Decreased expression and function of adipocyte hormone-sensitive lipase in subcutaneous fat cells of obese subjects

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Abstract Decreased lipolytic effect of catecholamines in adipose tissue has repeatedly been demonstrated in obesity and may be a cause of excess accumulation of body fat. However, the mechanisms behind this lipolysis defect are unclear. The role of hormone-sensitive lipase was examined using abdominal subcutaneous adipocytes from 34 obese drug-free and otherwise healthy males or females and 14 non-obese control subjects. The enzyme catalyzes the rate-limiting step of the lipolysis pathway. The maximum lipolytic capacity of fat cells was significantly decreased in obesity when measured using either a non-selective beta-adrenergic receptor agonist (isoprenaline) or a phosphodiesterase resistant cyclic AMP analogue (dibutyryl cyclic AMP). Likewise, enzyme activity, protein expression, and mRNA of hormone-sensitive lipase were significantly decreased in adipocytes of obese subjects. The findings were not influenced by age or gender. The data suggest that a decreased expression of hormone-sensitive lipase in subcutaneous fat cells, which in turn causes decreased enzyme function and impaired lipolytic capacity of adipocytes, is present in obesity. Impaired expression of the hormone-sensitive lipase gene might at least in part explain the enzyme defect.—Large, V., S. Reynisdottir, D. Langin, K. Fredby, M. Klannemark, C. Holm, and P. Arner. **Decreased expression and function of adipocyte hormone-sensitive lipase in subcutaneous fat cells of obese subjects.** ***J. Lipid Res.*** **1999.** 40: **2059–2065.**

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Obesity is a major health problem in the industrialized world. Abdominal obesity is particularly hazardous because of its strong association with diabetes mellitus and other atherosclerosis-promoting disorders. Excess caloric intake and physical inactivity are important contributing factors to the development of obesity. However, other mechanisms such as defects in energy expenditure are probably involved as well (1).

Lipid mobilization from fat stores is an important part of energy turnover. A number of in vivo and in vitro studies have shown that the ability of catecholamines to stimulate the mobilization of lipids from subcutaneous adipose tissue (which is the major fat depot) is impaired in obese subjects (2, 3 as reviewed). This could be an important mechanism contributing to the development of obesity, as catecholamines are the only hormones with a pronounced stimulatory effect on lipolysis in fat cells of humans (4, 5) and lipid mobilization is a key event in the regulation of energy expenditure. Recent studies on young subjects have suggested that a blunted lipolytic action of catecholamines is an early event in obesity (6). The pathophysiological mechanisms behind lipolytic catecholamine resistance in obesity are not fully understood. The catecholamines activate lipolysis through $beta_1$-, $beta_2$-, and $beta_3$-adrenoceptors and inhibit lipolysis through $alpha_2$-adrenoceptors (4). The beta-adrenoceptors mediated an increased cyclic AMP formation whereas the opposite effect is mediated by the $alpha_2$-adrenoceptor subtype. The activation of the cAMP-dependent protein kinase (protein kinase A) leads to the phosphorylation and activation of hormone-sensitive lipase (HSL) which catalyzes the rate-limiting step of lipolysis.

Alterations in the function of $beta_2$-adrenoceptors and $alpha_2$-adrenoceptors have been demonstrated in subcutaneous fat cells of obese subjects (7, 8). It is still unknown whether more distal defects in catecholamine signal transduction, near the final steps in the cyclic AMP pathway, i.e., at the protein kinase A or HSL level, exist in obesity. However, elderly male subjects with the insulin resistance syndrome, which usually accompanies obesity, have a doc-

Abbreviations: HSL, hormone-sensitive lipase; mRNA, messenger ribonucleic acid; cAMP, cyclic adenosine monophosphate; BMI, body mass index.

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umented post-receptor defect in catecholamine-induced lipolysis in abdominal subcutaneous fat cells (9). Furthermore, it was recently observed that normal-weight subjects with a heredity for obesity had a marked resistance to catecholamine-induced lipolysis in abdominal subcutaneous fat cells, which could be linked to a decrease in the enzymatic activity of HSL (10). Finally, an association between obesity and polymorphism in the HSL gene has recently been demonstrated (11). Therefore, it is possible that an adipocyte HSL defect is involved in the etiology of obesity. In the present study, this hypothesis was tested in 34 obese otherwise healthy subjects and 14 non-obese control subjects. Subcutaneous fat cells were incubated in vitro and the maximum lipolytic capacity of fat cells was determined. In addition, the enzymatic activity, the protein expression, and the mRNA levels of HSL were determined in adipocytes.

MATERIAL AND METHODS

The study population consisted of 48 male or female subjects (age ranged between 19 and 72 years), born in Sweden and living in the Stockholm area, with a large variation in body mass index (BMI). Only one man was elderly (72 years). The remaining men were <60 years. Four women were menopausal. Obesity was defined as a BMI higher than 28 kg/m^2. The subjects were recruited in two ways. The first cohort (4 men, 2 women) consisted of healthy drug-free non-obese volunteers (BMI 21–25 kg/m^2). The second cohort (25 men, 17 women) consisted of subjects (BMI 22–60 kg/m^2) who were referred to the department for surgical treatment of uncomplicated gallstone disease or obesity using gastric banding. All the latter subjects were healthy (except for obesity, gallstone, and hernia). None of them was on regular medication. Obese subjects with known overt complications of obesity such as established hypertension, diabetes, or dyslipidemia were excluded from the study. All subjects underwent some regular physical activity (a minimum of one moderate-length walk per week). None of them was completely sedentary or, on the contrary, involved in athletic performances. The study was approved by the hospital's Committee on Ethics and the subjects gave informed consent. The subjects were assigned to an obese (n = 34, BMI 28–60 kg/m^2) and a non-obese (n = 14, BMI 21–26 kg/m^2) group.

Subjects in the first cohort were investigated as out-patients in the morning after an overnight fast. Height, weight, and waist-to-hip ratio (WHR) were determined. Blood pressure was measured in the supine position by an automated sphygmomanometer and a venous blood sample was obtained. Thereafter, a subcutaneous fat biopsy (1–3 g) was obtained under local anesthesia from the abdominal region (12).

The subjects in the second cohort were examined as in-patients in the morning of the day before the operation after an overnight fast. The examination was performed exactly the same way as for the first cohort except for the fat biopsy. The latter (1–5 g of subcutaneous fat) was taken from the abdominal surgical incision at the beginning of the surgical procedures. All patients were operated early in the morning (between 8–10 am). They only received saline intravenously from 10 pm the day before operation until the adipose tissue biopsy was removed. General anesthesia of the subjects in the second cohort has been described in detail (12) We have previously shown that the regulation of catecholamine-induced lipolysis in abdominal subcutaneous fat cells is not statistically different in the same subject when the fat sample is obtained under local as compared to general anesthesia (12).

It was necessary to use two cohorts in the present study in order to include a sufficient number of non-obese subjects. After the study had started (1995) the surgical procedure for cholecystectomy changed from open surgery to laparoscopic surgery. For ethical reasons sufficient amounts of adipose tissue could not be removed from the laparoscopic incision.

The venous blood sample was submitted to analysis of plasma glucose, triglycerides, cholesterol, and HDL-cholesterol by the hospital's routine chemistry laboratory (which is an accredited one) and analysis of plasma insulin by our own laboratory using a commercially available kit (Pharmacia Upjohn, Uppsala, Sweden). The mean values for these measurements did not differ significantly between the non-obese subjects in the first and second cohorts. This was also true for age, sex distribution, BMI, WHR, systolic and diastolic blood pressure, and for the lipolytic rates or the HSL enzyme activity measured in fat cells. Therefore, the non-obese subjects in the two cohorts were grouped together in all further examinations.

Preparation of isolated fat cells and determinations of fat cell size and number

Isolated fat cells were prepared through incubation with collagenase according to Rodbell (13). Fat cell size was determined as follows. An aliquot of cells, suspended in an albumin buffer solution, was placed on a glass slide and the diameter of 100 cells was determined by examination with a microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with a caliper scale. Mean fat cell volume and weight were then calculated according to the methods developed by Hirsch and Gallian (14). The total lipid content in each incubation was determined gravimetrically after organic extraction. The number of fat cells in each incubation (see Lipolysis experiments below) can be estimated by dividing the total lipid weight by the mean cell weight. This method was compared with a more tedious method (15), where the number of fat cells is directly determined by counting cells in small aliquots of appropriately diluted fat cell suspensions. The correlation between the two methods was excellent (r = 0.97; n = 10) (6). Fat cell size was not determined in one lean subject.

Lipolysis experiments

The lipolysis assay was conducted exactly as described in detail previously (12). In brief, a diluted (5,000–10,000 cells/ml) suspension of fat cells was incubated for 2 h at 37°C in duplicate samples, with or without increasing concentrations of either isoprenaline (10^{-9}–10^{-5} mol/l), which is a non-selective beta-adrenoceptor agonist or dibutyryl cAMP (10^{-5}–10^{-2} mol/l), which is a phosphodiesterase-resistant cyclic AMP analogue that activates protein kinase A. Dibuturyl cAMP was not used in one obese subject. Glycerol release to the incubation medium was used as an index of the lipolysis rate and was determined using an automated bioluminescence assay (16). Under these conditions glycerol release can be detected within 15 min of incubation and is linear with incubation time for at least 4 h.

Both lipolytic agents caused a concentration-dependent increase of glycerol release, reaching a plateau before the highest drug concentration was reached. The maximum lipolytic capacity (responsiveness) of each agent was determined as the rate of glycerol release at the maximum effective drug concentration. No attempt were made to analyse the drug sensitivity (i.e., half maximum effective concentration), which reflects receptor events (17). We completely focused on the lipolytic capacity of fat cells which reflects the HSL expression (18) as HSL was the target of the study. The lipolysis rates in the presence or absence (basal lipolysis) of agonists were related to the number of incubated cells or to the amount of lipids extracted from the incubated fat cells. Lipolysis was also expressed as responsiveness of dibutyryl cyclic AMP or isoprenaline divided by the basal value.

Determination of HSL enzyme activity and protein expression

Because of the limited amount of tissue available, the protein measurements could not be performed in all subjects. However, enzyme activity was measured in all subjects. About 300 mg of frozen (at −70°C) tissue samples was homogenized in 0.6 ml of 0.25 mol/l sucrose, 1 mmol/l dithioerythritol, 1 mmol/l EDTA, 20 μg/ml leupeptin, 20 μg/ml antipain, and 1 μg/ml pepstatin A, at pH 7.0 and 4°C. Fat-depleted infranatants were obtained after centrifugation at 12,000 *g* and 4°C for 3 h. Tissue was homogenized and processed further simultaneously.

An aliquot of the infranatant was used for the determination of the maximum enzyme activity of HSL. The assay was performed exactly as described previously, using a diolein analogue 1(3)-mono[^{3}H]-oleoyl-2-oleoylglycerol as substrate (19, 20). Under the present incubation conditions (pH 7.0 and no apoC-II present) this substrate is only catalyzed by hormone-sensitive lipase and not by lipoprotein lipase. One unit of enzyme activity is defined as 1 μmol of oleic acid released per minute at 37°C. Because the phosphorylated and dephosphorylated forms of the enzyme have the same activity towards diglyceride substrates in this type of assay system, only the total amount of activatable enzyme in the sample is measured. The HSL activity was measured simultaneously in all samples and analyzed in triplicates. The lipase activity was related to the total protein concentration of the infranatant, which was measured using the BCA protein assay (Pierce, Rockford, IL). In unpublished methodological experiments, we have demonstrated that HSL activity measured in tissue that has been frozen at −70°C is not significantly different from the activity measured in fresh tissue from the same subject.

Another part of the infranatant was used for the determination of HSL protein expression by Western blot analysis as described previously (18). Samples from the obese or non-obese groups were always run together on the same blot, usually 1–2 non-obese and 4–5 obese per blot. The Western blot procedure was performed either with the same quantity of total proteins (200 μg) or with the same amount of total HSL activity (0.5 mU). Methodological experiments revealed that quantitative detection (see below) of HSL protein showed linearity when different amounts of activity corresponding to at least 2 mU of total enzymatic activity were used in the protein determination. Aliquots of the infranatants were adjusted to final concentrations of 0.008% bromophenol blue (w/v), 1% sodium dodecyl sulfate (w/v), and 7% glycerol (v/v) and applied to 8% polyacrylamide gels according to the Laemmli method (21) under reducing conditions (3% mercaptoethanol) and in the presence of molecular weight standards (7.1–208 kDa). Electrophoresis was carried out and proteins were transferred to nitrocellulose paper. The blot was first incubated for 2 h in blocking buffer (20 mmol/l of Tris-base, 137 mmol/l NaCl, pH = 7.6 and 5% (w/v) non-fat dry milk). The blot was then incubated with 14 ml buffer (20 mmol/l of Tris-base, 137 mmol/l NaCl, 2.5% (w/v) non-fat dry milk, 2.5% (w/v) Tween 20) containing first a polyclonal chicken anti-rat HSL (1:1000) for 1 h and then an anti-chicken IgG peroxidase conjugate (1:8000) for 1 h. Finally, the blot was incubated with enhanced chemiluminescence detection reagents (ECL development kit) for 1 min prior to exposure to Hyperfilm-ECL for 10–20 s. The relative amounts of immunodetectable HSL contained in each lane were determined by scanning with a high resolution color scanner JX-325 (Sharp, Japan) and Image Master 1-D software (Pharmacia LKB Biotechnology, Uppsala, Sweden), on an NEC computer. A standard of recombinant rat HSL (19) was included in each gel. The same batch of the standard (5 mU/lane) was used throughout the study. The protein reading of the samples was expressed as a function of the density of the standard.

Assay of mRNA levels

Because of limited amounts of adipose tissue available, this assay could not be performed in all subjects. Fat cells stored at −70°C were used. All samples were processed simultaneously in the mRNA assay. Steady state mRNA levels of HSL were measured using a solution hybridization assay. This technique has been described in detail previously (18). Packed adipocytes (200 μl) were homogenized and total RNA was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany). The integrity of the RNA was checked by electrophoresis in a 1% agarose gel containing ethidium bromide.

A 150-bp fragment of the HSL complementary DNA was cloned into a pBluescript vector and linearized using EcoRI. Antisense RNA produced using T3 RNA polymerase was labeled by incorporating [^{35}S]UTP into the reaction mixture. An unlabeled sense RNA complementary to the probe was synthesized and used as control.

The radiolabeled probe was hybridized to the samples or the sense RNA for 18 h at 70°C in a buffer containing 25% formamide. The samples were then treated with ribonucleases A and T1 for 45 min at 37°C, and precipitated for 30 min at 4°C after addition of trichloroacetic acid. The ribonuclease-resistant precipitated RNA-RNA hybrids were collected under vacuum on glass fiber filters (Whatman Inc., Clifton, NJ), before scintillation counting. Each sample was determined in duplicate and the HSL mRNA level was related to the amount of mRNA for the "housekeeping" gene γ-actin, which was also measured by solution-hybridization in the extract, using a 236-bp probe, provided by Mats Gåfvels (Huddinge, Sweden).

Drugs and chemicals

Dibutyryl cyclic AMP, *Clostridium histolyticum* collagenase type I, anti-chicken IgG peroxidase conjugate, antipain, pepstatin A, and leupeptin were obtained from Sigma (St. Louis, MO). Noradrenaline and (−) isoprenaline hydrochloride came from Hässle (Mölndal, Sweden). Kaleidoscope Standards were purchased from Bio-Rad (Hercules, CA) and 1(3)-mono[^{3}H]oleoyl-2-oleoylglycerol was synthesized by Dr. L. Krabisch at the Department of Cell and Molecular Biology, Lund University (Lund, Sweden). Nitrocellulose paper Hybond-C extra, ECL development kit for Western blot analysis and Hyperfilm-ECL were from Amersham (Buckinghamshire, UK). RNases A and T1 were both from Boehringer-Mannheim, Mannheim, Germany and [^{35}S]UTP was from New England Nuclear (Boston, MA). All other chemicals were of the highest grade of purity commercially available.

Statistical methods

Values presented are mean ± standard error of the mean (SEM). Values between groups were compared by Student's unpaired or *t*-test. In some cases, linear regression analysis and chi square analysis were performed. All statistical calculations were made using a commercially available computer program (Stat View Abacus Concepts, Berkeley, CA).

RESULTS

The clinical data are shown in **Table 1**. BMI, WHR, fat cell volume, diastolic blood pressure, and plasma glucose, insulin, and triglycerides were increased and HDL cholesterol decreased in the obese state. Plasma cholesterol and systolic blood pressure were not influenced by obesity.

Glycerol release from fat cells, when expressed in three different ways, was compared between the groups (**Table 2**).

TABLE 1. Clinical characteristics of the lean and obese subjects

	Non-obese (n = 14)	Obese (n = 34)	*P*
Age, yr	40 ± 3	37 ± 2	NS
Sex, M/F	8/6	21/13	NS
BMI, kg/m^2	23.5 ± 0.4	43.1 ± 0.7	<0.0001
Fat cell volume, pl	438 ± 16	776 ± 16	<0.0001
WHR	0.90 ± 0.02	0.96 ± 0.02	<0.05
p-Glucose, mmol/l	5.1 ± 0.1	6.7 ± 0.5	<0.05
p-Insulin, mU/l	8.6 ± 1.7	22.2 ± 2.5	<0.01
p-Chol, mmol/l	5.2 ± 0.2	5.7 ± 0.3	NS
p-HDL-Chol, mmol/l	1.4 ± 0.14	1.2 ± 0.04	<0.05
p-TG, mmol/l	1.3 ± 0.3	2.0 ± 0.2	<0.05
SBP, mmHG	131 ± 3	141 ± 3	NS
DBP, mmHG	77 ± 2	84 ± 2	<0.05

Values are mean ± SEM. They were statistically compared by Student's unpaired *t*-test; NS, not significant; BMI, body mass index; WHR, waist–hip ratio; p, fasting plasma; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; M, male; F, female.

The basal rate of glycerol release did not differ between the groups when expressed per g lipid but was increased in the obese group when expressed per cell. The maximum values for isoprenaline- and dibutyryl cyclic AMP-induced glycerol release were significantly decreased by 40–50% in the obese group, when expressed per g lipid but did not differ between the groups in a statistically significant way when expressed per cell number. The effects of isoprenaline and dibutyryl cyclic AMP were also expressed as a function of basal glycerol release (stimulated divided by basal). These agents, at maximum effective concentrations, increased the rate of glycerol 9- to 10-fold in the non-obese group and only 4-fold in the obese groups (P = 0.001 or lower).

The correlation between fat cell volume and the basal maximum isoprenaline-induced glycerol release and maximum dibutyryl cyclic AMP-induced glycerol release was investigated in the whole material. There was a significant correlation between fat cell volume and the lipolytic parameters when the latter were expressed per cell (r = 0.33–0.53, P = 0.0001–0.02) but not when they were expressed per g lipid ($r < 0.21$, $P > 0.18$).

The correlation between cell volume and glycerol release per cell number is depicted in **Fig. 1**. For basal lipolysis the glycerol values were evenly distributed above and below the regression line. For dibutyryl cyclic AMP-induced lipolysis, 23 of the values in obese subjects were distributed below the regression time and 10 above this time, whereas for the non-obese subjects 8 values were above and 5 were below the regression time. This difference between lean and obese was significant (Chi square 3.86, $P < 0.05$).

TABLE 2. Comparison of lipolytic parameters in subcutaneous adipose tissue of 34 obese and 14 non-obese subjects

Lipolytic Parameter	Non-obese	Obese	*P*
Basal (μmol/g lipid/2 h)	1.1 ± 0.2	1.5 ± 0.1	NS
Basal (μmol/10^7 cells/2 h)	5 ± 1	11 ± 1	<0.01
Isoprenaline (μmol/g lipid/2 h)	7.9 ± 0.9	4.8 ± 0.4	<0.001
Isoprenaline (μmol/10^7 cells/2 h)	31 ± 3	41 ± 4	NS
Isoprenaline/basal	9.5 ± 1.9	3.9 ± 0.4	<0.001
dcAMP (μmol/g lipid/2 h)	6.9 ± 0.8	4.1 ± 0.3	<0.001
dcAMP (μmol/10^7 cells/2 h)	26 ± 3	35 ± 3	NS
dcAMP/basal	8.9 ± 2.0	3.8 ± 0.5	=0.001

Lipolysis was examined as described in Material and Methods. Data are mean ± SEM. Values are glycerol release in the absence (basal lipolysis) or presence of a maximum effective isoprenaline or dibutyryl cyclic AMP (dcAMP) concentration. They were compared by Student's unpaired *t*-test.

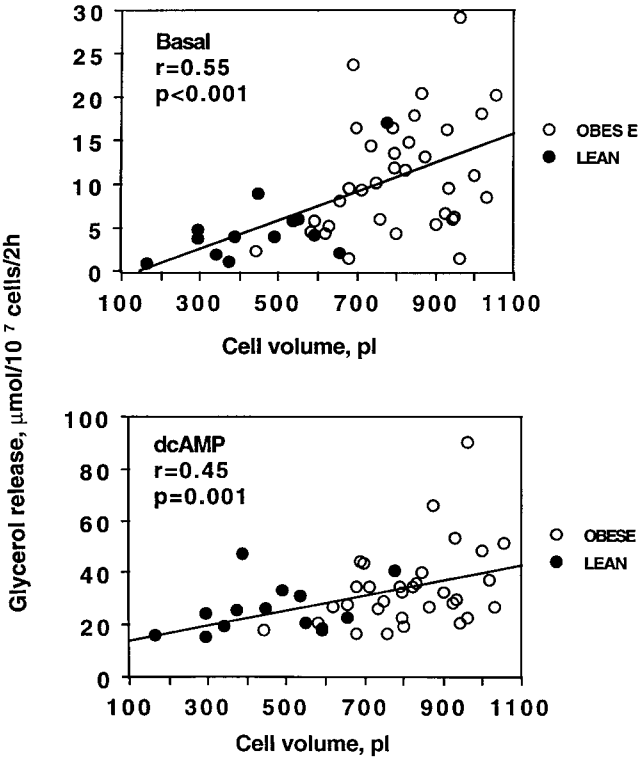

Fig. 1. Correlation between glycerol release from fat cells under basal conditions or induced by dibutyryl cyclic AMP (dcAMP) and fat cell volume. Values were examined using linear regression analysis. Obese subjects are open symbols and lean subjects are filled symbols.

The mean results of enzymatic activity and expression of HSL are depicted in **Table 3**. Obese subjects had much lower enzymatic activity of HSL than non-obese subjects ($P < 0.001$). The values of HSL protein expression were also decreased in obesity when a fixed amount of total protein was added to the assay systems ($P < 0.05$). A representative Western blot for the protein expression of fat cells from four obese and two non-obese subjects (using a fixed amount of total protein) is shown in **Fig. 2**. An immunoreactive specific 86 kDa protein corresponding to human HSL was detected. The relative densities of the HSL bands were much lower in three of the four obese subjects in comparison with the two non-obese ones. HSL protein expression was also measured using the same amount of total HSL activity (0.5 mU) (Table 3). When HSL protein was determined in this way, the mean values were almost identical in the two groups. Concerning HSL mRNA levels related to the mRNA level of γ-actin (Table 3), the values were statistically significantly higher in the obese as compared with the non-obese group.

TABLE 3. HSL activity, protein, and mRNA expression in fat cells of obese and non-obese subjects

	Fat Cell Lipolysis Capacity		
	Non-obese	Obese	*P*
HSL activity (mU/mg total protein)	10.1 ± 2.6 (n = 14)	5.8 ± 0.7 (n = 34)	<0.001
HSL protein (OD/mm^2/200 μg total protein)	5.3 ± 1.6 (n = 10)	2.8 ± 0.5 (n = 29)	<0.05
HSL protein (OD/mm^2/0.5 mU HSL)	0.8 ± 0.3 (n = 8)	0.7 ± 0.3 (n = 20)	NS
mRNA ratio HSL/γ actin	9.7 ± 1.0 (n = 10)	7.0 ± 0.7 (n = 21)	<0.05

Values are mean ± SEM. They were statistically compared by Student's unpaired *t*-test; NS, not significant; n, number of subjects.

A correlation analysis between lipolysis and HSL enzyme activity was performed using linear regression. As demonstrated in **Fig. 3**, HSL activity correlated with maximum isoprenaline-induced glycerol release ($r = 0.51$, $P < 0.001$) and with maximum dibutyryl cyclic AMP-induced glycerol release ($r = 0.52$, $P < 0.001$) when lipolysis was expressed per g of lipid. However, no such correlation was found when lipolysis was expressed per cell ($r < 0.3$, graph not shown). As demonstrated in **Fig. 4**, there was a strong correlation between maximum glycerol release induced by isoprenaline and dibutyryl cyclic AMP in the whole cohort ($r = 0.95$, $P < 0.001$).

The possible influence of age on lipolysis data was investigated by regression analysis in women and men separately. Age did not correlate with basal, isoprenaline-induced or noradrenaline-induced glycerol release where expressed per g lipid or per cell in men or women (r from 0.03 to 0.32, P from 0.20 to 0.83). Neither men nor women showed a correlation between age and ratio of isoprenaline or dibutyryl cyclic AMP responsiveness of lipolysis versus basal lipolysis (r from 0.07 to 0.14, $P > 0.8$).

The influence of obesity on lipolysis was also investigated according to gender. In women, essentially the same results were obtained as for the whole material presented in Table 2. Thus, basal glycerol release/g lipid and isoprenaline- or dibutyryl cyclic AMP-induced glycerol release per cell did not differ between obese and non-obese women whereas basal glycerol release/cell was significantly increased and isoprenaline- and dibutyryl cyclic AMP-induced glycerol/g lipid as well as isoprenaline responsiveness/basal lipolysis and dibutyryl cyclic AMP responsiveness/basal lipolysis were decreased in obese females. The findings with lipolysis in the whole cohort in Table 2 also hold true for men except in one case. The decreased dicyclic AMP-induced glycerol release per g lipid in obese as compared to non-obese subjects reached only a border line level of significance ($P = 0.063$) and may be due to the few lean males investigated (n = 5). Twenty-one obese men and 13 obese women were investigated. There were no statistically significant differences between these men and women as regards the lipolysis and HSL parameters (data not shown).

Waist–hip ratio or fasting plasma insulin did not correlate in a significant way with any of the results of HSL (values not shown).

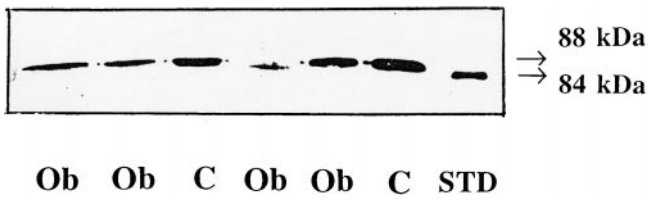

Fig. 2. Western blot of HSL from human adipose tissue. Fat-depleted infranatants (200 μg total protein) were subjected to SDS-PAGE and electroblotted onto nitrocellulose membranes. Immunoreactive proteins were detected with an anti-HSL antibody and enhanced chemiluminescence detection (for more details, see Methods). The position of the protein detected by the anti-HSL antibody has been calculated from the mobilities of reference proteins. This blot shows the results for three pairs of subjects. The last lane contains recombinant rat HSL (84 kDa) as standard (STD).

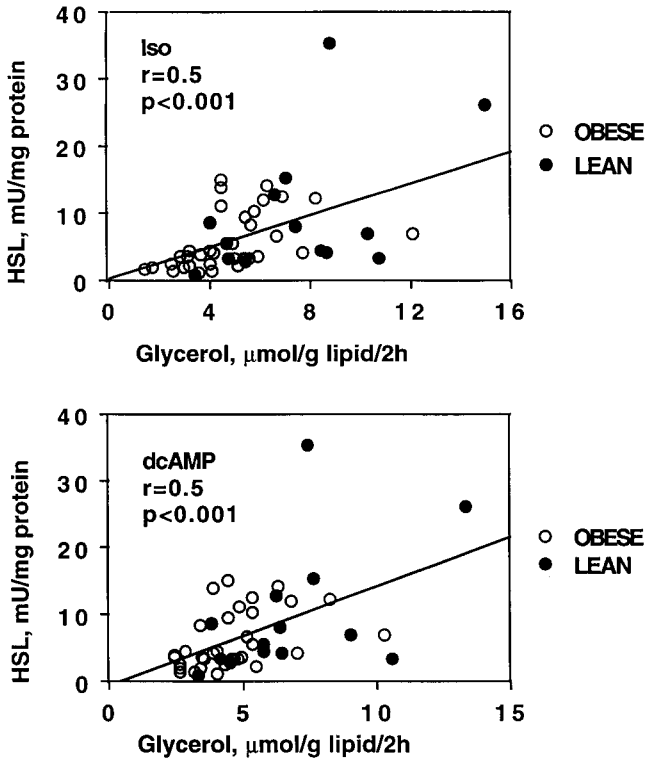

Fig. 3. Correlation between maximum glycerol release from fat cells induced by isoprenaline (Iso) or dibutyryl cyclic AMP (dcAMP, lower graph) and hormone-sensitive lipase (HSL) enzyme activity. See legend to Fig. 1 for further details.

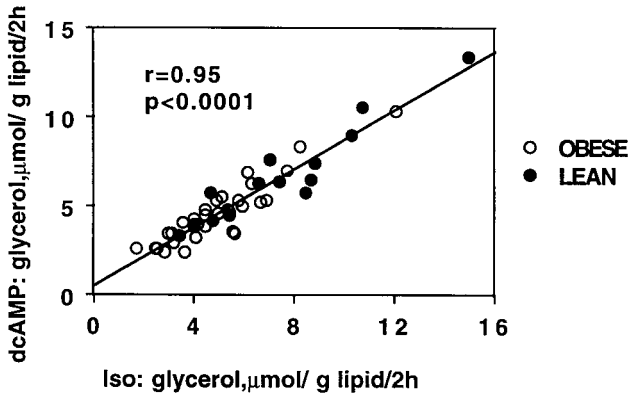

Fig. 4. Correlation between basal glycerol release and glycerol release induced by a maximum effective concentration of dcAMP. See legend to Fig. 1 for further details.

DISCUSSION

In this study we demonstrate that a defect in subcutaneous adipocyte expression and function of HSL is present in obesity. This HSL defect is accompanied by a decreased lipolytic capacity of adipocytes when lipolysis is expressed per g lipid of fat cells. This expression seems more relevant to lipolytic capacity than expression per cell because fat cell volume is increased in obesity and there is a relationship between cell size and lipolysis rate per cell but not per g lipid as shown in this study and in several earlier investigations (reviewed in reference 2). In the calculation of glycerol release per cell, the weight of the fat cell is utilized in the formula (14). A large fat cell from an obese subject has a much higher weight than a small adipocyte from a lean subject. Therefore cell size markedly influences the magnitude of the lipolysis rate when calculated per cell. As a consequence it is difficult to sort out whether differences in lipolysis rates/cell between obese and non-obese subjects only reflect differences in cell size or whether size-independent factors are present as well. However, in spite of the correlation between cell size and lipolytic capacity per cell, the lipolytic capacity was similar when mean rates in obese and non-obese subjects were compared (Table 2). Furthermore, as regards individual values, most obese subjects had lower lipolytic capacity per cell than what was expected from the regression analysis (Fig. 1). If the cell size dependency of lipolysis in obese fat cells should be "normal," a higher rate of lipolysis in obese than in non-obese subjects is expected when lipolytic capacity is expressed per cell. Furthermore, lipolytic capacity per lipid weight of adipocytes (but not per cell) correlated with the HSL enzymatic activity, which further argues for the use of lipolysis rates per weight of fat cells in comparative studies with HSL. However, it might be argued that obese subjects have fewer fat cells per g of lipid than the non-obese, hence a lower rate of lipolysis. In order to answer this question we also expressed the maximum lipolytic capacity as a function of basal rate of lipolysis. This expression is independent of fat cell number and size. The same ratio will be obtained whether glycerol release is expressed per cell or per g of lipid. There was a marked difference between the groups using stimulated/basal values as the mode of lipolysis expression. Isoprenaline and dibutyryl cyclic AMP increased the basal rate of lipolysis about 10-fold in the non-obese and about 4-fold in the obese group. When all the different ways to calculate lipolysis are considered together, the data strongly suggest that the lipolytic capacity of fat cells is decreased in obesity.

The results with basal lipolysis are the opposite of those concerning stimulated lipolysis. Basal rate was increased in obesity when expressed per cell but not different between the obese and non-obese states when expressed per g of lipid. This is in agreement with numerous earlier studies as reviewed (2). There is no clear explanation for the discrepancy between findings with basal and stimulated lipolysis. However, basal lipolysis has probably no relevance for HSL which becomes activated (i.e., phosphorylated) after catecholamine stimulation (22).

As reviewed in detail, a number of factors influence lipolysis regulation in human fat cells, such as age, gender, and menopause (23–25). For several reasons, however, we do not believe that our data are influenced by a poor selection of subjects. First, only one man was elderly and only four women were menopausal. Second, the groups were well matched for age and gender. Third, there was no significant correlation between age and any of the lipolysis parameters. Fourth, the findings with men and women, when analyzed separately, were essentially the same as when the whole material was analyzed together.

Almost identical findings were obtained when lipolysis was stimulated either at the level of all beta-adrenoceptors (with isoprenaline) or at the protein kinase A level (with dibutyryl cyclic AMP) and there was an excellent correlation between isoprenaline- and dibutyryl cyclic AMP-stimulated rates of lipolysis. These data strongly suggest that variations in lipolytic capacity between individuals are determined at or beyond the level of protein kinase A.

We observed that both the enzyme activity and the amount of HSL protein (contained in the same amount of total protein) were decreased in the fat cells from obese subjects. However, the amount of HSL protein determining 0.5 mU of specific HSL activity did not differ between the obese and non-obese groups. These data clearly point to an important role for the expression of the HSL protein for decreased HSL lipolytic capacity of fat cells in obesity. A defect in HSL protein expression of adipocytes could therefore be involved in the etiology of obesity. It should, however, be stressed that other distal steps (beyond cyclic AMP) in lipolytic activation also may govern the lipolytic capacity of fat cells such as phosphorylation and dephosphorylation of HSL as well as translocation of HSL (see references 2 and 4 for review).

The mechanisms behind the decrease in HSL protein in the investigated obese subjects could involve some factors relating to synthesis and/or breakdown of the protein. Unfortunately, there are no available techniques to directly study HSL synthesis or breakdown in the present type of study. We therefore measured steady state HSL

mRNA levels in relation to the mRNA level for a reporter gene (γ-actin) using a sensitive solution hybridization assay. We found that the HSL mRNA levels were significantly decreased in fat cells from obese subjects. Thus, a decreased synthesis of the HSL protein at the transcriptional level is a likely factor behind the findings of decreased HSL expression in adipocytes from obese subjects. However, the difference between groups was smaller for mRNA than for protein or enzyme activity of HSL. It is therefore possible that other variations in HSL turnover are present in obesity. However, structural variations in HSL are probably not involved, as earlier studies of DNA from obese subjects have failed to demonstrate important structural variations in the HSL protein (11). Our finding that the amount of HSL protein per unit of HSL enzyme activity was not altered in obesity also argues for a normal structure of the HSL protein in obesity.

When all present data are pooled, it is tempting to speculate that a defect in the regulation of the gene expression of HSL in subcutaneous adipocytes is present in obesity. Such a defect may lead to low adipocyte content of HSL protein, so that the enzymatic activity of HSL decreases which, in turn, decreases the lipolytic capacity of fat cells. As maximal lipolysis corresponds to a full phosphorylation/activation of HSL, one might propose that a decreased expression of HSL leads to decreased lipolysis capacity. Indeed previously published results show that there is a strong correlation between lipolysis capacity and HSL expression in human fat cells (18). Thus, decreased HSL expression may at least in part explain the well-documented resistance to the lipolytic effect of catecholamines in obesity (2). However, it is possible that multiple lipolysis defects co-exist in obesity. Previous studies have demonstrated increased function of alpha$_2$-adrenoceptors and decreased expression and function of beta$_2$-adrenoceptors in subcutaneous fat cells from obese subjects (7, 8). Unfortunately, the amount of adipose tissue that was available was not sufficient to also study the adrenoceptors in our investigated subjects.

In summary, the present study suggests that a decreased expression and function of HSL is present in fat cells of obese subjects and may cause a decreased lipolytic capacity of these cells. Impaired expression of the HSL gene might at least in part cause the HSL defect in obesity.

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