Using antibodies directed against ATGL, Zimmerman et al. (16) suggest that ATGL is responsible for 75% of the cytosolic acylhyrolase activity in white adipose tissue of HSL-deficient mice. ATGL could therefore participate together with HSL in adipose tissue lipolysis. Carboxylesterase 3 (or triacylglycerol hydrolase) was partially purified from mouse white adipose tissue and showed to possess lipase activity (19). Its contribution to lipolysis is not known. Here, we studied the relative contribution of HSL and other lipases to adipose tissue lipolysis and sought to determine whether alterations of adipocyte lipolysis and HSL expression were present in obesity.

RESEARCH DESIGN AND METHODS

Lipase enzymatic activities. A series of (5-(2H)-isoxazolonyl) ureas has been developed by Bayer as potent inhibitors of HSL (20). The selectivity of compound 59 (4-isopropyl-3-methyl-2-[1-(3-(*S*)-methyl-piperidin-1-yl)-meth-

anoyl]-2*H*-isoxazol-5-one), thereafter named BAY, was evaluated on enzymatic activities of purified lipase preparations using 1(3)-monooleoyl-2-0-monooleyl glycerol (MOME) for rat and human HSL, monoolein for mouse monoglyceride lipase, and triolein for bovine lipoprotein lipase and porcine pancreatic lipase as substrates (21,22). MOME is a diacylglycerol analog. It allows measurement of diacylglycerol hydrolase activity and is not a substrate for monoacylglycerol lipase. Cos7 cells were transfected with the pcDNA3, pcDNA3-human HSL, and pcDNA3-human ATGL vectors (17,23). Triolein hydrolysis was measured on cellular extracts.

Generation and analysis of HSL-null mice. HSL-null mice were generated by targeted disruption of the HSL gene in 129SV-derived embryonic stem cells (14,24). The animals were killed after an overnight fast according to Institut National de la Santé et de la Recherche Médicale animal care ethical guidelines. Adipose tissue samples from wild-type and HSL-null mice were homogenized in 4 vol homogenization buffer (0.25 mol/l sucrose, 1 mmol/l EDTA, pH 7.0, 1 mmol/l dithioerythritol, 20 μg/ml leupeptin, and 20 μg/ml antipain) and centrifuged at 15,000*g* at 4°C for 30 min, to obtain fat-free infranatants on which in vitro enzymatic activities were performed (21). Protein concentrations were determined using the Bio-Rad Protein Assay. Isolated adipocytes were obtained by digestion of visceral fat pads with Liberase Blendzyme 3 (Roche) in Krebs-Ringer bicarbonate buffer containing albumin (KRBA) (3.5 g/100 ml), glucose (108 mg/100 ml), and HEPES (238 mg/100 ml) at pH 7.4 under vigorous shaking at 37°C. Then, the fat cells were filtered through a nylon screen and washed with KRBA buffer to eliminate Liberase. Isolated adipocytes were brought to a 1/20 dilution in KRBA buffer with 1 unit/ml adenosine deaminase (Roche) and 100 nmol/l phenylisopropyladenosine (Sigma-Aldrich) for lipolysis assays and incubated with the pharmacological agents in a final volume of 100 μl for 90 min at 37°C under gentle shaking. Glycerol and FFAs were measured by a spectrophotometric assay (25) and the NEFA C kit (Wako), respectively. Total lipid was determined gravimetrically after solvent extraction (26).

Study cohorts. A first cohort included 14 obese men and 67 obese women aged 36 ± 8 years with BMI 37 ± 5 kg/m^2 and 29 nonobese women and 13 nonobese men aged 36 ± 9 years with BMI 24 ± 3 kg/m^2. In the morning after an overnight fast, a venous blood sample was obtained for analysis of serum leptin, plasma insulin, and plasma glucose (7,9,10); and a large subcutaneous fat biopsy (3–5 g) was obtained from the abdominal region by needle aspiration under local anesthesia. A second cohort included 80 healthy obese women with BMIs of 31–52 kg/m^2 and ages of 21–63 years. A third cohort included subjects from the European multicenter study NUGENOB (Nutrient-Gene Interactions in Human Obesity; www.nugenob.org). The 24 obese women were 31 ± 7 years of age with BMI 37 ± 5 kg/m^2. The third cohort subjects followed a 10-week program based on energy-restricted diet providing 600 kcal below the daily total energy expenditure. After dietary intervention, BMI decreased to 34 ± 5 kg/m^2 ($P < 0.0001$). For the second and third cohorts, biopsies of ~1 g subcutaneous abdominal adipose tissue were performed after an overnight fast. In a methodological study, we wanted to compare basal lipolysis in various adipose tissue preparations. Subcutaneous adipose tissue was obtained by needle biopsy from 251 healthy subjects (147 obese women, 56 nonobese women, 33 obese men, and 15 nonobese men). Finally, subcutaneous adipose tissue was also obtained during elective surgery under general anesthesia for nonmalignant disorders from subjects not selected on the basis of age, sex, or BMI. The studies were approved by the hospitals' committees on ethics in Toulouse and Stockholm. Individual informed consent was obtained.

Determination of mRNA levels. Total RNA was extracted using the RNeasy total RNA Mini kit (Qiagen). Integrity of total RNA was checked using an Agilent 2100 bioanalyzer (Agilent Biotechnologies). After reverse transcription of 1 μg total RNA, real-time quantitative polymerase chain reaction was performed on a GeneAmp 7000 Sequence Detection System (Applied Biosystems) or a iCycler IQ (Bio-Rad Laboratories). For each primer pair, a standard curve was obtained using serial dilutions of human adipose tissue cDNA before mRNA quantitation. 18S rRNA was used as a control to normalize gene expression.

Studies on freshly isolated human fat cells and adipose tissue pieces. One portion (~300 mg) of adipose tissue was used to study leptin release (27). The remaining adipose tissue was used for lipolysis experiments after isolation of fat cells by collagenase digestion (10). Glycerol in the medium was determined as described previously (28). FFAs were quantified using a sensitive chemiluminescence method (29). Glycerol and FFA release were expressed in relation to the lipid weight of the incubated cells, the amount of adipocyte proteins or per cell number. Fat-cell size and number were determined as described previously (10). The concentration of proteins did not differ between nonobese and obese subjects (108 ± 11 and 112 ± 14 ng/100 μl fat cells, $n = 8$ and 14, respectively). In the methodological study of basal lipolysis on 251 subjects, subcutaneous adipose tissue was cut into small pieces (10–20 mg) and incubated under basal conditions in the same type of albumin buffer as used for isolated fat cells (300 mg tissue/300 ml albumin buffer). Glycerol release was determined after 2 h of incubation and related to the amount of lipids.

Studies on human preadipocytes. Differentiation of preadipocytes was carried out as described previously (30). In brief, cells from the stromavascular fraction of collagenase-treated adipose tissue were isolated and differentiated in 22-mm wells using serum-free culture medium supplemented with 10 μmol/l rosiglitazone and 0.2 mmol/l 3-isobutyl-1-methylxanthine for 3 days and maintained in culture medium without rosiglitazone and the methylxanthine until the end of differentiation. On average, the cells of obese or nonobese subjects were investigated at day 15. Apparent fat-cell size of the differentiated preadipocytes was determined in the last 50 included subjects (12 nonobese and 38 obese subjects). The lipids formed microdroplets, which accumulated into one ellipsoid cluster. The area of each "lipid ellipse" was measured with a calibrated microscope, and mean area in each subject was determined from 10 to 26 measures and represented fat cell size. The average number of measurements in each obese and nonobese subject was similar (50 ± 25 and 60 ± 30, respectively). Lipolysis was performed as described previously (30). The glycerol concentration was determined in the medium. The average protein content was similar in obese (19 ± 6 μg/well) and nonobese (19 ± 8 μg/well) subjects. Leptin in the medium was measured using Quantikine Human Leptin Immunoassay (R&D Systems). We used cell protein content determined using BCA Protein Assays Reagent kit (Pierce) as denominator for lipolysis and leptin secretion. The protein content reflected the amount of adipocytes because the same level of differentiation was obtained in the two groups. On 7 nonobese and 14 obese subjects, it was possible to isolate 100 μg of proteins from three pooled incubation wells. These proteins were separated by SDS-PAGE and transferred to polyvinylidine fluoride membranes by Western blot. Two blots were performed. The blots were probed with antibodies against human HSL (9) and β_2-adrenoceptor (Santa Cruz). The proteins were detected with chemiluminiscence, and the band intensity was assessed by the National Institutes of Health Image program.

Statistical methods. The limiting factor in the first cohort was the amount of protein available for Western blot from the differentiated preadipocytes. Based on previous studies (30), we estimated that sufficient amounts of protein (100 μg) could be obtained from ~20% of the subjects and that it would be twice as easy to recruit obese rather than nonobese subjects because biopsies of very large amounts of adipose tissue need to be performed. Using published data on HSL expression level in adipose tissue (31), we calculated that a 50% difference in protein expression could be detected with 80% power at $P < 0.05$ with 14 obese and 7 nonobese subjects. This meant a total recruitment of >80 obese and 40 nonobese subjects. Values are means ± SD in text and means ± SE in figures. The data were compared by Wilcoxon, Mann-Whitney, or Kruskal-Wallis test.

RESULTS

Effect of an inhibitor of HSL on lipases and lipolysis. The role of HSL and non-HSL lipases in acylglycerol hydrolysis and adipose tissue lipolysis was investigated using BAY, an isoxazolone inhibitor of HSL (20). BAY specificity was determined using purified preparations of lipases (Fig. 1*A*). Human and rat HSL were potently inhibited in a concentration-dependent fashion, whereas no effect was observed on pancreatic lipase, lipoprotein lipase, and monoglyceride lipase. Importantly, BAY had no effect on extracts from Cos7 cells expressing ATGL, a recently identified adipose tissue triglyceride hydrolase that may participate in fat mobilization (Fig. 1*B*) (16). The effect of BAY was tested on the enzyme activity of adipose tissue extracts from wild-type and HSL-null mice. In wild-type mice, BAY caused a concentration-dependent decrease of triolein hydrolysis (Fig. 1*C*). At 1 μmol/l, BAY induced a 90% inhibition in extracts from wild-type mice, whereas no inhibition was observed in HSL-null mice (Fig. 1*D*). The residual enzyme activity observed in HSL-null animals was similar to the triglyceride hydrolase activity seen after BAY inhibition in wild-type mice. Thus, BAY does not inhibit non-HSL triglyceride lipases. Lipolysis assays were performed on isolated adipocytes from wild-type and HSL-null mice (Fig. 2). Inactivation of the HSL gene revealed that there was only a partial ablation of

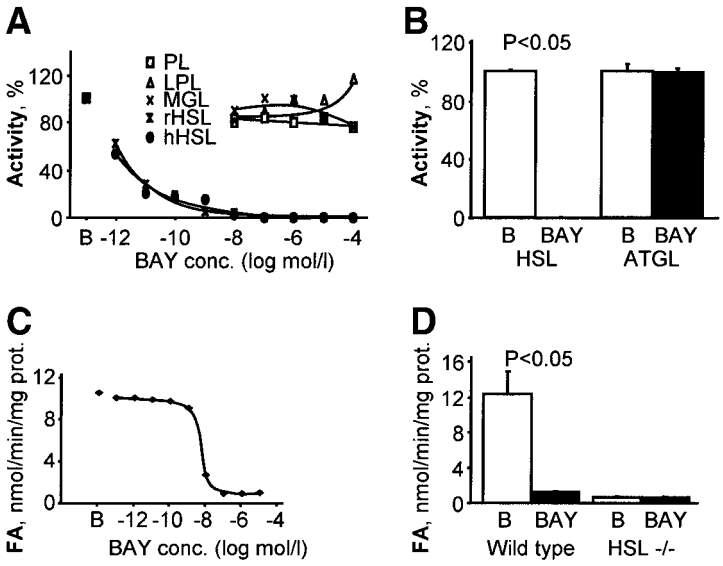

FIG. 1. Selectivity of BAY for inhibition of HSL. *A*: Concentration-response inhibition by BAY of purified human HSL (hHSL) or rat HSL (rHSL), pancreatic lipase (PL), lipoprotein lipase (LPL), and monoglyceride lipase (MGL) enzymatic activities ($n = 3$). *B*: BAY (10^{-6} mol/l) inhibition of triglyceride hydrolase activity in Cos7 cells expressing human HSL or human ATGL ($n = 3$). *C*: Concentration-response inhibition by BAY of mouse adipose tissue extract triglyceride hydrolase activity ($n = 3$). *D*: BAY (10^{-6} mol/l) inhibition of triglyceride hydrolase activity in wild-type and HSL-null mouse adipose tissue extracts ($n = 4$).

lipolysis in fat cells. BAY at 10 nmol/l partially inhibited isoprenaline-induced glycerol release, whereas 1 μmol/l BAY had an almost full inhibitory effect (Fig. 2*A*). BAY caused a concentration-dependent inhibition of isoprenaline-induced glycerol release in fat cells from wild-type mice, whereas basal lipolysis was not influenced (Fig. 2*B*). In HSL-null mice, isoprenaline did not induce lipolysis, and BAY had no inhibitory effect. Similar results were obtained for glycerol and FFA release (Fig. 2*B* and *C*). These inhibition data show that β-adrenoceptor–mediated lipolysis is entirely mediated by HSL in mouse fat cells, whereas a non-HSL lipase contributes to basal lipolysis.

Lipolysis studies on freshly isolated human fat cells are presented in Fig. 3. Isoprenaline concentration-response curves were shifted to the right using maximally effective antilipolytic concentrations of insulin and prostaglandin E2 with a preserved maximal lipolytic effect of the β-adrenoceptor agonist (Fig. 3*A*). This represents the classical decrease of agonist potency induced by antilipolytic molecules acting at the receptor level. In contrast, blockade of HSL activity by BAY caused an inhibition of the maximal glycerol release induced by isoprenaline. These data are consistent with the notion that the enzyme catalyzes the rate-limiting step of adipocyte lipolysis. At 1 μmol/l BAY, basal glycerol release was markedly reduced in contrast to the findings in mice. We also compared the effect of increasing concentrations of BAY on the inhibition of basal, atrial natriuretic peptide–induced (10^{-7} mol/l), and isoprenaline-induced (10^{-7} mol/l) glycerol release (Fig. 3*B*). Lipolysis was reduced in a concentration-dependent manner by BAY. Half-maximal effective concentration of BAY was $\sim 10^{-8}$ mol/l. We also investigated, in parallel, inhibition of glycerol and fatty acid release (Fig. 3*C*). BAY inhibited isoprenaline- and atrial natriuretic peptide–induced glycerol and fatty acid release. However, basal glycerol release but not basal fatty acid release was blunted by the inhibitor. Experiments investigating the effect of BAY on human fat cell lipolysis were independently performed in two laboratories and gave similar results (data not shown). To determine whether the procedure to isolate fat cells modified basal lipolysis, basal glycerol release from subcutaneous adipose tissue pieces and isolated adipocytes were compared (supplementary Fig. 1, online appendix [available at http://diabetes.diabetesjournals.org]). A strong positive correlation was observed between the two preparations ($r = 0.6$; $P < 0.001$).

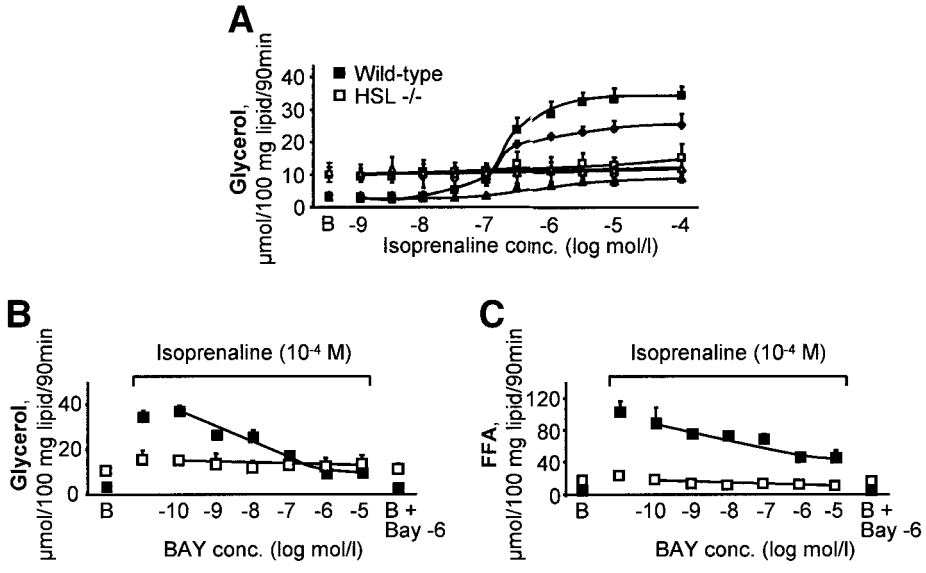

FIG. 2. Lipolysis in isolated adipocytes from wild-type and HSL-null mice. *A*: Glycerol release ($n = 5$). Concentration-response curves of isoprenaline were performed without BAY (squares), with 10^{-8} mol/l BAY (diamonds), or with 10^{-6} mol/l BAY (triangles). *B*: Glycerol release ($n = 3$). Concentration-response inhibition of BAY was measured on lipolysis induced by 10^{-4} mol/l isoprenaline. *C*: FFA release ($n = 3$). Concentration-response inhibition of BAY was measured on lipolysis induced by 10^{-4} mol/l isoprenaline.

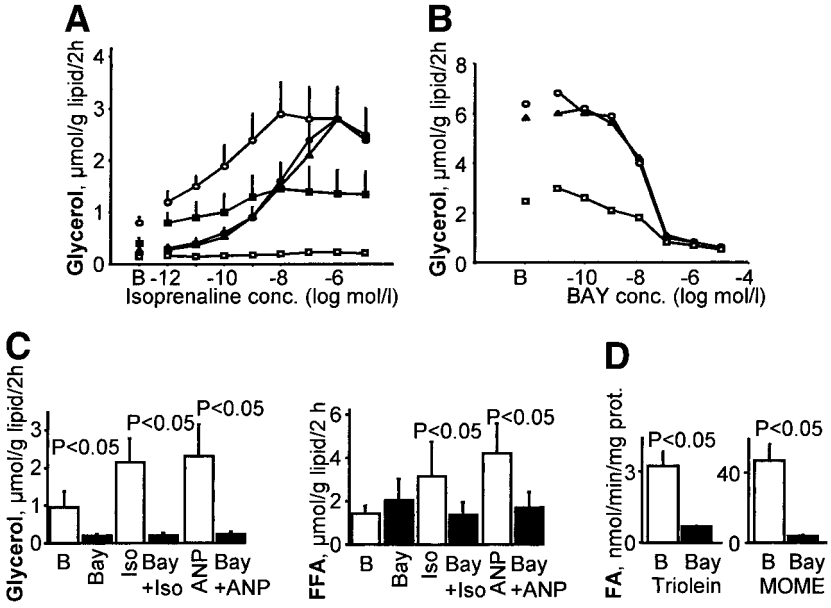

FIG. 3. Effect of BAY on human fat cell lipolysis. *A*: Antilipolytic effects of BAY (■, 10^{-10} mol/l; □, 10^{-7} mol/l), insulin (●, 10^{-8} mol/l), and prostaglandin E2 (△, 10^{-5} mol/l) on isoprenaline-induced glycerol release. ○, dose-response curve of isoprenaline without antilipolytic agents (n = 4). *B*: Effect of BAY on basal (□), isoprenaline-induced (○, 10^{-7} mol/l), and atrial natriuretic peptide–induced (▲, 10^{-7} mol/l) glycerol release (n = 3). *C*: Inhibition by BAY (10^{-5} mol/l) of basal, isoprenaline-induced (Iso, 10^{-7} mol/l), and atrial natriuretic peptide–induced (ANP, 10^{-7} mol/l) glycerol and FFA release (n = 6). *D*: BAY (10^{-6} mol/l) inhibition of triglyceride (triolein) and diglyceride (MOME) hydrolase activity in human adipose tissue extracts (n = 4).

However, the rate of glycerol release was ~50% more rapid from isolated cells than tissue pieces. A similar relationship was obtained when nonobese and obese were analyzed separately (r = 0.54 and r = 0.57, respectively). Although the true basal lipolysis rate may be overestimated because collagenase isolation may relieve antilipolysis induced by inhibitory factors produced by cells of the stromavascular fraction, mechanistic studies of basal lipolysis on isolated fat cells are valid. BAY inhibited a large fraction of triglyceride hydrolysis and the entirety of diglyceride hydrolysis in human adipocyte extracts (Fig. 3*D*). These results suggest that HSL mediates the hydrolysis of triglycerides under stimulated conditions and the hydrolysis of diglycerides under both stimulated and basal conditions.

HSL and ATGL gene expression in human adipose tissue. We studied the relationship between ATGL and HSL gene expression in human adipose tissue. ATGL and HSL mRNA were found to be highly expressed in mature adipocytes but not in the stromavascular fraction that contains preadipocytes, endothelial cells, resident macrophages, and lymphocytes (Fig. 4*A*). ATGL and HSL mRNA expression increased concomitantly during the course of conversion of human preadipocytes to adipocytes (Fig. 4*B*). The expression of the two lipase transcripts was investigated in subcutaneous adipose tissue from a cohort of 80 obese women. HSL and ATGL mRNA levels were strongly correlated (r = 0.8, $P < 0.0001$) (Fig. 4*C*). On an independent group of 24 women, the positive correlation was confirmed in habitual dietary conditions (r = 0.6, $P < 0.005$) but also observed after a 10-week hypocaloric diet (r = 0.9, $P < 0.0001$). The individual variations induced by the hypocaloric diet were highly correlated (r = 0.8, $P < 0.0001$) (Fig. 4*D*). The relationship in the expression of the two genes is specific because no correlation was observed between ATGL or HSL mRNA and the transcript for adiponutrin, a protein with high homology to ATGL also expressed in adipose tissue (data not shown) (18). It appears therefore that HSL and ATGL are tightly coregulated in human adipose tissue.

Comparison of nonobese and obese subjects. Having established the essential role of HSL in catecholamine- and

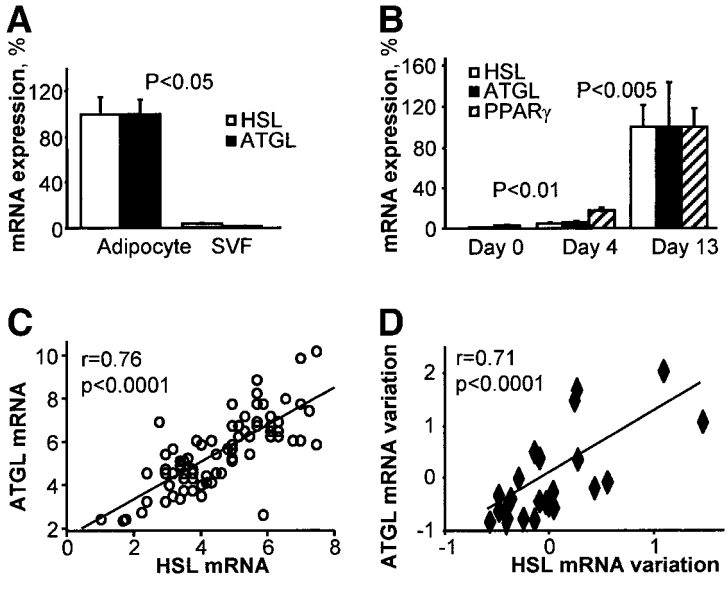

FIG. 4. HSL and ATGL gene expression in human adipose tissue. HSL and ATGL mRNA levels were quantified by reverse transcription-quantitative PCR and normalized with 18S rRNA levels. *A*: HSL and ATGL mRNA levels in isolated adipocytes and stromavascular fraction (SVF) from human adipose tissue (n = 6). *B*: Expression of HSL, ATGL, and peroxisome proliferator–activated receptor γ (PPARγ) mRNA during human preadipocyte differentiation (n = 5). *C*: Relationship between HSL and ATGL mRNA levels in subcutaneous adipose tissue from 80 obese women. *D*: Relationship between HSL and ATGL mRNA variation during a hypocaloric diet in subcutaneous adipose tissue from 24 obese women. HSL and ATGL mRNA levels were measured before and after a 10-week hypocaloric diet. The variations are calculated as follows for each individual: (mRNA level after the diet − mRNA level before the diet)/mRNA level before the diet.

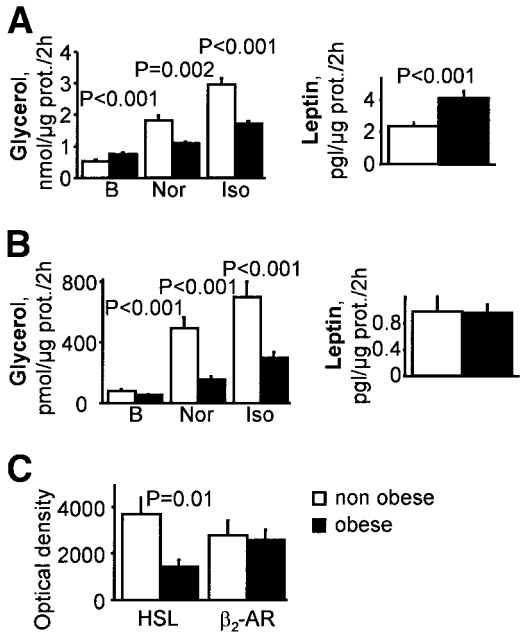

FIG. 5. Lipolysis, leptin secretion, and HSL expression in mature adipocytes and differentiated preadipocytes from obese and lean subjects. *A*: Glycerol and leptin release in isolated mature adipocytes from 42 lean and 81 obese subjects. *B*: Glycerol and leptin release in preadipocytes differentiated in primary culture from 42 lean and 81 obese subjects. *C*: Protein expression of HSL and β_2-adrenoceptor (β_2-AR) in differentiated human preadipocytes from 14 obese and 7 lean subjects determined by Western blot analysis.

natriuretic peptide–stimulated human fat cell lipolysis, we investigated whether defects of adipocyte lipolysis and HSL expression were found in human obesity. A population of 81 obese and 42 nonobese subjects was studied. Circulating leptin concentrations were 37 ± 33 ng/ml in the obese subjects and 12 ± 12 ng/ml in the nonobese subjects ($P < 0.001$). Corresponding values for plasma insulin were 12.5 ± 6.9 and 6.6 ± 3.6 mU/l ($P < 0.001$) and for plasma glucose, 5.4 ± 1.9 and 4.9 ± 0.5 mmol/l, respectively ($P < 0.001$).

Lipolysis data from mature fat cells and preadipocytes differentiated in primary culture were compared in Fig. 5. We also measured leptin secretion as a control adipocyte function. In freshly isolated mature fat cells, catecholamine-induced (norepinephrine) and β-adrenoceptor–induced (isoprenaline) lipolysis were decreased by 40% ($P = 0.002$ and $P < 0.001$, respectively) in obesity (Fig. 5*A*). Leptin secretion was increased by 80% ($P < 0.001$). We expressed lipolysis data per protein content because, first, the protein concentration is similar in large mature adipocytes from obese subjects and small mature adipocyte from lean subjects (32); and, second, it allowed a comparison of data between mature fat cells and differentiated preadipocytes. Lipolysis data were also expressed as glycerol release per amount of incubated lipids (supplementary Table 1, online appendix). Results were as described for lipolysis per protein content. When results were presented per cell number, catecholamine-induced lipolysis was not different between nonobese and obese subjects, although the basal rate was twofold increased in obesity ($P < 0.001$). The discrepancy between per cell number as denominator compared with other denominators is due to the large increase in cell size observed in obesity. This well-known phenomenon and its interpretation have been extensively discussed (9). In the present study, fat cell volume (picolitres) was 490 ± 195 in nonobese and 800 ± 150 in obese ($P < 0.001$) subjects. To avoid the confounding influence of the mode of expression on lipolysis data, catecholamine-induced lipolysis was also expressed as a ratio of basal lipolysis. Norepinephrine- and isoprenaline-induced lipolysis was blunted in obese subjects (supplementary Table 1, online appendix). Similar results were observed when a subgroup analysis of the women was performed (data not shown). Taken together, these data confirm and extend to a large cohort of subjects earlier observations of impaired catecholamine-induced lipolysis and increased leptin secretion in fat cells from subcutaneous adipose tissue of obese subjects (7,27).

To find out whether the lipolysis defect is primary or secondary to obesity, we used preadipocytes prepared from the same biopsies as the mature fat cells. The fraction of preadipocytes that differentiated into adipocytes was similar in the nonobese and in the obese subjects: 60 ± 20% and 57 ± 18%, respectively. The size of the cells was also similar in the obese and in the nonobese subjects: $1.8 \pm 0.4 \times 10^{-9}$ m^2 and $1.5 \pm 0.3 \times 10^{-9}$ m^2, respectively. Differentiated preadipocytes displayed similar leptin secretion in obese and nonobese subjects, whereas norepinephrine- and isoprenaline-stimulated lipolysis was reduced by 60–70% in the obese subjects ($P < 0.001$) (Fig. 5*B*). As a ratio of stimulated to basal lipolysis, a similar pattern was found (supplementary Table 1, online appendix). Thus, a blunted lipolysis without a change in leptin secretion in preadipocytes from obese subjects suggests that altered lipolysis is not secondary to the obese state.

We have previously shown a decreased HSL expression in mature subcutaneous fat cells of obese subjects (9). The very limited amount of stromavascular cells precluded protein analysis on preadipocytes of all subjects. However, power calculation indicated that we could detect differences between obese and nonobese subjects with 14 obese and 7 nonobese subjects (see STATISTICAL METHODS). HSL expression was decreased by 60% in cells from obese subjects (Fig. 5*C*). There was no difference between groups in the protein expression of the β_2-adrenoceptor.

DISCUSSION

The hydrolysis of the triglycerides stored in the fat cell is a complex phenomenon involving lipases and proteins associated with the lipid droplet (4,33). Based on the present data and earlier reports, the following model for lipase activation can be proposed in human fat cells (Fig. 6). ATGL and HSL both possess the capacity to hydrolyze triglycerides in vitro (16,34). However, only HSL shows a significant diglyceride lipase activity (35). Although HSL has the capacity to hydrolyze monoglycerides in vitro, monoglyceride lipase, which is not under hormonal control, is required to obtain complete hydrolysis of monoglycerides in vivo (36). Triglycerides are hydrolyzed at a lower rate than diglycerides, indicating that the first step of lipolysis is rate limiting (37). In human fat cells, catecholamines and natriuretic peptides stimulate lipolysis through β-adrenoceptors and the natriuretic peptide receptor A, respectively. Stimulation of the two pathways leads to an increase in cAMP and cGMP levels, respectively (4). Both protein kinases A and G phosphorylate and activate HSL at least in part through translocation of the enzyme from the cytosol to the lipid droplet (38–40). In line with the role of HSL in triglyceride hydrolysis under

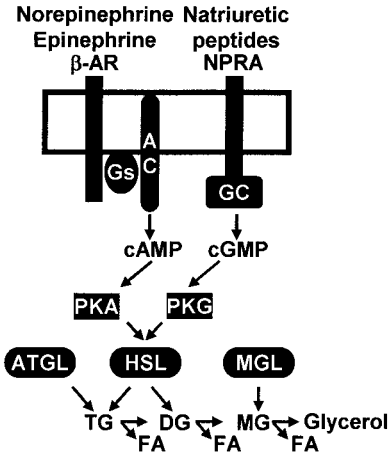

FIG. 6. Model for stimulatory pathways in human adipose tissue lipolysis. For the sake of clarity, important nonenzymatic modulators of the lipolytic process such as perilipins are not shown. AC, adenylyl cyclase; AR, adrenoceptor; DG, diglyceride; FA, fatty acid; GC, guanylate cyclase; Gs, stimulatory G protein; MG, monoglyceride; MGL, monoglyceride lipase; NPRA, natriuretic peptide receptor A; PKA, protein kinase A; PKG, protein kinase G; TG, triglyceride.

stimulated conditions, ATGL is not phosphorylated by protein kinase A and does not appear to be acutely regulated by isoprenaline (16). Our data clearly demonstrate that the two major and independent activation pathways converge on HSL. However, our data show that non-HSL lipases contribute to the hydrolysis of triglycerides into diglycerides under basal conditions. Although ATGL seems to play a predominant role in basal lipolysis, it cannot be excluded that other adipose tissue enzymes with the capacity to hydrolyze triglycerides play a role (16,17,19). The coordinated variation in ATGL and HSL gene expression during adipocyte differentiation and in various dietary conditions suggests that the two genes belong to a common regulatory network with tight transcriptional control.

Primary alterations in the ability to mobilize lipids stored in adipose tissue could occur in obesity. Moreover, a primary defect in leptin secretion could be present and induce a leptin-resistant state with inadequate feedback signals between caloric intake and energy demand. In freshly isolated fat cell preparations, we could confirm earlier studies demonstrating increased leptin secretion and decreased catecholamine-induced lipolysis in obesity (7,27). In differentiated preadipocytes, catecholamine-induced lipolysis was markedly reduced although leptin secretion was normal. These findings were obtained in a very large cohort (81 obese and 42 nonobese subjects). Because the preadipocytes were differentiated in vitro and kept in serum-free medium for 2 weeks, it is very likely that any confounding environmental influence could be excluded (41). This notion is further supported by the finding that basal lipolysis was increased in freshly isolated fat cells but decreased in differentiated preadipocytes of the obese subjects. It has recently been demonstrated that obesity is characterized by a low-grade inflammatory state of adipose tissue with an increase in macrophage number (42,43). It may be argued that macrophage-derived cytokines could influence lipolysis in adipose tissue of the obese subjects and explain the differences between lean and obese subjects. However, data from catecholamine-induced lipolysis were similar in freshly isolated fat cells and differentiated preadipocytes. From the different cell types of the stromavascular fraction, only preadipocytes survive in the serum-free conditions of culture (41), excluding the confounding contribution of factors produced by the other cells on the differentiation process. One cannot exclude stable epigenetic effects resulting from paracrine or endocrine effects on preadipocytes during the early development of adipose tissue. This appears unlikely because lipolysis was selectively altered in differentiated preadipocytes from obese subjects, whereas the level of differentiation, cell size, leptin secretion, and expression of β_2-adrenoceptors were not affected. Our data therefore favor an adipocyte-autonomous defect of lipolysis in obesity.

The impaired HSL expression most likely contributes to the lipolytic defect observed in obesity. As demonstrated here, HSL activity is rate-limiting for catecholamine-induced lipolysis. We showed earlier that the level of HSL expression is related to the lipolytic capacity in mature fat cells (11). The protein expression of this enzyme was markedly reduced, whereas the protein expression of the β_2-adrenoceptor, another potential candidate (7), was not altered in differentiated preadipocytes from obese subjects. The coregulation between HSL and ATGL gene expression observed in different dietary conditions suggests that the expression of ATGL may be altered in obesity. A lower expression of ATGL has been reported in white adipose tissue of genetically obese mice (18). It remains to be determined whether other lipases are important in adipocyte lipolysis and whether coregulation with HSL is observed. The link between HSL and obesity is also supported by genetic studies that show association with obesity and impaired lipolytic activity of subcutaneous fat cells (44–46). The physiological significance of lipolysis and HSL defects in obesity may be seen in two ways. A lipolytic defect could contribute to the development of obesity through impairment in the mobilization of fat stores. However, data from HSL-null mice do not support this hypothesis. The animals are lean and resistant to genetic and diet-induced obesity (12,15,47). Alternatively, the defect may constitute an early, possibly primary, event in obesity that protects against excessive FFA release. Accordingly, HSL deficiency in mice causes a reduction in plasma FFA levels (12,13). The lipid profile of HSL-null mice somewhat resembles that of patients treated with nicotinic acid, which acts, at least in part, through inhibition of adipose tissue lipolysis (3,48). In that context, a strong rationale can be seen for the development of adipose tissue HSL inhibitors such as BAY in the treatment of the metabolic syndrome (20,49,50).

In summary, this study demonstrates that HSL is the major lipase catalyzing the rate-limiting step in stimulated lipolysis in humans, whereas ATGL participates in basal lipolysis. Decreased catecholamine-induced lipolysis but not leptin production in subcutaneous adipose tissue of obese subjects is an early, possibly primary, defect that is linked to decreased protein expression of HSL.

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