β-Adrenergic Responsiveness of Adenylate Cyclase in Human Adipocyte Plasma Membranes in Obesity and After Massive Weight Reduction

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The aim of this study was to find out how β-adrenergic responsiveness of adipocytes is altered in obesity and by weight loss and to investigate what mechanisms lead to potential alterations in responsiveness. Crude plasma membranes were prepared from adipocytes of massively obese and normal-weight individuals, as well as previously obese patients that had lost an average of 38% of their initial weight after bariatric surgery. Stimulation of adenylate cyclase by isoproterenol varied considerably in fat cell plasma membranes from different individuals. Crude fat cell plasma membranes from obese patients were less responsive to isoproterenol than those from normal-weight subjects, whereas those from postgastroplasty patients were hyperresponsive. The response was correlated negatively with cell size and positively with β-adrenergic receptor density and with the ratio of β-receptors and stimulatory G-proteins (Gs). There was no correlation with Gs content. However, differences in receptor density between small and large cells or normal-weight, obese, and post-bypass patients could not explain the observed differences in responsiveness to isoproterenol between the different groups.

FAT CELL LIPOLYSIS is linked to obesity in several different ways. It has to adapt to increased fat mass and weight loss.[1-4] In rare cases such as pseudohypoparathyroidism type 1a, changes in lipolysis may even be important in the development of obesity.[5] Lipolysis and concomitant re-esterification may be an important futile cycle. It has been reported to be important in the increased thermogenesis after exercise and may therefore have a role in energy expenditure.[6] There are also regional differences in adipose tissue metabolism, and such differences are probably important in the development of the metabolic syndrome.[7-17]

Lipolysis is controlled by intracellular cyclic adenosine monophosphate concentrations, and changes in adenylate cyclase regulation may therefore be important in generating the changes and differences in adipocyte metabolism described earlier. Adenylate cyclase is under dual control, being regulated by both stimulatory and inhibitory receptors acting via stimulatory and inhibitory G-proteins (Gs).[18] In adipocyte plasma membranes, the major positive regulation occurs via the β-adrenergic receptor, and adenosine and α_2-adrenergic receptors inhibit the cyclase.[19]

Changes in the inhibitory arm of cyclase regulation have been suggested to be responsible for the inhibition of lipolysis in obese Zucker rats.[20] Indeed, the inhibitory arm has been reported to be altered in adipocytes in obese humans.[4,21] Less is known about the importance of potential changes in the stimulatory arm of adenylate cyclase regulation. The stimulatory arm has also been suggested to be blunted in obesity in both experimental animals[22] and man.[21] Decreases in both β-receptor number[23] and stimulatory G-protein (Gs) concentrations[22,24,25] or functional activity[26] have been reported, but their roles are not clear at present.

The aim of the present study therefore was to find out how β-adrenergic responsiveness of human fat cells is altered in obesity and to study the possible molecular mechanisms of any changes.

SUBJECTS AND METHODS

Tissue Donors

Samples of lower-abdominal subcutaneous adipose tissue were excised under general anesthesia from 10 obese subjects (body mass index [BMI], 46.3 ± 5.6 kg/m^2, mean ± SEM) and nine normal-weight subjects (BMI, 25.0 ± 1.4 kg/m^2), and from seven patients whose BMI had decreased from a mean of 56.4 (range, 42.3 to 86.0) to 33.9 (range, 28.9 to 41.4) kg/m^2. The ages (mean ± SEM) of normal-weight, obese, and postobese patients were 48 ± 6, 46 ± 4, and 45 ± 4 years. Obese patients were undergoing gastric bypasses or gastroplasties, and the other patients had cholecystectomies, hysterectomies, or plastic surgery. Patients with diabetes, hormone treatments, or β-adrenergic–blocking agents were excluded. All patients were white, and all were female except one obese, one postobese, and four normal-weight patients. The conclusions reached were not altered if the male patients were excluded from the study.

Informed consent was obtained from each tissue donor. The project was approved by the ethics committees of the Milton S. Hershey Medical Center and Helsinki University Central Hospital.

Isolation of Fat Cells and Crude Plasma Membranes

Fat cells were isolated by a modification of the method reported by Rodbell[27] in the presence of collagenase (0.5 mg/mL) under constant shaking at 2 Hz at 37°C in a buffer containing 125 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $CaCl_2$, 2.5 mmol/L $MgCl_2$, 1 mmol/L KH_2PO_4, 4 mmol/L glucose, 2% bovine serum albumin, and 25 mmol/L Tris at pH 7.4. After 60 minutes, the cells were filtered through a nylon cloth and washed three times with the same buffer without collagenase. Cell size was estimated by direct microscopy of isolated cells. The cells were homogenized, and crude plasma membranes were isolated as described elsewhere.[28]

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Total protein concentrations of these preparates were assayed by the method reported by Lowry et al.[29]

Adenylate Cyclase Assay

Adenylate cyclase activities of fresh adipocyte crude membranes were assayed in a total volume of 500 μL in the presence of 150 mmol/L NaCl, 10 mmol/L KCl, 10 mmol/L $MgCl_2$, 1 mmol/L EDTA, 2 mmol/L dithiothreitol, 1 mmol/L adenosine triphosphate, 100 μmol/L papaverine, 1 mmol/L guanosine triphosphate ([GTP] except in experiments with guanosine 5′-[γ-thio]triphosphate [GTPγS]), bovine serum albumin (1 mg/mL), 50 mmol/L Tris hydrochloride, pH 7.5, and adenosine deaminase (1 μg/mL). 7-Deacetyl-6-(*N*-acetylglycyl)-forskolin (20 μmol/L) or different concentrations of isoproterenol or GTPγS were added as shown. Adenosine deaminase was centrifuged to remove ammonium sulfate and then redissolved in the assay buffer immediately before use. After 20 minutes at 37°C, the incubation was terminated by boiling the tubes for 2 minutes. Boiled samples were then centrifuged, and the supernatants were assayed for cyclic adenosine monophosphate using kit no. 6301 from Advanced Magnetics (Cambridge, MA).

Receptor Binding Experiments

β-Adrenergic receptors in six fresh samples were characterized by Scatchard analysis using 5 to 400 pmol/L [^{125}I]iodocyanopindolol as the radioactive antagonist and 10 μmol/L isoproterenol as the displacing ligand in 250 μL 50-mmol/L Tris, 10 mmol/L $MgCl_2$, and 50 μmol/L GTP at pH 7.4. After 45 minutes at 37°C, the samples were poured onto Whatman G/FC glass fiber filters and washed twice with 5 mL water under a vacuum of 15 mm Hg. K_d values were 40 ± 11 pmol/L (mean ± SEM). Receptor densities reported in this assay were obtained in one assay using frozen membranes and 400 pmol/L [^{125}I]iodocyanopindolol, which saturates β_1- and β_2-receptors but has a lower affinity to β_3[12,19]; the significance of β_3 in human white fat cells has been questioned.[12,19]

Determination of Gs

Antiserum MINNI was raised in a rabbit against a conjugate of keyhole limpet hemocyanin and the peptide CRMHLRQYELL that, with the exception of the *N*-terminal cysteine, corresponds to the C-terminal sequence of Gs α-subunit.[30,31] This antiserum was used to quantify Gs in Western blots, using a bacterial lysate containing recombinant Gsα (15% of total protein) as the standard. This lysate was a gift from Dr Joel Linden (University of Virginia, Charlottesville, VA).

Reagents

Adenosine deaminase (type VIII from calf intestine), collagenase (type II from *Clostridium histolyticum*), (−)-isoproterenol HCl, and bovine serum albumin were purchased from Sigma (St Louis, MO), and 7-deacetyl-6-(*N*-acetylglycyl)forskolin was from Calbiochem (San Diego, CA). GTP and GTPγS were from Boehringer (Mannheim, Germany), and [^{125}I]iodocyanopindolol was from New England Nuclear (Boston, MA).

Statistical Analysis

Student's two-tailed *t* test of unpaired values, two-way repeated-measures ANOVA, and linear regression analysis were performed using the MacIntosh StatView 512 program (Apple, Cupertino, CA). No corrections were made for multiple comparisons.

RESULTS

Adipose tissue samples were obtained from patients with widely varying BMIs as already described. Fat cells were isolated, and crude plasma membranes were prepared. Total adenylate cyclase activities (as measured in the presence of 20 μmol/L 7-deacetyl-6-(*N*-acetylglycyl)forskolin) did not have a statistically significant correlation with the stimulation of cyclase or BMI (results not shown). Neither were there any statistically significant differences between lean, obese, and previously obese subjects.

Stimulation of adenylate cyclase by different concentrations of isoproterenol was investigated. Stimulation of the cyclase was expressed in two independent ways. First, activity measured in the presence of forskolin (which stimulates cyclase maximally independently of receptor systems) in each plasma membrane sample was taken as 100%, and dose-response curves were on a scale from zero activity to 100%. This method expresses the stimulation independently of basal, unstimulated activity. Second, stimulation was estimated by dividing the isoproterenol-stimulated value by basal activity (-fold over basal). This method expresses the stimulation independently of maximal activity. Both methods produced essentially similar results: activities expressed in the two different ways were tightly correlated at all concentrations ($.0004 < P < .004$). Individual dose-response curves to isoproterenol were variable. The cyclase was least responsive in adipocyte plasma membranes from obese donors and was hyperresponsive in membranes from postobese individuals (Fig 1). Repeated-measures ANOVA produced a *P* value of .008 for the comparison between all three groups and .03 for the comparison between the lean and obese when activities were expressed as in Fig 1. Stimulation (percent of maximum) at 100 nmol/L isoproterenol was correlated with BMI ($R = .494$ and $P = .01$); however, the correlation was much better ($R = -.632$, $P = .004$) when the weight-loss group was omitted.

A good correlation was also found between cell size and response to isoproterenol. The linear correlations of stimulation and cell volume at all concentrations expressed in either way were significant at $.003 < P < .02$, with the exception of 100 nmol/L expressed as an increase in activity over the basal level. Dose-response curves of membranes prepared from individuals with median cell size greater and less than 400 pL (Fig 2) were different at a *P* of .002 (two-way repeated-measures ANOVA). Cell size was clearly better correlated with the response to isoproterenol than BMI or grouping to normal-weight, obese, and post-bypass patients.

β-Adrenergic receptors were quantified as described earlier. There was a positive linear correlation of β-adrenoceptor content per milligram of protein with the response to isoproterenol. When calculated as a percentage of the maximum, the correlation was statistically significant at 10 μmol/L isoproterenol ($P < .04$). The correlation of stimulation expressed as an increase in activity over the basal level with the receptor density at 10 μmol/L isoproterenol

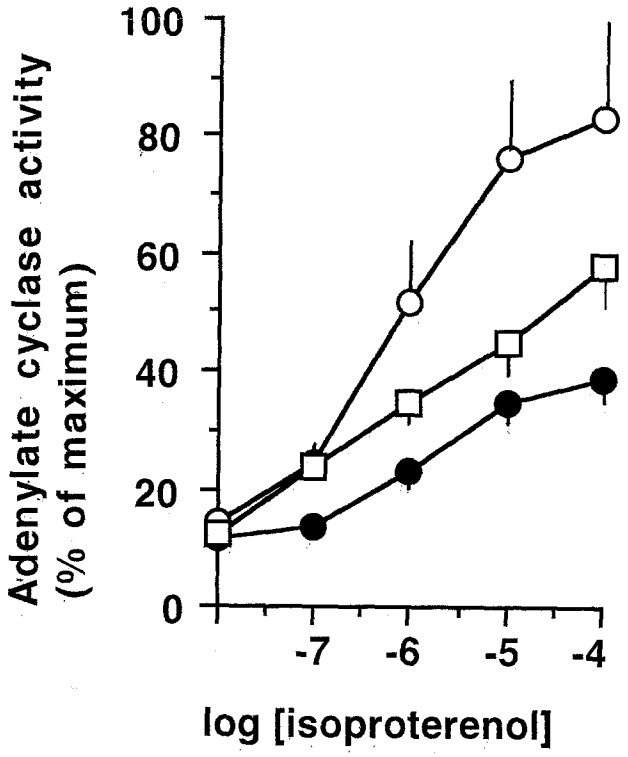

Fig 1. Stimulation of adenylate cyclase by isoproterenol in adipocyte plasma membranes isolated from normal-weight, obese, and previously obese patients. Plasma membranes were isolated and incubated with different concentrations of isoproterenol or 20 μmol/L 7-deacetyl-6-(*N*-acetylglycyl)forskolin. Adenylate cyclase activities were expressed as a % of the value measured in the presence of the forskolin analog. Values are the mean ± SEM. (□) Normal-weight; (●) obese; (○) weight-loss.

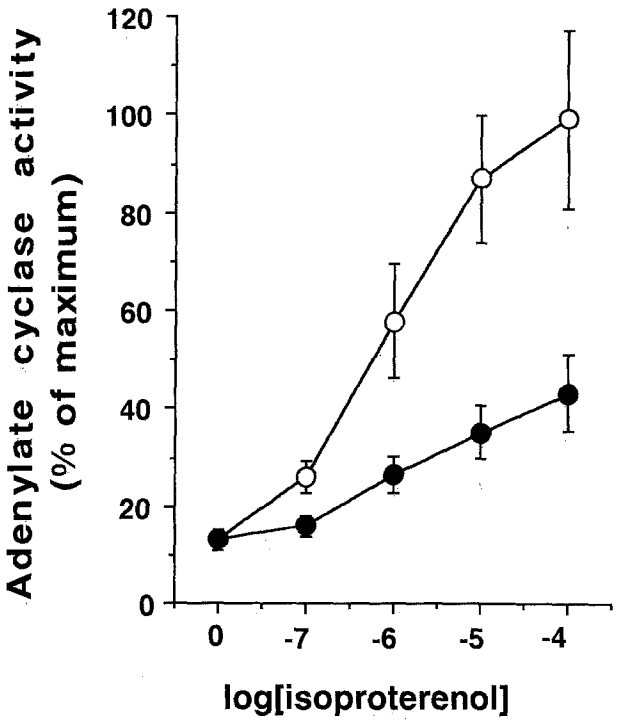

Fig 2. Stimulation of adenylate cyclase by isoproterenol in adipocyte plasma membranes isolated from large and small fat cells. Membranes were isolated and incubated as described in text and Fig 1. Adenylate cyclase activity is expressed on a scale from 0% to 100%, which refers to the value measured in the presence of 20 μmol/L 7-deacetyl-6-(*N*-acetylglycyl)forskolin. (○) Cells < 400 pL; (●) cells > 400 pL.

is shown in Fig 3 ($R = .683, P = .004$); correlations at 1 and 100 μmol/L were significant at P values of .04 and .01, respectively.

Gs was quantified using antiserum MINNI and recombinant Gsα standard as previously described.[30,31] There was no correlation of Gs with the response to isoproterenol at any concentration (Table 1). An excellent correlation was observed between response and the ratio of β-adrenergic receptor density and Gs (Fig 4). The correlations at all concentrations were significant at $.004 < P = .05$, with the exception of 100 nmol/L expressed as an increase in activity over the basal level.

GTPγS was used to investigate the interaction of Gs and adenylate cyclase in samples from 16 patients. This nucleotide analog is not hydrolyzed by G proteins and activates them independently of receptor systems. When the patients were divided into eight high responders and eight low responders according to stimulation by isoproterenol, there were no differences in stimulation of cyclase by GTPγS (100 pmol/L to 10 μmol/L) between the two groups; neither was there a statistically significant difference between obese, normal-weight, and postobese subjects (results not shown).

This study revealed no statistically significant differences in receptor density per milligram of protein or per Gs between the three groups.

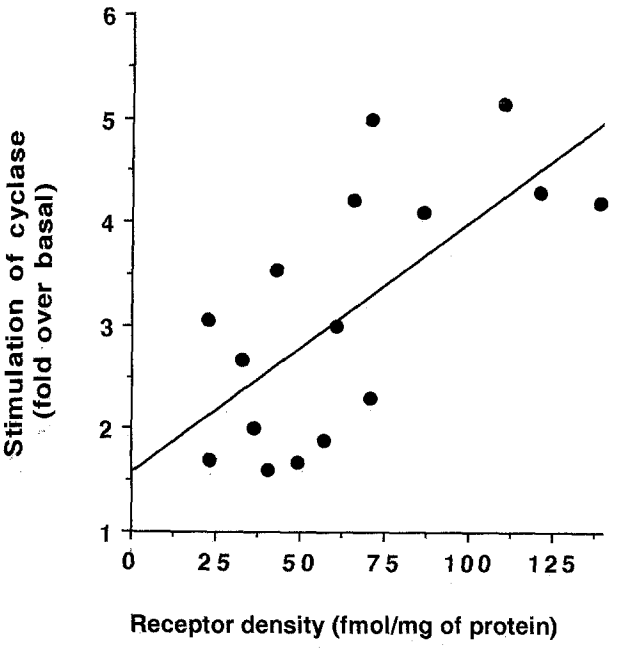

Fig 3. Correlation between stimulation of adenylate cyclase by 10 μmol/L isoproterenol and β-adrenoceptor density. Cyclase activities in the presence of isoproterenol were divided by basal activity.

Table 1. Correlations of Different Parameters With the Response of Adenylate Cyclase to Isoproterenol

Parameter	*R*	*P*
β-Receptors/mg protein	.518/.683	.0076/.0036
Gs/mg protein	.138/.071	.653/.818
β-Receptors/Gs	.725/.745	.0076/.0035

NOTE. Adenylate cyclase of crude plasma membranes was stimulated by 10 μmol/L isoproterenol. Stimulation of adenylate cyclase was expressed as a percentage of the maximum (first figures) and as an increase in activity over the basal level (second figures).

DISCUSSION

There was wide variation in the responsiveness of adenylate cyclase in fat cell plasma membranes between different individuals. Fat mass in different human subjects varies within a scale of two orders of magnitude. Despite this, the concentration of glycerol and free fatty acids in the plasma of massively obese subjects is only slightly or moderately increased.[32] Since the turnover of these compounds is close to normal in obesity,[33] it is clear that lipolysis must be inhibited *in situ* in adipose tissue of obese as compared with normal-weight individuals. Despite this, whole-body lipolysis is increased because of the increased adipose tissue mass. The stimulatory arm of adenylate cyclase regulation seems to be attenuated in intact fat cells isolated from obese individuals.[20] In genetically obese mice, the response of adenylate cyclase to β-adrenergic stimulation has been shown to be blunted.[22] The present results confirm and extend the finding of low β-adrenergic responsiveness in obesity, demonstrating directly a low response of adenylate cyclase to isoproterenol in fat cell plasma membranes from obese humans. Membranes isolated from the weight-loss group were actually hyperresponsive to isoproterenol, which is in agreement with recently published in vivo data.[3] The strongly positive effect of weight loss on isoproterenol sensitivity suggests that the low responsiveness in obese patients was not the cause of their obesity.

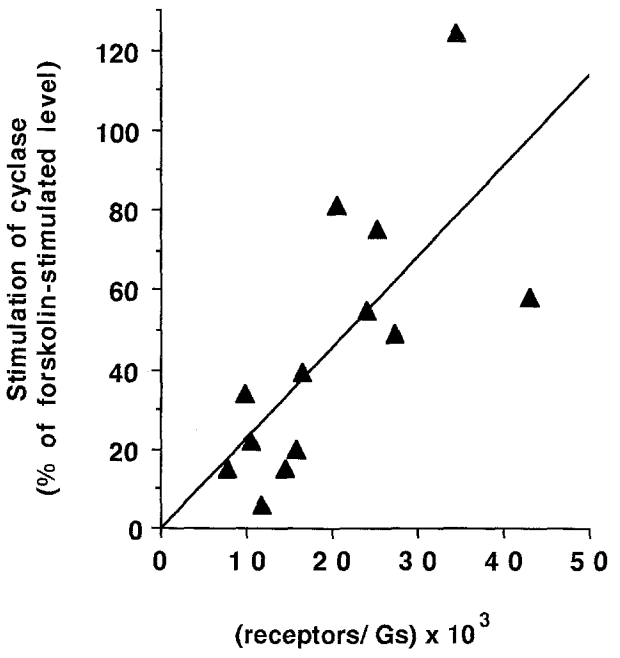

Fig 4. Correlation between stimulation of adenylate cyclase by isoproterenol and the ratio of β-adrenoceptors and Gs. Plasma membranes were isolated and incubated with different concentrations of isoproterenol or 20 μmol/L 7-deacetyl-6-(*N*-acetylglycyl)forskolin as described in text. Adenylate cyclase activities were expressed as a percentage of the value measured in the presence of the forskolin analog.

Several mechanisms could be involved in the generation of the difference in responsiveness, ranging from receptor number[23] and affinity to decreased levels[25] and functional activity[26] of Gs. Pseudohypoparathyroidism type 1a, associated with defective Gs, leads to blunted lipolysis and obesity.[5] It therefore seems clear that Gs can be rate-limiting for lipolysis under some conditions. Table 1 shows the correlations of isoproterenol responsiveness to β-receptor number and Gs per milligram of protein and to receptor number/Gs. The data suggest that the major determinant of responsiveness to isoproterenol in human adipocyte plasma membranes is receptor density, not Gs concentration. The number of copies of Gs in crude plasma membranes was on average 63 times higher than the number of β-adrenergic receptors (range, 23 to 130), which is in accordance with findings in other systems.[34] Therefore, it may be that receptors are always saturated with Gs, which might explain why receptor density seems to be the major determinant of isoproterenol responsiveness in human crude adipocyte plasma membranes.

It has been suggested that the β-adrenergic receptor density of human fat cells is variable, and that individuals with a low number of receptors compensate for their lower sensitivity by higher catecholamine concentrations.[35] Of course, the reverse explanation, desensitization of tissues because of primarily high catecholamine levels, is equally possible. Cell volume has been suggested to be important in the regulation of adenylate cyclase and lipolysis.[36-38] The present results with the stimulatory arm of cyclase regulation support this suggestion, whereas our previous results[4] show that this is not true with the inhibitory regulation by adenosine and adenosine analogs. Of course, it is at present impossible to know whether small cells will have more β-adrenergic receptors or if cells with more β-receptors will become smaller.

The present results show that stimulation of adenylate cyclase by isoproterenol is blunted in crude adipocyte plasma membranes of obese as compared with normal-weight individuals, whereas the cyclase is hyperresponsive after massive weight loss. The response was correlated negatively with cell size and positively with β-adrenergic receptor density, but no correlation was observed between receptor density and BMI. This suggests that downregulation of receptors is not the cause of the observed alterations in responsiveness of fat cells in obesity.

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