Hormone-Sensitive Lipase in Human Adipose Tissue, Isolated Adipocytes, and Cultured Adipocytes

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Summary

Employing a perfusion technique, the activity of hormone-sensitive lipase (HSL) in human adipose tissue and isolated adipocytes was found to be significantly stimulated by 5 μ M epinephrine (5-fold, $P < 0.001$) or 0.5 mM theophylline (2-fold, $P < 0.03$). The stimulatory effect of both, however, was not cumulative; instead, theophylline appeared to blunt the epinephrine effect. The two effectors together produced a 3-fold increase in activity over basal ($P < 0.01$). Basal lipolysis was highly and significantly correlated with epinephrine and/or theophylline stimulated lipolysis in the tissue as well as in the isolated cells.

The activity of the enzyme was examined in cultured human adipocytes using a cell-free system. The basal activity of HSL in the 20,000 $\times$ g supernatant (S_{20}) fraction of cultured cells grown in fat-enriched medium was significantly higher than the value in the same fraction of cells grown in regular medium (6.06 ± 1.49 versus 2.78 ± 0.89 nmole glycerol/min/mg protein, mean $\pm$ S.D., $P < 0.01$), and was similar in the S_{20} fractions of cells grown in the enriched medium and the original tissue (6.06 ± 1.49 versus 5.44 ± 2.73 nmole glycerol/min/mg protein, mean $\pm$ S.D., $P > 0.83$). When the tissue and cells were stimulated *in vitro* before fractionation, the HSL activity in the S_{20} fraction of the original tissue increased 4-fold over basal ($P < 0.001$), whereas that in the cultured cells increased 2-fold ($P < 0.01$) regardless of the culture medium employed. In the S_{20} fractions derived from basally incubated samples, but not in those derived from the epinephrine-stimulated samples, the HSL enzyme was slightly but significantly ($P < 0.002$) activated by exogenous addition of ATP, cyclic AMP, and protein kinase.

The data suggest that the activity of HSL is retained in cultured human adipocytes and could be enhanced under conditions of culture which favor lipid accumulation and adipose conversion.

Speculation

Although the hormone-sensitive lipase enzyme protein in adipose tissue of the rat and the chicken has been extensively purified, the human enzyme remains difficult to purify. The finding in this study, that human adipose tissue hormone-sensitive lipase activity is retained in culture, should facilitate the purification and characterization of the enzyme in man, promote the study of the cellular mechanisms involved in the synthesis, transport and degradation of the enzyme and enhance the search for possible genetic heterogeneity of the enzyme among individuals suspected to have genetic obesity.

The enzymes involved in lipid metabolism in adipose tissue have been extensively studied (22, 29, 30, 33, 38, 39, 40, 46, 49); however, the so-called hormone sensitive lipase (HSL) has proved difficult to obtain in sufficiently high purity for characterization. In the crude preparation, it appears tightly bound to large lipid-

rich particles from which it is difficult to separate (34). Recently, it has become possible to extensively purify and characterize the enzyme in the adipose tissue of the rats and the chicken (14-16, 24, 34, 35). The pH optima for the extensively purified (2000-fold) rat enzyme and the partially purified (35-fold) human enzyme were 7.0 and 7.4, respectively (24, 25, 26). *In vitro* studies with human adipose tissue and isolated cells suggest that HSL is, indeed, very active in the human adipose tissue but the literature on the subject appears to suggest that there is no uniform system for reporting specific activity of human HSL. Some report activity per wet weight (6, 7, 19, 32, 43, 44, 47), others per lipid content (8, 9, 36), others per isolated cell (8, 10-13) and still others per packed cell volume (13, 20, 41); only an occasional study (8) expresses HSL activity on more than one basis.

Few studies have reported on the HSL activity of cultured rat adipocytes (18) and none on the enzyme in cultured human cells; although by contrast, lipoprotein lipase activity of cultured human and rat adipocytes has been studied (17, 18, 27, 45, 50). In this study, we have devised a new but simple perfusion technique, designed to minimize the known inhibitory effect of free fatty acids on adipose tissue lipolysis (4, 5, 23, 28). We have employed this technique to examine the activity of HSL in whole human adipose tissue and in the adipose cells isolated from the tissue. We have also studied the activity of HSL in cultured human adipocytes.

MATERIALS AND METHODS

Adipose tissue (0.5-5 g) was obtained from the subcutaneous tissue of the abdominal wall (15 samples), the upper thigh (6 samples) and the ankle (2 samples) from 23 children aged 1-12 years. The children were free of nutritional or metabolic diseases. They were undergoing scheduled elective operative procedures and the samples were excised at the onset of operation and transferred immediately to the laboratory.

The tissues were divided into three portions of similar weight. The first two portions were incubated with collagenase (52) to isolate adipocytes as previously described (1, 2). The isolated cells from the first portion were cultured also as previously described (2, 3). Isolated cells were counted in an inverted cell counting chamber on plastic coverslips. The cell yield was $8.0 \pm 1.7 \times 10^5$ cells per g wet tissue and $1.3 \pm 0.3 \times 10^6$ cells per ml packed cells (mean $\pm$ S.D., $n = 21$). Glass coverslips were found unsuitable for adipose cell counting as the cell membrane is extremely fragile on contact with glass. The third tissue portion was cut into small (approximately 50 mg) pieces. The cut pieces and the isolated cells from the second portion were each divided into a number of equal subportions determined by the original tissue size and the types of studies anticipated. On each sample, however, cell culture was attempted. Basal and hormone stimulated lipolytic rates were determined on the whole tissue and on the isolated cells. Preliminary experiments using various concentrations of epinephrine

(10^{-2} to 10^{-7} M) and theophylline (10^{-2} to 10^{-6} M) singly and in combination established that maximal stimulation was obtained with 5 μ M epinephrine alone; theophylline alone at an optimal concentration of 0.5 mM also caused significant stimulation of lipolysis although less than that obtained with 5 μ M epinephrine (see "Results"). Because the addition of theophylline to epinephrine did not enhance the stimulatory effect of epinephrine at its optimal concentration (5 μ M), subsequent stimulation experiments were performed with 5 μ M epinephrine alone (see "Results").

Studies on tissues and isolated cells. In an attempt to minimize the problem of free fatty acid inhibition of lipolysis in stimulated tissue and cells, an apparatus (Fig. 1) was devised. This apparatus, which permitted several experiments to be performed at one time, consisted of multiple 12-ml graduated polypropylene minicolumns (53) each fitted with a 35 μ filter at its tip and attached by polyethylene tubing (2.5 mm ID) to a 20-channel peristaltic cassette pump (54). Channels were capable of delivering medium at rates from 0.25–500 ml per h. The intake end of the tubing was

inserted into a flask containing the incubation medium, with or without effectors (epinephrine and/or theophylline). The tubing was passed through the pump and the exit end was attached to a butterfly needle (25 gauge) inserted into the cap of the column. Incubation medium (200 μ l) was placed into the column and cut pieces of tissue (250 mg wet weight) or 250–300 μ l of packed cells (approximately 25×10^5 cells) were gently introduced on top of the medium. A 25-gauge syringe needle was inserted into the column tip and placed into a 3-ml plastic sample vial which could be changed at desired sampling intervals. The tissues or cells were then perfused under constant pressure with medium at rates which were readily controlled. Each sample collected was accurately measured in order to determine the precise flow rate in each channel at any particular moment. The entire system was placed in an incubator at 37°C in an atmosphere of 5% CO₂ and 95% air. From preliminary studies, a perfusion rate of 5–10 ml per h was found to be ideal and all perfusion experiments were performed in this range. After equilibrating for 10 min at 37°C, samples were collected at 1–5-min intervals for the first 15 min and at 15-min intervals thereafter for as long as 1 h. The collected samples were either immediately assayed or kept frozen at –70°C for later assay of glycerol by the method of Chernick (21). Preliminary studies (results not shown), in which we compared the standard incubation method with the perfusion method, established that there was no difference in the lipolytic rates of a few tissue samples under basal or epinephrine-stimulated conditions; however, we chose to employ the perfusion technique in our studies because of its simplicity and the opportunity it offered to study the same tissue sample under several different conditions.

In some experiments after the perfusion, the tissues were removed, washed with buffer, quickly blotted dry, weighed and homogenized under ice in an equal volume of 50 mM sodium phosphate buffer, pH 6.9 containing 1 mM EDTA. The homogenate was centrifuged at $20,000 \times g$ at 4°C for 20 min. The aqueous supernatant (S_{20}) fraction below the upper fat cake was removed for immediate assay or kept frozen at –70°C for later assay of HSL by a modification of the method of Khoo and Steinberg (33) described below.

Studies on the cultured cells. The cultured cells were first subcultured after an initial period of 1–4 wk. They were subsequently subcultured at confluence and the medium changed every 3–4 days. After the fourth passage, some cells were maintained in the regular medium which consisted of RPMI 1640 with 10% fetal calf serum (FCS), penicillin (1 unit/ml) and streptomycin (1 μ g/ml). Others were grown in a fat-enriched medium, made up of M199 fortified with 20% FCS, 0.75 mM linoleic acid, 10 mM glucose 200 μ units insulin/ml, penicillin, and streptomycin. The cultured cells were studied at weekly intervals after the fourth passage for up to 8 wk.

Hormone stimulation of the cultured cells was performed as follows. Replicate monolayer cells in T-150 flasks were drained of medium and washed three times with serum-free medium. Then 15 ml of serum-free RPMI 1640 with 5% BSA with (stimulated) and without (basal) epinephrine (5 μ M) were added. The cells were incubated for 30 min; immediately washed six times with 50 mM sodium phosphate buffer, pH 6.9, and then lysed by freezing and thawing four times in distilled water (3 ml/flask). The lysate was centrifuged at $20,000 \times g$ at 4°C for 20 min. The aqueous supernatant (S_{20}) fraction was removed for immediate assay or kept frozen at –70°C for later assay of HSL by a method modified from Khoo and Steinberg (33) described below.

Activation of HSL enzyme in the S_{20} fractions. We adapted the method of Khoo and Steinberg (33) to the measurement of HSL in the S_{20} fraction of both the tissue homogenate and cultured cell lysates. The enzyme source was incubated (activated) at room temperature for 10 min in a total volume of 100 μ l of a mixture containing 25 mM Tris-HCl buffer, pH 8.0, 5 mM magnesium acetate, 1 mM EDTA, 2 mM dithiothreitol, 0.5 mM ATP, 0.01 mM cyclic AMP, 150 g/ml protein kinase (cyclic AMP dependent) and 25–50 μ g protein. The nonactivated enzyme mixture was similarly incubated, but in the absence of ATP, cyclic AMP, and

Fig. 1. *Top:* side view of the perfusion apparatus showing, on the left, 50-ml flasks containing perfusion medium. A polyethylene tube, emerging from each flask, passes through the peristaltic pump (center), set to deliver at desired flow rate. On the right, each polyethylene tube emerges from the pump and is inserted, by a 25-gauge butterfly needle, into the cap of a 12-ml polypropylene minicolumn containing 0.2 ml medium and tissue fragments or cells. Each column is fitted with a 35- μ filter at its tip. The medium, after bathing the cells or tissue fragments, is collected in 3-ml sample vials, which are changed at desired intervals. The volume of each vial is accurately measured to determine the precise flow rate in each channel. *Bottom:* Front view of the apparatus showing additional details of the cassettes, the pump, the minicolumns, and the collecting sample vials. The pump is capable of holding a maximum of ten cassettes, each of which can accept two polyethylene tubes (internal diameters, 0.5 mm) for a maximum of 20 channels. The picture shows ten channels.

protein kinase. The controls lacked the enzyme protein but contained all other ingredients.

Assay of HSL enzyme in the S_{20} fractions. After 10 min activation, as described above, 200 μ l of a substrate mixture was added, which contained 10^{-4} M triolein sonicated in 5% gum arabic and 0.5% BSA dissolved in 50 mM sodium phosphate buffer, pH 7.4. The glycerol formed in this reaction mixture was monitored spectrophotometrically by monitoring the decrease in NADH absorbance at 340 nm by a modification of the method of Pinter *et al.* (42). In this modification, the assay was performed by adding to the 300 μ l of enzyme-substrate mixture, 1.5 ml of a reaction mixture containing 3 mM magnesium sulfate, 1 mM potassium chloride, 0.25 mM ATP, 0.2 mM NADH, 2 mM PEP (tri-monocyclohexylammonium salt), 6 units/ml LDH, and 1.3 units/ml pyruvate kinase in 50 mM triethanolamine-HCl buffer, pH 7.4. The total reaction mixture was shaken rapidly and initial absorbance (A1) read at 340 nm at room temperature in a Hitachi digital recording spectrophotometer model 191. Then 18 μ l of glycerol kinase (150 μ g/ml) was added to initiate the reaction which was then monitored by recording absorbance (A2) at 5-min intervals for 30 min at room temperature. Glycerol standards (0–200 nmole/ml) in 50 mM sodium phosphate buffer, pH 7.4, containing 5% gum arabic and 0.5% BSA were analyzed simultaneously with each set of determinations. The reaction was usually complete within 5 min in the absence of new glycerol formation; however, new glycerol formed by the action of HSL enzyme on triolein could be followed for as long as 2–3 h. The rate of glycerol formation between 15–30 min was used for data analysis.

From preliminary studies employing a pH range from 6.5–8.0, maximal activity of HSL was found at pH 7.4 and this pH was employed in all subsequent determinations. Also from preliminary investigations, triolein at concentrations from 10^{-2} to 10^{-8} M was employed. Maximal enzyme activity was observed over a broad range (10^{-6} to 10^{-4} M) of triolein concentration; triolein at a concentration of 10^{-4} M was employed in all subsequent determinations in order to ensure an adequate amount of substrate in the reactions. The enzyme activity was also linear over a wide range (5–100 μ g) of enzyme protein content in the reaction mixture. Therefore, the enzyme substrate mixture was designed to contain 25–50 μ g protein in all experiments.

Protein in the S_{20} fractions was assayed by the method of Lowry *et al.* (37). Specific activity, expressed as nmole of glycerol formed or released per min, was determined per g tissue wet weight, per 10^6 cells or per mg protein in the S_{20} fraction.

The data were analyzed by paired *t* test and significance assigned where the *P* value was less than 0.05. Also, when appropriate, regression analysis of paired data was performed.

RESULTS

Whole tissue and isolated adipocytes. Figure 2 demonstrates that incubating whole tissue or isolated cells with epinephrine (5 μ M) or theophylline (0.5 mM) or both produced significant increases in the specific activity of HSL. In the whole tissue, epinephrine alone caused a 5.4-fold increase over basal activity and was more effective as a stimulant than theophylline alone, which produced a 1.8-fold increase or a combination of both which produced a 3.3-fold increase. In isolated cells, similar results were obtained. Epinephrine alone, which produced a 4.5-fold increase over basal, proved more effective as a lipolytic stimulant than theophylline alone, which caused a 1.7-fold increase or a combination of both which caused a 2.5-fold increase over basal levels.

In Figure 3, a significant direct relationship is demonstrated between basal and effector-stimulated lipolysis for 5 μ M epinephrine and/or 0.5 mM theophylline in the whole tissue and in the isolated cells.

The cultured cells. Figure 4 shows the appearance of a typical cultured adipose cell line after 6 wk in culture when it has become essentially lipid-free (a). The same cell line shows progressive lipid

Fig. 2. Effect of epinephrine and theophylline on lipolysis in whole adipose tissue and in cells isolated from adipose tissue. Cells (or tissues) were incubated for 60 min, as described in "Materials and Methods," in the absence of any effectors (a) or in the presence of 5 μ M epinephrine (b) 0.5 mM theophylline (c) or both (d). Glycerol release was measured, as described in "Materials and Methods." Results are expressed as mean $\pm$ S.D. for the number of samples indicated at the bottom of each bar. Values below the graph indicate the *P* value for the paired *t* test comparing the means between the two treatments joined by each pair of arrows.

reaccumulation when grown in the presence of free fatty acids for 24 (b) and for 7 days (c).

The basal specific activity of HSL in the S_{20} fraction of lysates of cells grown in fat-enriched medium was similar to that in the S_{20} fraction of the original tissue and was twice that in the S_{20} fraction of lysates of cells grown in the regular medium (Fig. 5).

When the cultured cells were stimulated *in vitro* for 30 min with 5 μ M epinephrine and then lysed, the specific activity of HSL in the S_{20} fraction of lysates of cells grown in fat-enriched and in regular medium doubled. The HSL activity in the S_{20} fraction of the stimulated original tissue increased 4-fold over basal, attaining values which were twice those in the S_{20} fractions of lysates of cells grown in the fat-enriched medium and four times those in the S_{20} fraction of lysates of cells grown in the regular medium (Fig. 5).

Enzyme activation. As shown in Table 1, HSL was slightly (8–15%) but significantly activated in all S_{20} fractions of tissue homogenates and cell lysates incubated basally *in vitro*. Upon activation, there was a mean increase of 11% in the enzyme activity of basally incubated tissue ($n = 10$, $P < 0.001$), a mean increase of 15% in enzyme activity of basally incubated cells grown in regular medium ($n = 10$, $P < 0.002$) and a mean increase of 8% in the enzyme activity of basally incubated cells grown in fat-enriched medium ($n = 6$, $P < 0.03$). On the other hand, when the tissues and cells had been epinephrine-stimulated *in vitro* before homogenization and lysis respectively, the increases in activity found on activation of the enzyme in the respective S_{20} fractions were very small (3–7%) and not significant: whole tissue ($n = 10$, $P > 0.1$),

Fig. 3. Relationship between basal and effector-stimulated lipolysis in whole adipose tissue and in cells isolated from adipose tissue. Whole adipose tissue or isolated adipose cells were incubated as described in "Materials and Methods" in the absence (basal) or presence of 5 μ M epinephrine and/or 0.5 mM theophylline. Basal and effector-stimulated lipolysis were plotted for each tissue or cell sample. In isolated cells and in whole tissue respectively, basal lipolysis was compared with epinephrine-stimulated lipolysis (*a* and *b*), with theophylline-stimulated lipolysis (*c* and *d*) and with lipolysis stimulated by both (*e* and *f*). For each set of values compared, the regression equation derived has been shown on the regression line and the number of samples compared (*n*), the correlation coefficient (*r*) and the *P* value have also been shown.

cells in fat-enriched medium ($n = 6$, $P > 0.05$) cells in regular medium ($n = 10$, $P > 0.05$).

DISCUSSION

Adipose tissue perfusion technique has been employed at least twice previously (5, 48) and found useful in helping to establish the time relationship between the attainment of maximal intracellular cyclic AMP concentration and maximal cellular lipolytic rate (5). It has also been employed to establish the concentration of adenosine necessary to induce adenosine lipolytic inhibition in adipose tissue (48). The particular perfusion method we have employed is different from that previously employed by others (5, 48) although it shares in the advantage that it is a simple technique which allows several studies to be performed on the same samples simultaneously and consecutively. The value of HSL obtained using this technique is similar to the values obtained using the standard incubation technique on the same samples (results not shown). Our data are in general agreement with previously reported levels of HSL in human adipose tissue and cells, whether expressed per g wet tissue (6, 7, 19, 32, 43, 47) or per cell (8, 10–13, 31).

Studies of adipose tissue HSL have generally been limited to

the whole tissue and isolated cells. In this study, we have attempted to establish the fate of this enzyme in cultured cells as well. Higher levels of HSL activity in cultured cells were found in those cells grown in the fat-enriched medium; however, whether cultured in regular or enriched medium, significant levels of basal HSL activity were retained in the cells. These basal levels increased significantly when the cells were stimulated *in vitro* by epinephrine. It is interesting that the cells with the highest levels of basal and epinephrine-stimulated HSL activities were those grown in the fat-enriched medium and had showed the morphologic changes associated with adipose conversion as seen by us (Fig. 4) and also previously described by others (50, 51). The cells grown in the enriched medium had basal levels of HSL activity similar to those in the original tissue and, although they did not respond to epinephrine stimulation as markedly as did the original tissue, nevertheless, they responded briskly by doubling, on stimulation, their HSL activity over basal.

In this study, we noted that although theophylline by itself significantly stimulated lipolysis nearly 2-fold over basal, it appeared to blunt or reduce the epinephrine effect on adipose tissue and cells when combined with epinephrine. This observation was consistently noted throughout and, at the present time, there is no ready explanation for it, especially in view of the assigned role of

Fig. 4. (a) Cultured adipocytes from a 7-year-old girl (Oil-Red-O stained) 6 wk after initial culture and subcultured four times in regular medium (see "Materials and Methods"). The cells have assumed the usual fibroblast appearance and little intracellular neutral lipid remains ($\times 100$). (b) Cultured adipocytes as in (a), but grown for 24 h in fat-enriched medium (see "Materials and Methods"). The cells (stained) remain fibroblast-like in appearance, but now show small intracellular neutral lipid droplets ($\times 100$). (c) Cultured adipocytes as in (b), grown for 7 days in fat-enriched medium. Note that the cells have become more rounded in appearance, massively engorged with large amounts of intracellular neutral lipid. The nucleus in some cells appears to be located at the periphery ($\times 100$). Further exposure to fat-enriched medium for an additional 7 days caused no further intracellular lipid-accumulation.

theophylline as a phosphodiesterase inhibitor. It would be expected to enhance lipolysis by inhibiting the enzyme which degrades cyclic AMP, thus effecting an accumulation of intracellular cyclic AMP. But, as noted earlier, there is a significant time lag between the attainment of maximal intracellular cyclic AMP concentration and the onset of maximal lipolysis in rat adipose cells (5), suggesting that factors other than intracellular cyclic

AMP concentration may be important to the lipolytic process. Although our data did not establish a direct relationship between adipose tissue HSL activities and the ages of the donor children ($r = 0.30$, $n = 21$, $P > 0.05$) we did find a consistent and significant direct relationship between basal and stimulated (epinephrine and/or theophylline) HSL activities in the adipose tissue and cells of these children. The higher the basal activity of HSL in the adipose cell, the greater was the response to epinephrine and/or theophylline stimulation. In our own studies, we did not systematically measure the cell size; however, we did note that the smallest appearing cells (deriving from the heels and thighs) had the lowest basal and effector-stimulated HSL activities. In adults, a significant direct relationship has been previously reported between basal and hormone-stimulated lipolysis in human adipose tissue (31, 32). In addition, a direct relationship was also found by the same investigators (31, 32) between basal and hormone-stimulated lipolysis on the one hand and adipose cell size on the other.

Fig. 5. Effect of *in vivo* incubation with epinephrine on the hormone-sensitive lipase in the aqueous fractions of adipose tissue and cultured adipose cells grown on regular and fat-enriched medium. Whole tissues were incubated as described in "Materials and Methods" in the absence (a) or presence (a') of $5 \mu\text{M}$ epinephrine. The cells were thoroughly washed and homogenized and the S_{20} fractions (see "Materials and Methods") assayed for hormone-sensitive lipase as described. Isolated cells were cultured as described in "Materials and Methods." Ten lines were grown in regular medium, six of these in fat-enriched medium as well. For these experiments, paired flasks of cells grown in fat-enriched medium were incubated for 30 min in the absence (b) or presence (b') of epinephrine. Paired flasks of cells grown in regular medium were also incubated for 30 min in the absence (c) or presence (c') of epinephrine. The cells were washed carefully and lysed and the S_{20} fractions (see "Materials and Methods") were assayed for hormone-sensitive lipase. Results are expressed as mean $\pm$ S.D. for the number of cell lines indicated at the bottom of each bar. Each cell line was studied at least twice. Values below the graph indicate the P values for the paired t test comparing means between the two treatments joined by each pair of arrows.

Table 1. Activation of hormone-sensitive lipase activity in the cell-free system employing the S_{20} fraction of tissue homogenates and cultured cell lysates¹

		Cultured cells		
		Whole tissue	Fat-enriched medium	Regular medium
<i>S₂₀ fraction: basally incubated samples</i>		(10)	(6)	(10)
Minus activators*	<i>n</i> =	5.44 ± 2.78	6.06 ± 1.49	2.78 ± 0.89
Plus activators**		6.05 ± 3.15	6.55 ± 1.25	3.19 ± 0.90
	<i>P</i> =	< 0.001	< 0.03	< 0.001
<i>S₂₀ fraction: epinephrine stimulated samples</i>		(10)	(6)	(10)
Minus activators*	<i>n</i> =	19.39 ± 7.30	10.35 ± 3.69	4.57 ± 1.43
Plus activators**		19.99 ± 8.15	10.96 ± 4.07	4.92 ± 1.42
	<i>P</i> =	> 0.10	> 0.06	> 0.05

¹ Whole tissue and cultured cells were fractionated as described in "Materials and Methods" after incubation in the absence (basal) or presence of 5 μ M epinephrine for 30 min *in vitro*. The S_{20} fractions (see "Materials and Methods") were assayed in the absence (minus activators)* and presence (plus activators)** of ATP, cAMP, and protein kinase (see "Materials and Methods"). Results are expressed as mean $\pm$ S.D. for the number (*n*) of tissue samples or cell lines studied. The *P* value relates to the paired *t* test comparing the same samples treated with or without activators.

Further, James *et al.* (32) reported a direct relationship between basal and hormone-stimulated lipolysis and the degree of obesity.

Our data suggest that HSL in the crude S_{20} fraction can be activated. The degree of activation, however, appears low, attaining statistical significance when the preparation was derived from basal incubation but not when the preparation had been epinephrine-stimulated *in vitro*. The difference appears to be due to the fact that the % increase in activity due to activation was greater in the basal than in the epinephrine-stimulated samples. We expect that it would be possible to effect higher activation with greater purification of the enzyme protein.

Hormone sensitive lipase has proved difficult to purify and characterize; however, the enzyme has been extensively purified (2000-fold) in the rat adipose tissue and somewhat less so (300-fold) in the chicken adipose tissue. The enzyme from the rat tissue has been characterized with respect to its molecular weight (14, 15, 24), its isoelectric point (14, 15, 34), its Stokes radius (15), and a few of its inhibitors (24, 34). Activation of the rat enzyme was achieved by phosphorylation of the enzyme protein (15, 24). In man, it has been difficult to conduct the same studies because of the inordinately large quantity of fat tissue needed for such studies. It is encouraging to find that cultured human adipocytes retain high enough HSL activity so that they can be grown in sufficiently large quantity in order to obtain enough protein for purification and characterization. Some potential advantages are inherent in the use of the culture method to study the HSL activities in human adipose tissue, particularly in the individual patients who might be suspected to have genetic or familial obesity. It would enable us to undertake a search for genetic heterogeneity of HSL among such individuals. Finally, this method may be very useful in our efforts to clarify the cellular mechanisms involved in the synthesis, transport and degradation of hormone-sensitive lipase in the human adipose cell.

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