Hormone-sensitive lipase expression and activity in relation to lipolysis in human fat cells

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Abstract Hormone-sensitive lipase (HSL) catalyzes the rate-limiting step in adipocyte lipolysis. The activity of HSL is thought to be primarily regulated by reversible phosphorylation. However, the regulation of HSL activity by pre-translational mechanisms has been poorly studied. The present studies were undertaken to explore the relationship between the levels of HSL protein and mRNA expressions and the lipolytic capacity. The study was performed in human abdominal subcutaneous adipocytes with identical sizes but having either a high (HL) or low (LL) lipolytic capacity (n = 16). Basal and maximal lipolysis induced by catecholamines, an adenylyl cyclase activator forskolin, and a cyclic AMP analogue dibutyryl cAMP were 50% lower in LL- in comparison with HL-fat cells ($P < 0.05$ or better). No differences in drug sensitivity were found. HSL activity and quantity were about 50% lower in LL- compared with HL-fat cells ($P < 0.05$). Moreover, the mRNA ratio between HSL and γ-actin was 35% lower in LL- compared with HL-fat cells ($P < 0.05$). There was a strong linear correlation between the protein and enzymatic HSL measurements ($r^2 = 0.91$). In addition, the maximum lipolytic capacity was significantly correlated with HSL activity ($r^2 = 0.75$) and HSL protein amount ($r^2 = 0.64$). It is concluded that hormone-sensitive lipase (HSL) expression, measured either as total HSL protein by Western blot analysis or as total amount of activatable HSL enzyme, is a major determinant of the maximum lipolytic capacity of human fat cells. In addition, HSL protein expression is at least, in part, determined by HSL mRNA expression.—Large, V., P. Arner, S. Reynisdottir, J. Grober, V. Van Harmelen, C. Holm, and D. Langin. **Hormone-sensitive lipase expression and activity in relation to lipolysis in human fat cells. *J. Lipid Res.* 1988.** 39: **1688–1695.**

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Hormone-sensitive lipase (HSL) is the enzyme responsible for the hydrolysis of triacylglycerol from the lipid droplet of adipocytes into glycerol and non-esterified fatty acids (1). Non-esterified fatty acids are bound to the carrier protein albumin and transported via the blood stream to target tissues (mainly liver and muscle). The control of human adipose tissue lipolysis is determined by a number of agents. HSL activity is inhibited by insulin, adenosine, prostaglandins, and catecholamines (in the latter case via α_2-adrenoceptors), and activated by agents that promote an increase of intracellular level of cAMP, such as catecholamines via β-adrenoceptors (2).

The activity of HSL is thought to be primarily regulated by phosphorylation–dephosphorylation. Other factors may, however, contribute in an important way to the lipolytic function of HSL rather than phosphorylation (3). Sztalryd and Kraemer (4) reported an increase in HSL activity after 3 days of fasting in rats that is paralleled by increases in HSL protein and HSL mRNA levels. These findings suggest that HSL activity is not regulated exclusively by post-translational control mechanisms in rat adipose tissue and that pre-translational mechanisms appear to predominate, at least during prolonged food deprivation (4). In humans, little is known about the functional importance of variations in HSL expression. As the rate-limiting enzyme in adipose tissue lipolysis, any variation in the amount of HSL protein could have an impact on the capacity of adipose tissue to hydrolyze triacylglycerols. A 2-fold increase in HSL mRNA level associated with increased plasma free fatty acid levels was found in adipose tissue of cancer patients (5). Furthermore, a decrease in maximally stimulated lipolysis was shown in fat cells of patients with familial combined hyperlipidemia (6) and in subjects with heredity for obesity (7). These lipolytic defects were associated with a decrease in HSL enzyme activity.

The hallmark of HSL, which distinguishes this enzyme from all other known triacylglycerol lipases, is the control of its activity through phosphorylation (8). As there is no satisfactory method to directly probe the proportion of

Abbreviations: HSL, hormone-sensitive lipase; dc AMP, dibutyryl cyclic adenosine monophosphate; IgG, immonoglobulin G; LL, low lipolysis; HL, high lipolysis.

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HSL in the active form (i.e., the phosphorylated form), the activation of HSL through reversible phosphorylation is studied indirectly by measuring the glycerol released from the adipocytes. In parallel, it is possible to determine total HSL activity using a diacylglycerol analogue as substrate (9), and to evaluate the protein and mRNA amounts.

In this study, we have investigated the relationship between HSL activity, protein and mRNA levels, function, and lipolysis in human abdominal subcutaneous adipocytes from two groups of subjects with either a high or low maximum lipolytic capacity per cell. It is well known that fat cell size has a major influence on the rate of lipolysis in human fat cells (2). In order to avoid this confounding factor, HSL investigations were performed on fat cells with matched cell size.

MATERIAL AND METHODS

Subjects

Eight pairs of obese subjects were selected according to values of maximum isoprenaline-induced lipolysis per cell in vitro. Both subjects of one pair had the same sex and similar fat cell volumes but differed in rate of adipocyte lipolysis (Table 1). The subjects with a low lipolytic rate constituted the low lipolysis (LL) group, the subjects with a high lipolytic rate constituted the high lipolysis (HL) group. The ratio between the mean lipolytic rate of the two groups was about two.

An abdominal subcutaneous fat biopsy was obtained during elective surgery after an overnight fast. The subjects underwent gastric banding because of obesity, involving a laparotomy. They were drug-free and otherwise healthy. Subjects with overt known complications to obesity such as established hypertension, diabetes, or dyslipidemia were excluded prior to examination. General anesthesia was induced by a short-acting barbiturate and maintained by nitrous oxide and fentanyl. The samples of adipose tissue were taken at the beginning of the surgical procedures, within minutes after the skin incision was made, and 20–30 min after the induction of general anesthesia. The study was approved by the hospital's Committee on Ethics. In the morning of the day before the anesthesia, blood pressure was measured and a venous blood sample was obtained after an overnight fast for the determination of glucose and lipids, by the hospital's routine chemistry laboratory and determination of plasma insulin using a commercial RIA kit (Pharmacia-Upjohn, Uppsala, Sweden).

Fat biopsies were immediately transported to the laboratory in saline at 37°C and about 300 mg of the tissue was frozen in liquid nitrogen in order to determine HSL activity and to perform Western blot analysis. The rest of the tissue was used immediately for in vitro lipolysis experiments.

Preparation of isolated fat cells and determination of fat size and number

Isolated fat cells were prepared through incubation with collagenase according to Rodbell (10). 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 (11). 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 (12), 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 ($r = 0.97$; $n = 10$) (13). In other methodological experiments, also performed in our laboratory, subcutaneous fat cell size was compared between isolated fat cells and formaline-fixed tissue fragments that were obtained from the same subject (14). The mean fat cell size did not differ significantly between the methods and there was a strong relationship between the values obtained with the two ways of determining fat cell size ($r = 0.85$).

Lipolysis experiments

Isolated fat cells were incubated as reported in detail earlier (15). In brief, about 2000 adipocytes were incubated in duplicate in 0.2 ml of an appropriate buffer for lipolysis in the presence or absence of increasing concentrations of several agents acting at different levels of the lipolytic cascade. We used the natural catecholamine noradrenaline acting on all adrenoceptor subtypes, the agonist isoprenaline acting on the three β-adrenoceptors, the selective β_1-adrenoceptor agonist dobutamine, the selective β_2-adrenoceptor agonist terbutaline, the partial selective β_3-adrenoceptor agonist CGP 12177, forskolin, a direct activator of adenylyl cyclase, and dibutyryl cyclic AMP (dcAMP). The latter molecule is a phosphodiesterase-resistant cyclic AMP analogue that activates the cyclic AMP-dependent protein kinase. Glycerol release in the incubation medium was determined after a 2-h incubation at 37°C, using an automated bioluminescence assay (16). Lipolysis rates in the presence or absence of maximum effective agonist concentrations were expressed per cell number. All agonists caused a dose-dependent increase in glycerol release that reached a plateau at the highest agonist concentrations. The sensitivity to agonist action was defined as the pD2 value, i.e., the negative logarithm of the EC_{50} value (effective agonist concentration causing 50% of maximal effect).

Assay of HSL activity

Frozen tissue samples (about 300 mg) were crushed in liquid nitrogen and then homogenized with a knife homogenizer in 0.6 ml of 0.25 M sucrose, 1 mM dithioerythritol, 1 mM 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.

The HSL activity assay was performed essentially as described previously, using the diolein analogue 1(3)-mono[^{3}H]oloeyl-2-oleoylglycerol as substrate (17, 18). One unit of enzyme activity is defined as 1 μmole of oleic acid released per minute at 37°C. As the phosphorylated and dephosphorylated forms of the enzyme have the same activity towards diglyceride substrates, only the total amount of activatable enzyme in the sample is measured. All samples were analyzed in triplicate and lipase activity was related to the total protein concentration of the infranatant, which was measured using the BCA protein assay (Pierce, Rockford., IL). In uncharted methodological experiments, we demonstrated that HSL activity is not significantly different in the same subject when measured on tissue that had been frozen at −70°C compared to when measured on fresh tissue.

Western blot analysis

Aliquots of the infranatants (200 μg of total proteins) 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 (19) under reducing conditions (3% mercaptoethanol). Prestained molecular weight standards (Kaleidoscope Standards) were used as references. Electrophoresis was carried out at a constant current (50 mA/gel) for 3 h. Proteins were then transferred to nitrocellulose paper (60 V, 16–18 h). The blot was incubated for 2 h in blocking buffer (20 mm Tris-base, 137 mm NaCl, pH = 7.6, and 5% (w/v) non-fat dry milk). Then the blot was incubated with a polyclonal chicken anti-rat HSL (1:1000) for 1 h. Finally, the blot was incubated with an anti-chicken IgG peroxidase conjugate (1:8000) for 1 h and then briefly incubated with enhanced chemiluminescence detection reagents (ECL development kit) prior to exposure to Hyperfilm-ECL for 10–20 sec. 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.

In order to be able to compare the values for HSL density from one blot to another, a standard of recombinant rat HSL (18) was included in each gel. The same batch of diluted standard (5 mU/lane) was used throughout the study.

Competitive immunoprecipitation experiment

Two different experimental approaches were utilized. In the first, a human full-length HSL cDNA clone was subcloned into the mammalian expression vector pcDNA3 (InVitrogen, Leek, The Netherlands). COS cells were transfected with the expression vector using lipofectin (Gibco-BRL, Cergy Pontoise, France). Cell extracts were prepared 72-h post-transfection; 0.5 μg of affinity-purified rabbit anti-rat HSL (0.7 mg/ml) was then preincubated overnight with 200 μl of transfected COS cell extract at 4°C on a rocking table. After this preincubation, 180 μl (corresponding to 2 mU of HSL activity) of adipose tissue fat-free infranatant was added and the incubation was continued for 3 h at 4°C on a rocking table. After adding 100 μl of protein-A–Sepharose, the incubation was continued for an additional hour. After five washes with 1 ml of TBS (20 mm Tris-HCl, 137 mm NaCl, pH = 7.4) containing 0.1% N-lauroyl-sarcosine, the immunoprecipitates were subjected to the Western blotting procedure described above. Three controls were included in this experiment. In the first, the infranatant was incubated with antibodies that had not been preincubated. In the second, no infranatant was added. Finally, 200 μl of cell extract from non-transfected COS cells was incubated with HSL antibodies.

In the second experimental approach, 10 μl of affinity-purified rabbit anti-human HSL antibodies (0.4 mg/ml) was preincubated overnight at 4°C on a rocking table with different amounts of recombinant human HSL corresponding to 0, 5, 10, 15, and 20 mU in lanes 1, 2, 3, 4, and 5, respectively. After this preincubation, 140 μl (corresponding to 2.5 mU of HSL activity) of adipose tissue fat-free infranatant was added and the incubation was continued for 3 h at 4°C on a rocking table. After adding 100 μl of protein-A–Sepharose, the incubation was continued for an additional hour. After five washes as described above, the immunoprecipitates were subjected to the Western blotting procedure. One control was included in lane 6, which contained only the human antibodies without either pre-incubation with recombinant human HSL or incubation with adipose tissue fat-free infranatant. Lane 7 contained a standard of recombinant rat HSL.

Assay of mRNA levels

Steady-state mRNA levels of HSL were measured using a solution hybridization assay. This technique has been described in detail previously (20, 21). 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. The RNA concentration was measured spectrophotometrically.

A 150-bp fragment of human HSL cDNA (nt 62 to 212 downstream ATG), 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), terbutaline sulfate from Draco (Lund, Sweden), dobutamine hydrochloride from Lilly (Indianapolis, IN), and CGP 12177 from Ciba Geigy (Basel, Switzerland). Kaleidoscope Standards were purchased from Bio-Rad (Hercules, CA) and 1(3)-mono[^{3}H]oleoyl-2-oleoylglycerol was prepared by Dr. L. Krabisch at the Department of Cell and Molecular Biology, Lund University (Lund, Sweden) (22). The recombinant rat and human HSL, the affinity-purified rabbit anti-rat HSL, and the affinity-purified rabbit anti-human HSL antibodies were synthesized by Dr. C. Holm 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). Protein-A–Sepharose was purchased from Pharmacia Biotech (Uppsala, Sweden). 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 analysis

Values are given as the mean ± standard error of the mean (SEM). Statistical analysis was determined using Student's paired *t*-test and correlation using linear regression analysis.

RESULTS

Table 1 shows the selection of eight pairs of obese subjects, according to sex, maximum isoprenaline-stimulated lipolysis, and fat cell volume. Glycerol release at the maximum effective isoprenaline concentration was defined as the maximum lipolytic capacity. The study included six female and two male pairs of subjects with fat cell volume ranging from 738 to 1068 picoliters. The subjects of a pair were selected with an about 2-fold difference in values of maximum isoprenaline-induced lipolysis ($P < 0.001$), with a mean fat cell volume almost identical in the two subjects.

TABLE 1. Selection of pairs of subjects with identical sex and similar fat cell volume but different maximum isoprenaline-induced lipolysis rates per cell in vitro

Pairs	Sex	Maximum Isoprenaline Response		Fat Cell Volume	
		LL	HL	LL	HL
		μmol/10⁷ cells/2 h		*picoliter*	
1	F	14.5	25.3	738	758
2	F	18.4	39.6	747	796
3	F	18.0	52.1	798	812
4	M	20.4	40.6	829	822
5	F	32.8	55.9	899	824
6	F	25.0	52.4	923	996
7	M	23.3	61.9	1046	1044
8	F	23.9	63.4	1068	1018
Mean ± SEM		22.1 ± 2.0	48.9 ± 4.6[a]	881 ± 46	884 ± 41

LL, low lipolysis group; HL, high lipolysis group; F, female; M, male.

[a] $P \leq 0.001$: HL versus LL group.

Table 2 shows the clinical data of the 16 subjects. The body mass index (BMI) ranged from 37.0 to 52.6 kg/m^2, and waist-hip ratio ranged from 0.82 to 1.08. There was no statistically significant difference in the clinical characteristics between the two groups.

The pharmacological properties of noradrenaline, isoprenaline, specific β-adrenoceptor subtype agonists, forskolin, and dcAMP are shown in **Table 3**. Basal lipolysis rates were 14.3 ± 2.0 μmol/10^7 cells in the HL group and 6.3 ± 0.9 μmol/10^7 cells in the LL group ($P < 0.01$). Maximum lipolysis values for all compounds were 2-fold higher in HL-compared to the LL-group ($P < 0.05$ or better). This significant difference also remained after subtraction of the values for basal lipolysis (data not shown). On the other hand, the pD2 values for noradrenaline, dobutamine, terbutaline, and CGP 12177 did not differ statistically between the two groups.

Figure 1A showed Western blot analysis for three pairs of subjects. Two immunoreactive bands were detected, a major band at about 88 kDa and, in some samples, a minor band at about 84 kDa. The 84 kDa band observed in the present study does not seem to be an artefact as the reproducibility of the lower band determination from the same fat-free infranatant was higher than 80% (data not shown). In order to determine the nature of the 84 kDa immunoreactive protein, a competitive immunoprecipitation experiment, followed by Western blot analysis was performed (Fig. 1B). Lane 1 showed an immunoprecipitation with an infranatant containing the two proteins. Both bands appeared on the blot. Lane 3 showed an immunoprecipitation with extract from COS cells transfected with an expression vector for human HSL. Only one band was detected corresponding in size to the upper band (88 kDa) detected in adipose tissue (lane 1). Lane 4 was a control immunoprecipitation performed with non-transfected COS cell extract. As expected, no immunoreactive proteins were detected. Lane 2 showed the result of an immunoprecipitation with an infranatant known to contain both proteins and anti-HSL antibodies preincubated with an excess of COS cell-expressed HSL. No immunoreactive band at 84 kDa was detected, indicating that the preincubation of the antibody with human HSL prevented the immunoprecipitation of this protein. These data strongly suggest that the 84 kDa protein is an HSL variant. To examine the relative affinity of the HSL antibodies for the 88 and 84 kDa proteins, we performed another competitive immunoprecipitation experiment with increasing amounts of recombinant human HSL in competition with a fixed amount of adipose tissue infranatant containing the two proteins (Fig. 1C). Lane 1 showed the immunoprecipitation of the infranatant alone and both immunoreactive bands appeared on the blot. In competition with increasing amounts of recombinant human HSL (lanes 2–5), we observed an increasing intensity of the band for the 88 kDa protein and a decreasing intensity of the band for the 84 kDa protein. In competition with 20 mU of recombinant human HSL (lane 5), no immunoreactive band at 84 kDa was detected. As expected, anti-human HSL antibodies neither preincubated with recombinant human HSL nor incubated with infra-

TABLE 2. Clinical characteristics

	LL	HL	*P*
Age (years)	38 ± 4	31 ± 2	NS
BMI (kg/m^2)	44.0 ± 1.3	44.7 ± 1.7	NS
Waist-hip ratio	0.97 ± 0.03	0.96 ± 0.02	NS
Glucose (mmol/l)	7.6 ± 1.0	5.5 ± 0.2	NS
Insulin (mU/l)	25.0 ± 4.2	23.7 ± 6.2	NS
Cholesterol (mmol/l)	5.3 ± 0.2	5.4 ± 0.3	NS
Triglycerides (mmol/l)	1.8 ± 0.3	2.3 ± 0.4	NS
Systolic BP (mm Hg)	137 ± 7	127 ± 5	NS
Diastolic BP (mm Hg)	83 ± 4	75 ± 3	NS

The values (mean ± SEM, n = 8) are compared using paired Student's *t*-test. LL, low lipolysis group; HL, high lipolysis group; BMI, body mass index; BP, blood pressure.

TABLE 3. Pharmacological properties of isoprenaline, noradrenaline, β-adrenoceptor agonists, forskolin, and dibutyryl cAMP (dcAMP) in human subcutaneous adipocytes from subjects with a high (HL) and low (LL) maximum lipolytic capacity

	Group	pD2	Maximum
			μmol/10⁷ cells
Isoprenaline	HL		48.9 ± 4.6
	LL		22.0 ± 2.0[b]
Noradrenaline	HL	7.20 ± 0.09	39.1 ± 5.1
	LL	7.74 ± 0.26	18.0 ± 1.5[b]
Dobutamine	HL	7.42 ± 0.16	44.0 ± 4.7
	LL	7.15 ± 0.19	23.0 ± 1.9[b]
Terbutaline	HL	7.24 ± 0.29	46.2 ± 5.2
	LL	6.91 ± 0.21	22.8 ± 1.8[b]
CGP 12177	HL	7.72 ± 0.65	25.3 ± 3.6
	LL	7.97 ± 0.43	12.5 ± 1.3[a]
Forskolin	HL		47.7 ± 5.5
	LL		23.0 ± 1.9[b]
dcAMP	HL		45.3 ± 5.8
	LL		23.2 ± 1.8[a]

Data are means ± SEM (n = 8); pD2 = −log (EC_{50}); maximum, maximum glycerol release per cell number.

[a] $P < 0.05$; [b] $P < 0.005$: HL versus LL group.

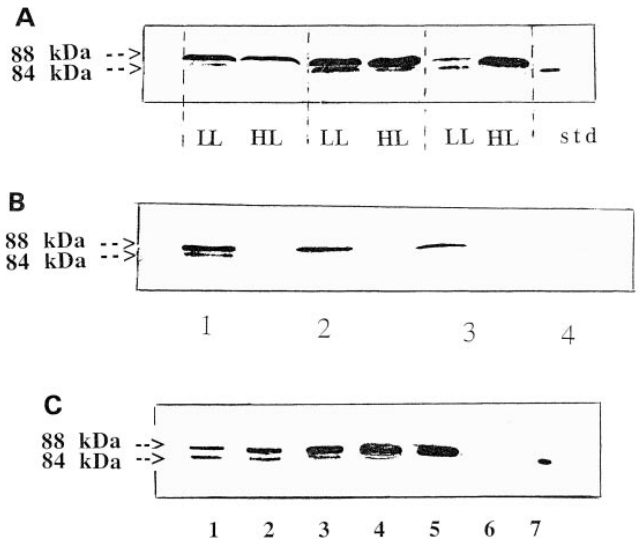

Fig. 1. Detection of HSL in human adipocytes. A: Western blot identification of HSL from human adipose tissue. Fat-depleted infranatants (200 μg total protein) were subjected to SDS-PAGE (8%) and electroblotted onto nitrocellulose membranes. Immunoreactive proteins were detected with an anti-HSL antibody and enhanced chemiluminescence detection (for details, see Methods). The position of the major proteins detected by the anti-HSL antibody was 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. B: A competitive immunoprecipitation experiment was performed as described in the Materials and Methods section, using polyclonal affinity-purified rabbit anti-rat HSL and protein-A–Sepharose. The immunoprecipitate experiments were analyzed by Western blotting using anti-rat HSL as primary antibody (for details, see Methods). Lanes 1 and 2, fat-depleted human adipose tissue infranatants. Material in lane 1 was incubated with antibodies that had not been preincubated with an excess of extract from COS cells expressing human HSL. Material in lane 2 was incubated with antibodies that has been preincubated with an excess of extract from COS cells expressing human HSL. Lane 3, extract from COS cells transfected with an expression vector for human HSL. Lane 4, Extract from non-transfected COS cells. C: A second competitive immunoprecipitation experiment was performed using affinity-purified rabbit anti-human HSL and protein-A–Sepharose. To study the relative affinity of the HSL antibodies for the 88 kDa and 84 kDa proteins, a fixed amount of antibodies was preincubated with different amounts of recombinant human HSL corresponding to 0, 5, 10, 15, and 20 mU in lane 1, 2, 3, 4, and 5, respectively, and then incubated with 2.5 mU of HSL activity of adipose tissue fat-free infranatant. After addition of protein-A–Sepharose, the immunoprecipitates were subjected to the Western blotting procedure described in Methods. One control was included in lane 6, which contained only the human antibodies, neither preincubated with recombinant human HSL nor incubated with adipose tissue fat-free infranatant. Lane 7 contained a standard of recombinant rat HSL.

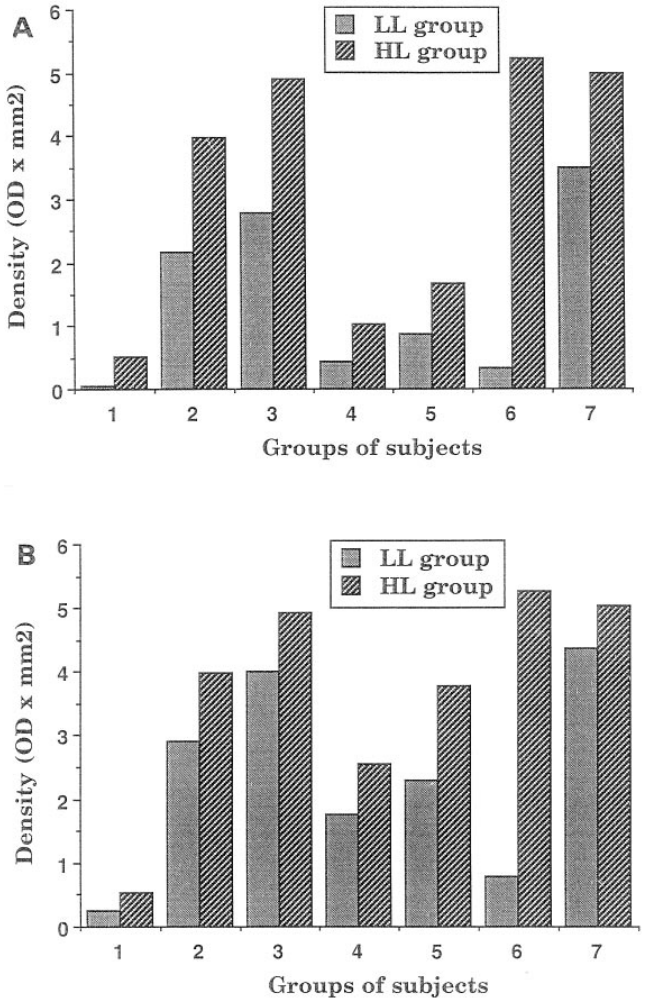

Fig. 2. Abundance of hormone-sensitive lipase in adipose tissue of the subjects from the LL (low lipolytic) and HL (high lipolytic) groups. A densitometer was used to determine the individual optic density of HSL bands obtained upon Western blot analysis. Densities of either the upper band alone (upper graph) or of both bands together (lower graph) were reported.

natant showed no immunoreactive bands at 84 and 88 kDa (lane 6).

Figure 2 showed the individual protein densities for 7 pairs of subjects. For one pair of subjects, the amount of HSL enzyme was too low to be quantified with this method. In each pair, the upper band density was higher for the subject with the high lipolysis rate in comparison with the subject with the low lipolysis rate ($P < 0.05$ for group comparison) (Fig. 2A). The mean upper band density was 50% lower in the LL-group compared with the HL-group (**Table 4**). This difference was also statistically significant when the mean densities of both bands were compared (Fig. 2B and Table 4). Furthermore, mean HSL activity was about 50% lower in the LL-group in comparison with the HL-group and the mRNA ratio between HSL and γ-actin was 35% lower in the LL-group in comparison with the HL-group (Table 4).

The considerable interindividual variation in HSL expression, observed in Fig. 2, was further studied by investigating the relationship between HSL activity, HSL protein amount, and maximum lipolytic capacity (**Fig. 3**). A highly significant correlation between enzyme activity and upper-band density was found. About 90% of the variation in the activity could be explained by variation in upper-band density (i.e., adjusted r^2). Moreover, a significant correlation between maximum lipolytic capacity and enzymatic activity was found. About 75% of the variation in maxi-

TABLE 4. Hormone-sensitive lipase (HSL) activity, protein, and mRNA levels in subjects with high (HL) or low (LL) maximum lipolysis rates

	LL	HL	*P*
HSL activity, mU/mg protein	1.26 ± 0.22	2.35 ± 0.42	0.017
HSL density, OD × mm^2			
Upper band	1.46 ± 0.51	3.20 ± 0.77	0.024
Both bands	2.34 ± 0.58	3.72 ± 0.64	0.041
HSL mRNA ratio, HSL/γ-actin	6.38 ± 0.52	8.67 ± 0.99	0.031

Data (means ± SEM) were statistically compared by paired Student's *t*-test; OD, optical density of the immunoreactive bands.

mum lipolysis could be explained by variation of HSL activity. There was also a significant correlation between upper-band density and maximum lipolytic capacity (r^2 = 0.64). When the densities of both bands were considered, the correlation coefficient was 0.82 between activity and density, and 0.60 between maximum lipolytic capacity and density (data not shown).

DISCUSSION

In this work, we studied the relationship between HSL enzymatic activity, protein and mRNA amounts, and the lipolytic function of human abdominal subcutaneous adipocytes with identical sizes but with either a high or low lipolytic capacity. The patient pairs were matched to have similar fat cell volumes. This was considered important as it is has been shown that there is a positive correlation between fat cell size and basal or catecholamine-stimulated rate of lipolysis in human fat cells, as recently reviewed (2). The LL-group showed a markedly lower basal and catecholamine-induced rate of lipolysis than the HL-group. These differences were maintained when lipolysis was stimulated at different levels of the lipolytic cascade: β-adrenoceptors, adenylyl cyclase, and cAMP-dependent protein kinase. A variation in the lipolytic action of the non-metabolizing cyclic AMP analogue (dcAMP) can only be explained by variations in lipolysis activation at, or beyond, the level of cAMP-dependent protein kinase. Quantitative analysis of the relationship between cellular cAMP concentrations and lipolysis has shown that maximal glycerol release rates are obtained with a rather limited increase in cAMP levels (23). This strongly suggests that phosphorylation and activation of HSL is the rate-limiting step of the lipolytic cascade and that the difference in the lipolytic capacity between the LL- and HL-groups was localized at this step.

Strong evidence for a major role of HSL expression in determining the lipolytic capacity of human fat cells came from direct measurements of HSL activity and protein level. The results showed 50% lower levels of HSL activity and protein, associated with a 50% lower maximum lipolysis rate in the LL-group in comparison with the HL-group. In the whole material, there was a strong linear correlation between HSL protein expression and HSL activity (r^2 = 0.91). This was an expected finding based on the fact that the assay used to analyze HSL activity did not discriminate between phosphorylated and unphosphorylated HSL, but was rather a determination of the total amount of activatable enzyme. The maximum lipolytic capacity was significantly correlated with HSL activity (r^2 = 0.75) and protein (r^2 = 0.64), strongly suggesting that HSL protein expression is a major determinant of the maximum lipolytic capacity of human fat cells.

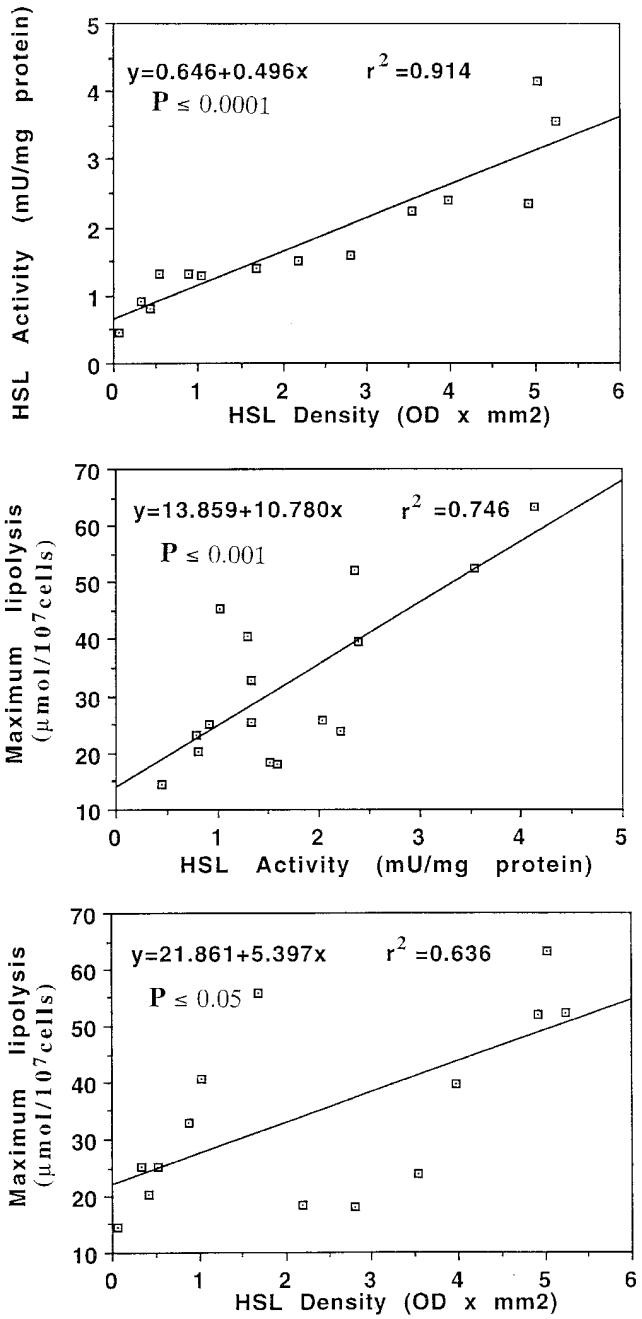

Fig. 3. Relationship between upper band HSL density and activity (upper panel), HSL activity and maximum lipolysis capacity (center), and upper band HSL density and maximum lipolysis capacity (lower panel) in the whole material. The correlation coefficient (r^2) was calculated using linear regression analysis.

The mechanisms behind the variation in HSL protein expression between the investigated subjects could involve some factors related 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 mRNA levels for both the HSL and the "house-keeping" gene γ-actin, with a sensitive solution hybridization assay. These measurements showed that steady-state HSL mRNA level expressed per amount of the "reporter gene" γ-actin, was 35% lower in the LL-group as compared with the HL-group. Thus, low transcriptional activity of the HSL gene and/or high degradation rate of mRNA is probably one explanation for the decreased HSL protein levels in adipocytes with the low lipolytic capacity. This does not exclude that other variations in HSL protein turnover are present in fat cells with high or low lipolytic capacity.

The immunodetection of HSL using Western blot analysis occasionally revealed a lower 84 kDa protein band in addition to the main 88 kDa band. The identification of the human HSL protein as an 88 kDa protein on SDS-PAGE, using polyclonal antibodies against rat HSL, has been shown earlier (24). The human adipose tissue HSL has a slightly larger apparent M_r (88 kDa) than the enzyme from rat adipose tissue (84 kDa). The previous study (24) did not report a lower band for HSL from human adipose tissue, but the determination was performed on samples from a few subjects. The 84 kDa band observed in the present study does not seem to be an artefact as the reproducibility for determination of the lower band from the same fat-free infranatant was higher than 80% (data not shown). In order to assay the specificity of the immunoreactivity of the 84 kDa protein under more stringent conditions than those used for Western blot analysis, we performed competitive immunoprecipitation experiments, followed by Western blot analysis of the immunoprecipitates. The results obtained (Figs. 1B and 1C) strongly suggest that the 84 and 88 kDa proteins share common epitopes and are structurally related. The mechanism whereby the lower molecular weight HSL variant is generated is unknown and can only be speculated upon. It is possible that human adipocytes express two HSL variants. As there is no evidence for the existence of more than one HSL gene (25–27), the two variants are in that case most likely products of the same gene, generated through alternative splicing, alternative N-terminal processing, post-translational mechanisms, or some other mechanisms. Recently, an alternative splicing of the exon 6 was found that generates a catalytically inactive form of the HSL in human subcutaneous adipocytes (28). However, this short transcript had a molecular mass of 80 kDa with Western blot analysis, which is smaller than the short form of HSL that we found. Therefore, the 84 kDa protein probably does not correspond to the alternative splicing form of the exon 6. Another possibility is that the 84 kDa protein represents a proteolytic fragment of the 88 kDa protein. Unfortunately, these hypotheses are not possible to test experimentally at present. However, in this context, it is important to emphasize that the presence of the lower (84 kDa) HSL immunoreactive protein in some of the subjects analyzed does not change the interpretation of the results presented in this study, as the results were similar regardless of whether the 88 kDa protein was considered alone or together with the 84 kDa protein (Fig. 2).

In conclusion, this study showed that HSL protein and mRNA expressions are major determinants of the maximum lipolytic capacity of human fat cells. An assessment of HSL expression is therefore required in further studies aiming at deciphering the mechanisms underlying variation of adipose tissue lipolytic capacity in humans.

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