Lipolytic Catecholamine Resistance Due to Decreased β_2-Adrenoceptor Expression in Fat Cells

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Abstract

The existence of lipolytic β-adrenoceptor (BAR) resistance was investigated in vivo and in isolated abdominal subcutaneous adipocytes in 65 healthy and drug-free subjects. The concentration of isoprenaline (nonselective BAR agonist) causing half-maximum lipolysis effect (ED_{50}) varied bimodally and 10^6-fold between individuals but was almost constant in the same subject when measured two times at rest or before and 30 min after exercise. The subjects were categorized as having either high or low isoprenaline sensitivity. The former group had a 50% reduced in vivo lipolytic response to exercise and mental stress, despite a 50% increased plasma noradrenaline response ($P < 0.01$) and a 350% increased plasma adrenaline response ($P < 0.02$). In fat cells the lipolytic ED_{50} values for noradrenaline and terbutaline (BAR_2 agonist) were 10 times lower ($P < 0.001$) in low-sensitive subjects, but the maximum lipolytic actions of these agents (and of isoprenaline) were similar in both groups. The action on lipolysis of dobutamine (BAR_1 agonist), forskolin (stimulating adenylate cyclase), dibuturyl cyclic AMP (activating protein kinase), clonidine (α_2-adrenergic agonist), or phenyl isopropyladenosine (adenosine receptor agonist) were almost identical in high- and low-sensitivity subjects. ED_{50} for isoprenaline correlated with ED_{50} for terbutaline ($r = 0.75$), but not with ED_{50} for dobutamine. In high-sensitivity subjects the number of BAR_2 was almost threefold increased ($P < 0.002$) and the steady-state adipocyte mRNA level for BAR_2 was sixfold increased ($P < 0.005$). BAR_2 affinity as well as BAR_1 number, affinity and mRNA expression were similar in both groups. In 11 cholecystectomy patients (otherwise healthy) lipolytic ED_{50} for β agonists correlated in omental and subcutaneous fat cells ($r = 0.85$ for isoprenaline; $r = 0.95$ for terbutaline). In conclusion, lipolytic resistance to catecholamines is present in vivo in apparently healthy subjects due to reduced expression of BAR_2 in adipocytes. (*J. Clin. Invest.* 1992. 90:2175–2186.) Key words: β-adrenoceptors • catecholamines • exercise • glycerol • lipolysis • messenger RNA

Introduction

Peripheral hormone resistance is a common phenomenon in clinical medicine and involves many different hormone systems, including catecholamines. Hormone resistance is usually associated with disease. As reviewed, peripheral catecholamine resistance has been described in common clinical disorders such as hypertension (1) and heart failure (2). For the lipolytic action of catecholamines, however, hormone resistance also occurs during normal ontogenic development of catecholamine function (3, 4). Subcutaneous adipose tissue is an attractive model for catecholamine studies in humans. It is easily available and at least three different adrenoceptors are coupled to the same effector in the fat cells (see reference 5 for review). β_1- and β_2-Adrenoceptors ($BAR_{1,2}$)[1] stimulate and α_2-adrenoceptors inhibit lipolysis in human subcutaneous adipose fat cells through a chain of events involving coupling to adenylate cyclase through G proteins, which regulate cyclic AMP production and thereby the activity of hormone-sensitive lipase. The recently cloned BAR_3 (6) does not appear to be functionally expressed in human subcutaneous fat cells (7).

Previous studies have shown blunted catecholamine action in normal human fat cells at early and late stages in life in apparently healthy subjects. During the first years of life there is a marked resistance of the lipolytic action of catecholamines in fat cells due to enhanced α_2-receptor activity (3). In elderly subjects one also finds lipolytic resistance to catecholamines. In the latter case the mechanism is a decreased ability of cyclic AMP to stimulate hormone-sensitive lipase (4). We do not know at present whether there is a lipolytic catecholamine resistance independent of age or involving any of the BAR receptors. However, hypersensitivity of catecholamine-induced lipolysis has recently been demonstrated in vivo in formerly obese subjects compared to obese and never obese (8). Furthermore, the functional meaning of coexistence of several BAR subtypes in fat cells is not clear. However, these receptor subtypes may be independently regulated in human subcutaneous adipose tissue in that recent in situ studies have shown that BAR_2 and BAR_1 in this tissue have different sensitivity to acute homologous desensitization (9).

In the present study we have investigated the possible occurrence of lipolytic BAR-mediated catecholamine resistance in a large number of apparently normal subjects by comparing lipolysis regulation in vivo and in vitro. An exercise test was used as an in vivo measure of noradrenaline-induced lipolysis because BAR plays the major role in the activation of lipolysis in situ during exercise (10). A mental stress test was used to test adrenaline-induced lipolysis in vivo (11). The plasma levels of noradrenaline, adrenaline, and glycerol (lipolysis index) were measured during exercise and mental stress in nonobese subjects without medication. The action on lipolysis in abdominal subcutaneous adipocytes of agents acting on selective receptor

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1. *Abbreviations used in this paper:* BAR, β-adrenoceptor; ^{125}ICYP, ^{125}I-cyanopindolol; KPP, Krebs-Ringer phosphate (buffer); TNA, total nucleic acids.

and postreceptor steps in the lipolytic cascade has been investigated and compared with the gene expression and the stoichiometric properties of BAR_1 and BAR_2. The possible existence of regional variations in BAR subtypes were investigated by comparing omental and subcutaneous fat cells from cholecystectomy patients.

Methods

Subjects and experimental design. 65 healthy subjects of both sexes were investigated. All were drug-free. Subjects with obesity (body mass index > 30 kg/m^2), elevated blood pressure, fasting hyperglycemia, or fasting hyperlipidemia (total plasma triglycerides and cholesterol) were excluded from the study. Premenopausal women were investigated in the middle of the menstruation cycle. Clinical data are shown in Table I. After an overnight fast they rested for 15 min, whereafter a venous blood sample was obtained for the determination of plasma noradrenaline and adrenaline using high-pressure liquid chromatography and subsequent electrochemical detection (12). Then, a subcutaneous fat biopsy (0.5–2.5 g) was taken under local anesthesia from the region immediately to the left or to the right of the umbilicus, as described previously (13). In order to study intraindividual variations in adipocyte lipolysis, five subjects were investigated a second time after an 11–18-mo-long interval, when a new biopsy was taken from the contralateral side. As regards plasma catecholamines all noradrenaline values were above the detection limit whereas 6 of the 65 measured resting values for adrenaline were below the detection limit (0.03 nmol/liter).

The first 25 of the investigated subjects also underwent an exercise test, as described below. This was done 2–4 mo after the fat biopsy, except in 10 of the subjects who were investigated concerning the influence of temporary vigorous exercise on lipolysis regulation. In the latter subjects the preexercise biopsy was obtained exactly as described above. 15 min after this biopsy the subjects did the exercise test and immediately thereafter a new abdominal fat biopsy was taken from the contralateral side. The left and right sides were used in a randomized order. We have previously shown that the in vivo results of repeated exercise tests in the same subject are identical when performed separately or in connection with fat biopsies (14). 13 of the subjects underwent a mental stress test 3–6 mo after the biopsy as described below. Two of these subjects participated also in the exercise test.

The study also comprised six female and five male subjects who underwent elective cholecystectomy because of gallstone. None had jaundice or other diseases besides gall-bladder disease. All were drug free. The ranges for age and body mass index were 27–74 yr and 21.0–28.3 kg/m^2, respectively. The subjects fasted overnight and only saline was infused intravenously before the fat biopsy. The surgical procedures started at 8 a.m. General anesthesia was induced by a short-acting barbiturate and maintained by phentanyl and nitrous oxide. Subcutaneous adipose tissue was taken from an upper paramedian incision at the beginning of surgery and the omental fat specimens were taken from the major omentum 5–10 min later.

The participants were individually informed and their consent was obtained. The study was approved by the hospital's committee on ethics.

Isolation of fat cells. Adipose tissue was transported to the laboratory and the preparation of isolated adipocytes, using Rodbell's method (15), was started within 10 min after collection. The specimens were cut into fragments with weights ~ 5–10 mg. Adipocytes were isolated from the stroma cells by incubation with 0.5 g/l of collagenase for 60 min in 5 ml of Krebs-Ringer phosphate (KRP) buffer (pH 7.4) with 40 g/liter of dialyzed bovine serum albumin at 37°C in a shaking bath. The adipocytes were washed through a silk cloth three times with a collagenase-free buffer. Fat cell size was measured by direct microscopy and the mean adipocyte diameter and standard deviation were calculated from the diameter of 100 cells. Because of the high adipocyte lipid content (> 95%) and spherical shape one can estimate the mean adipocyte weight, volume, and cell surface area from the mean adipocyte diameter. Total lipid content in the incubate was measured by organic extraction. In this way, the number of fat cells in each incubate can be calculated by dividing the total lipid weight by the mean adipocyte weight. This procedure has been described elsewhere (16).

Exercise test. The experimental protocol has been described in detail previously (14). Briefly, after an overnight fast the participants rested for 5 min on a bicycle ergometer (model 380B, Siemens-Elema, Stockholm, Sweden). Then they exercised for 30 min at a load corresponding to two thirds of their maximum aerobic power ($VO_{2\,max}$). The latter was estimated from a submaximal work test performed 1–2 wk before the study. Blood samples were drawn from an indwelling catheter in a cubital vein for the determination of plasma insulin with a radioimmunoassay using a commercial kit (Pharmacia, Uppsala, Sweden), plasma catecholamines as described above, and glycerol (17) before and after 15 and 30 min of exercise. The heart rate was recorded continuously on a Mingograph ECG recorder (Siemens-Elema) during the exercise period. All plasma adrenaline values were above the detection limit.

Mental stress. After an overnight fast the subjects rested sitting in a bed for 30 min. During the resting period the test procedure was explained to the participant. A modified (18) filmed version of Stroop's color-word conflict test was used as described previously (19). In brief, color words are shown on a TV screen in different colors, the combination of words and colors being incongruous. The subject's task is to ignore the word and name the color of the print. In addition, there is a simultaneous auditory presentation of conflicting color words, which also has to be ignored. The duration of visually presented color words varies randomly between 0.4 and 1.0 s and that of audiolog-presented words between 0.7 and 1.8 s. The mental stress-test lasted 30 min. The heart rate and the plasma levels of insulin, catecholamines, and glycerol were determined as described for the exercise test before and 10, 20, and 30 min after the start of the mental stress. All values for adrenaline were above the detection limit.

Lipolysis assay. This assay has been described in detail (3, 4). In brief, adipocytes (1,000–2,000 cells) were incubated in duplicate with air as the gas phase in 0.2 ml of KRP buffer containing 40 g/liter of bovine serum albumin, 1 g/liter of glucose, 0.1 mg/liter of ascorbic

Table I. Characteristics of Subjects with High or Low Isoprenaline Sensitivity

Sensitivity group	Age	Sex (M/F)	BMI	Waist-to-hip (ratio)	P-NA	P-A	Fat cell weight	VO_{2max}	Training (yes/no)	Smoker (yes/no)
	yr		*kg/m^2*		*nmol/liter*		*ng*	*liter/min*		
High	36±3	19/14	23.0±0.4	0.938±0.011	1.5±0.1	0.15±0.02	444±34	2.26±0.17	17/16	15/18
Low	43±3	20/12	23.9±0.6	0.947±0.016	2.2±0.2	0.19±0.04	508±38	2.27±0.19	17/15	12/20
P	NS	NS	NS	NS	< 0.01	NS	NS	NS	NS	NS

P-NA and P-A are resting plasma levels of noradrenaline and adrenaline, respectively. VO_{2max} is the maximum aerobic power during an exercise test. The subjects were asked for their exercise/training habits. Yes represents a regular exercise program more than twice per week.

acid, and various concentrations of lipolytic and antilipolytic agents. The release of glycerol was used as an index of lipolysis. The glycerol concentration at the end of a 2-h incubation was determined in a cell-free aliquot with an ultrasensitive automatic bioluminescence method (17, 20). Glycerol release, which was linear with time for at least 4 h, was expressed per cell surface area as well as per number of fat cells incubated. The lipolysis dose–response curves were analyzed as regards responsiveness (maximum effect), sensitivity and intrinsic activity (maximal response in relation to maximum isoprenaline effect for lipolytic agents and maximum effect in relation to basal lipolysis for antilipolytic agents). When the responsiveness of various lipolytic agents was calculated, we corrected for the difference in basal lipolysis between subjects by reducing all values by the basal value. As regards antilipolytic agents, we used the relative-to-basal inhibition because of some interindividual variability in basal lipolysis (21). Sensitivity was estimated graphically from the individual dose–response curves resulting in a drug concentration giving half-maximal response (ED_{50}). Because these values may differ by more than one order of potency in the same group, we chose to calculate and present such data in their logarithmic form. The lipolytic interactions between selective BAR agonists and antagonists were evaluated as described previously (22) as regards the concentration ratio of agonist in the presence and absence of the antagonist and the equilibrium dissociation constant for the antagonist.

The following lipolysis-acting agents were added in various submaximal and maximal effective concentrations to the medium: noradrenaline (nonselective catecholamine), isopropylnoradrenaline (isoprenaline, nonselective BAR agonist), clonidine (α_2 agonist), terbutaline (selective BAR_2 agonist), dobutamine (selective BAR_1 agonist), forskolin (activator of adenylate cyclase), dibuturyl cyclic AMP (phosphodiesterase-resistant cyclic AMP analogue), and phenyl isopropyl adenosine (adenosine receptor agonist). In the experiments with clonidine and phenyl isopropyl adenosine the incubation buffer was supplemented with 1 U/ml of adenosine deaminase in order to remove traces of adenosine from the incubation medium, which may otherwise influence antilipolysis experiments (3, 4). In some experiments with terbutaline and dobutamine, the incubation medium was also supplemented with 10^{-8} mol/liter of either metoprolol (selective BAR_1 antagonist) or ICI 118,551 (selective BAR_2 antagonist). Because the amount of adipose tissue available was limited, it was not possible to use all the lipolytic-acting agents in all subjects. However, noradrenaline and isoprenaline were used in all of them.

Radioligand binding. The receptor binding studies have been described in detail (23, 24). These assays consumed large amounts of fat cells and could only be performed on 19 individuals. The radioligand used was the hydrophobic and nonselective β antagonist ^{125}I-cyanopindolol (^{125}ICYP). Freshly isolated fat cells, in a concentration of about 20,000/ml, were incubated in duplicate at 37°C in 0.5 ml of KRP buffer (pH 7.4) containing bovine serum albumin (5 g/liter), glucose (1 g/liter), and ascorbic acid (0.1 g/liter). The saturation experiments were conducted with increasing concentrations of ^{125}ICYP while a fixed concentration of ^{125}ICYP, with increasing concentrations of competing antagonists was used in the displacement experiments.

In the saturation experiments freshly isolated fat cells were incubated for 60 min with the following concentrations of ^{125}ICYP: 0, 10, 50, 100, 250, 500, and 750 pmol/liter. Incubations were performed in duplicate and in a third set of tubes the nonspecific binding was determined by the addition of 0.1 μmol/liter of propranolol and was estimated to be ~ 30%. The total amount of radioactivity added was measured in a fourth row of cell-free tubes. At the end of the incubations the cell-bound radioactivity was determined by the addition of ice-cold saline (4 ml × 3) and vacuum filtering through a GF/C filter (Whatman Inc., Clifton, NJ).

In the competition experiments a fixed concentration of 100 pmol/liter ^{125}ICYP was used in all incubations and the effect of increasing concentrations of the BAR_2-specific (25) antagonist ICI 118,551 was studied. ICI 118,555 was used in 10 different concentrations (0, 10^{-11} to 10^{-4} mol/liter). Nonspecific binding, defined as the radioactivity measured with 10^{-4} mol/liter ICI 118,551, was ~ 35%. In four subjects, fat cells were also incubated with 200 pmol/liter of ^{125}ICYP in the absence and the presence of the above-mentioned concentrations of ICI 118,551. The results were compared with those obtained using 100 pmol/liter of ^{125}ICYP.

Saturation experiments with ^{125}ICYP always gave a straight line in a Scatchard plot indicating that the radioligand binds to BAR_1 and BAR_2 with identical affinity. Displacement of ^{125}ICYP with ICI 118,551 revealed a shallow biphasic curve which fitted a two-site model significantly better than a one-site model, deriving from the fact that binding to both high-affinity (BAR_2) and low-affinity receptors (BAR_1) are identified by the selective antagonist.

The saturation experiments were evaluated by linear regression analysis of Scatchard plots (26). Displacement curves were analyzed by a nonlinear least squares regression method (27). The evaluation program (LIGAND) permits a statistical comparison between a one- and a two-site model and provides the best estimates for binding isotherms. From the best fitted two-site curve it is possible to estimate the proportion of high- and low-affinity receptors as well as the affinity (K_d) of the two receptors. Based on the information that no binding sites for BAR_3 are detected with ^{125}ICYP in human subcutaneous fat cells (7) the following method was used for the determination of total binding sites for BAR_1 and BAR_2. The maximal number of binding sites (Bo) was obtained from the saturation binding results. This value was multiplied with the fraction of high- and low-affinity binding sites, for ICI 118,551, respectively. The latter was obtained from the displacement binding experiments.

mRNA. The assays for BAR_1 and BAR_2 mRNA in fat cells have recently been described in detail (28) and were performed on adipocytes from the 19 subjects that were included in the radioligand binding investigation described above. This method consumed large amounts of fat cells so additional mRNA species could not be measured. Complementary oligonucleotide probes corresponding to nucleotide 739–789 for BAR_1 (29) and to nucleotide 772–822 for BAR_2 (30) were synthesized and cloned into pGEM-3. The plasmid was used for in vitro synthesis of cRNA, which was radiolabeled with [^{35}S]UTP.

Isolated fat cells were kept in −70°C. About 150 μl of adipocytes were homogenized and digested with protein and total nucleic acids (TNA) were extracted with phenol-chloroform. The amount of TNA and DNA in the extract was determined fluorometrically. The DNA method used (31) does not interfere with RNA, because DNA is allowed to bind to bisbenzymidazole. The amounts of BAR_1 mRNA and BAR_2 mRNA in the extract were determined in duplicate by solution hybridization (32) exactly as described in detail (28). Briefly, [^{35}S]UTP cRNA was hybridized at 70°C to TNA samples. Nonhybridized material was digested with RNase. RNase-resistant material was precipitated and collected on a glass filter. Sample TNA hybridization was compared with a known amount of in vitro synthesized mRNA strand complementary to the radioactive probe. The amount of mRNA was related to the amount of DNA in the TNA sample and expressed as molecules per cell, assuming a content of 6.4 pg of DNA/adipocytes. All determinations of either BAR_1 or BAR_2 mRNA were made in duplicate on the same occasion.

We have previously shown in human subcutaneous adipose tissue (28) as well as in human liver (24) that these probes hybridize to a single mRNA species with a size corresponding to about 2.5 kb for BAR_1 and to about 2.2 kb for BAR_2, using Northern blot analysis (29, 30). We have also shown that there is no cross-hybridization between the probes (24, 28). Finally, the steady-state mRNA levels have been found to be almost identical in isolated subcutaneous fat cells and in intact subcutaneous adipose tissue specimens (29). Some additional methodological experiments were presently performed. The concentration of TNA in a fixed volume (110 μl) of the extraction mixture was proportional to the amount of added fat cells. The amount of mRNA for BAR_1 and BAR_2 per microgram of TNA was constant when measured in TNA extracted from 25, 50, 100, and 150 μl of fat cells from the same donor. The coefficient of variation for the mRNA measurements of BAR_1 and BAR_2 was 10%.

Drugs and chemicals. Bovine serum albumin (fraction V) (lot No. 63F-0748) was obtained from Sigma Chemical Co. (St. Louis, MO). Collagenase prepared from *Clostridium histolyticum* was of Sigma type I. The BAR radioligand ^{125}ICYP (sp act 2,200 Ci/mmol) was purchased from Dupont/New England Nuclear (Boston, MA). Propranolol was supplied by Sigma Chemical Co. and clonidine by Boehringer Ingelheim (Rhein, FRG). Noradrenaline, isoprenaline and metoprolol came from Hässle (Mölndal, Sweden). Terbutaline was from Draco (Lund, Sweden), dobutamine from Eli Lilly & Co. (Indianapolis, IN), and ICI 118,551 from ICI Pharmaceuticals (Cheshire, UK). Adenosine deaminase came from Boehringer-Mannheim GmbH (Mannheim, FRG). Glycerol kinase from *E. coli* (Sigma no. G4509) and ATP monitoring reagent containing firefly luciferase (LKB Vallac, Turku, Finland) were used in the glycerol assay. All other chemicals were of the highest grade of purity commercially available. The same batches of hormones, collagenase, and albumin were used in all the experiments.

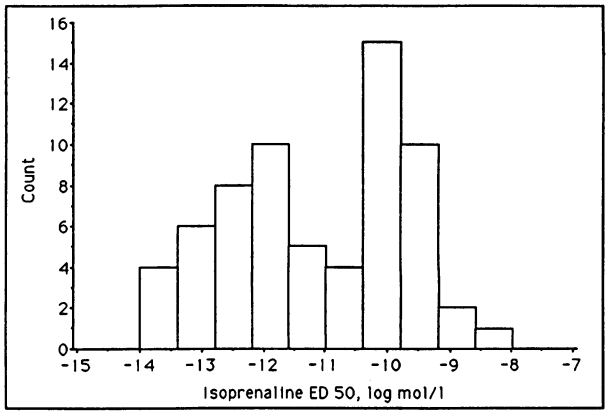
Statistical analysis. Data were analyzed for Gaussian distribution. The student's two-tailed unpaired *t* test or analysis of variance was used for comparison of data between groups. Standard error of the mean was used as a measure of dispersion. In some cases a linear regression analysis was performed using the method of least squares. The statistical difference for slope and/or position between two regression lines was tested using the *F*-distribution test. As regards half-maximal effective drug concentration in lipolysis studies and K_d values from radioligand binding studies, the data were transformed into logarithmic form in order to obtain normal distributions. The coefficient of variation was determined by calculating the standard deviation divided with the sample mean.

Results

The marked interindividual variations observed in Fig. 1 occurred in the ED_{50} values for isoprenaline-induced lipolysis; the extreme subjects differed ~ 10^6-fold. The cumulative frequency distribution of the ED_{50} values differed significantly ($P < 0.001$) from a unimodal normal normal distribution. Instead, the histogram suggests a biomodal Gaussian distribution, which is supported by the probit analysis, where a plot of all values yielded two interconnected straight lines. On the basis of these results, the subjects belonged to two different populations with either high isoprenaline sensitivity ($n = 33$) or low isoprenaline sensitivity ($n = 32$). The median ED_{50} value (10^{-11} mol/liter) became the natural cutoff point between these equally sized populations.

To study whether the differences observed in isoprenaline ED_{50} had any physiological importance, the results of the exercise test (Fig. 2) were divided according to high ($n = 12$) or low ($n = 13$) isoprenaline sensitivity. In both groups plasma catecholamines, pulse rate, and plasma glycerol levels (lipolysis index) rose gradually during exercise. However, in the low-sensitivity subjects, there was a 50% more marked rise in plasma noradrenaline ($P < 0.01$) in spite of a 50% reduced rise in glycerol ($P < 0.01$), as compared to the high-sensitivity subjects at the end of exercise. Both groups had an almost identical response as regards plasma adrenaline and pulse rate. Plasma insulin started at similar levels and decreased ($P < 0.05$) in an almost identical way in both groups. The insulin values in the high-sensitivity group were 4.0±0.7, 4.1±0.6, and 3.0±0.7 mU/liter at 0, 15, and 30 min of exercise, respectively. The corresponding values for the low-sensitive group were 4.3±0.7, 4.2±0.8 and 3.2±0.7 mU/liter.

The existence of in vivo lipolytic resistance was further investigated by plotting the plasma noradrenaline level versus the



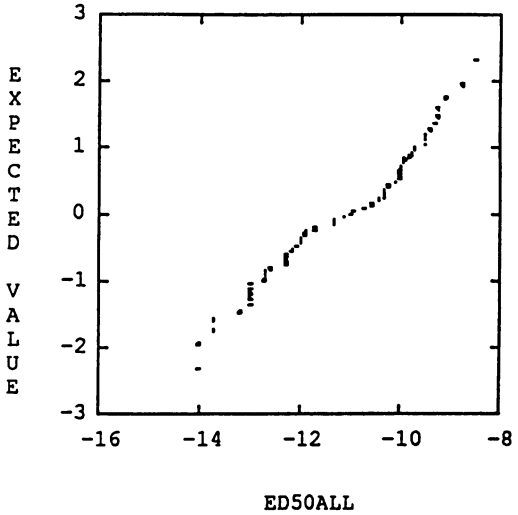


Figure 1. Concentrations of isoprenaline causing a half-maximum lipolytic effect in fat cells (ED_{50}). Fat cells were incubated with or without several concentrations of isoprenaline and glycerol release was determined. ED_{50} was determined from the dose–response curves. The upper graph shows a histogram of all values. The lower graph shows probit analysis of the cumulative distribution of all values. l, liter.

plasma glycerol level at the end of exercise (Fig. 3). A correlation ($r = 0.52$–0.53) between these two parameters was observed in the two groups and the slope of the regression line was significantly steeper for high- than for low-sensitive subjects ($F = 7.4$; $P < 0.001$). From the equations of these regression lines it could be predicted that a plasma noradrenaline value of 10 nmol/liter corresponded to a plasma glycerol value of 125 μmol/liter in the low- and 300 μmol/liter in the high-sensitivity group. At 15 nmol/liter of noradrenaline the difference in plasma glycerol was even greater; 150 vs. 500 μmol/liter.

The results of the mental stress test are shown in Table II. Six subjects had high BAR sensitivity and seven low BAR sensitivity. In both groups plasma catecholamines, plasma glycerol and heart rate increased gradually during the first 20 min of the stress test, when a plateau was reached (figure not shown). There was some individual variation as regards the time point when each parameter peaked. Therefore we only used the maximal values during mental stress in the statistical calculations. Plasma noradrenaline and the heart rate started at similar lev-

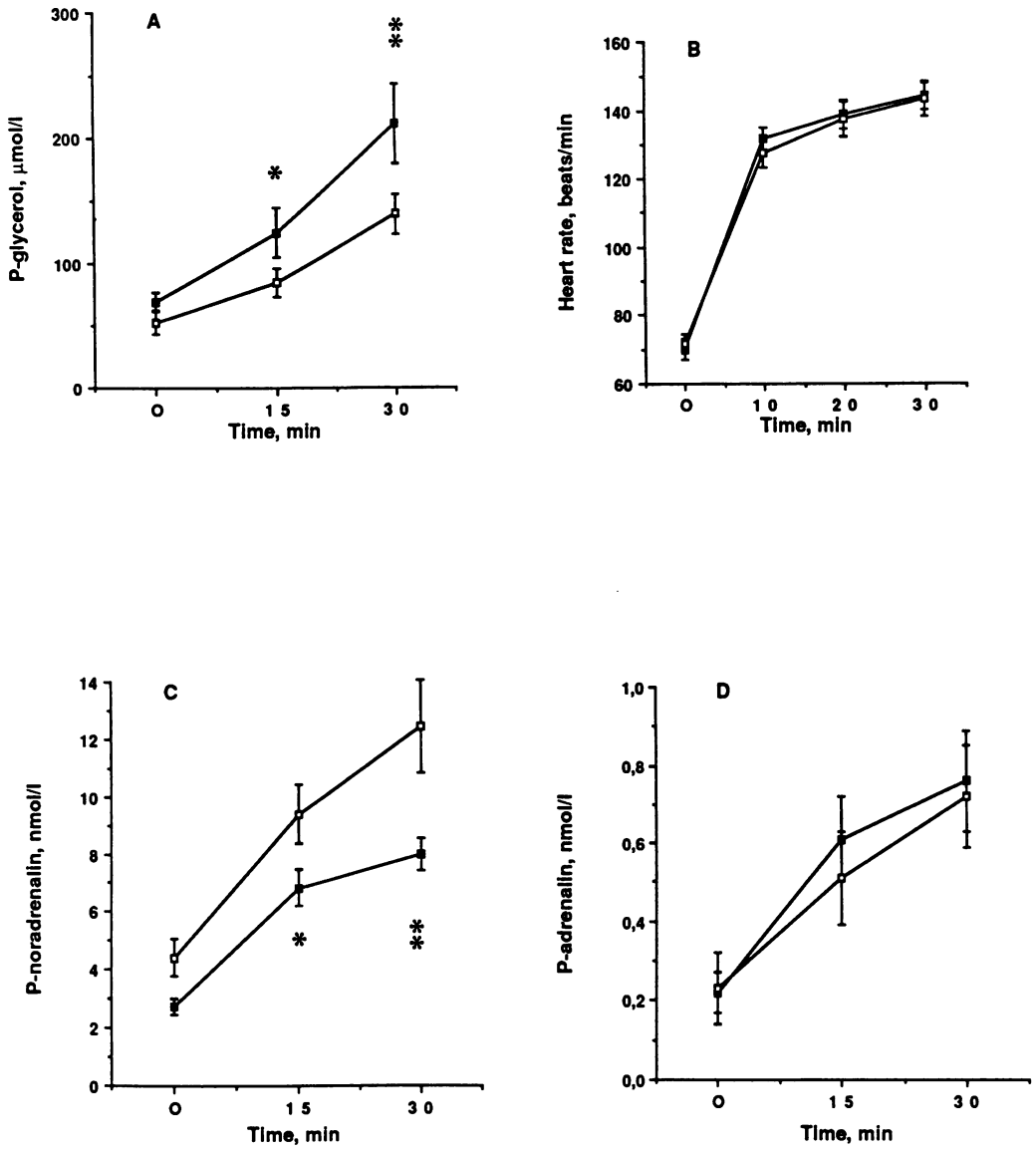


Figure 2. Results of an exercise test in 12 subjects with high (■) and 13 subjects with low (□) adipocyte isoprenaline sensitivity. Heart rate and plasma levels of glycerol, adrenaline, and noradrenaline were compared before and after 15 and 30 min of exercise. The values were compared using Student's unpaired *t*-test or analysis of variance of repeated measures. Both tests gave similar results. The results of the former test is indicated in the graph. $*P < 0.05$, $**P < 0.01$.

els and increased in the same order of magnitude in both groups. Plasma glycerol increased from a similar starting value to about 70 and 160 µmol/liter in the low- and high-sensitivity groups, respectively ($P < 0.01$). Plasma adrenaline started also at similar levels in both groups but increased to 0.43 nmol/liter in low-sensitivity subjects and to 0.12 nmol/liter in high-sensitivity subjects ($P < 0.02$). The plasma insulin levels were in the same order of magnitude in both groups and did not change during mental stress (data not shown).

The question whether isoprenaline sensitivity and responsiveness were stable in a certain individual despite the large interindividual variations was investigated in 13 subjects before and after exercise and in 5 subjects at rest, with an 11–18-mo interval (Fig. 4). The body weight was constant during this period (data not shown). In spite of large interindividual variations, the ED_{50} values obtained before and after exercise were almost unchanged in both high and low sensitive subjects. The coefficient of variation was 4%. Likewise, the resting values show small intraindividual variations. The coefficient of variation was 5%. There were also small intraindividual variations in the maximal lipolytic action of isoprenaline responsiveness (figures not shown). The coefficient of variation was 15% and 14% at rest and before vs. after exercise, respectively.

The possible existence of resistance to noradrenaline-induced lipolysis in vitro in subjects with low isoprenaline sensitivity was tested in Fig. 5. The mean curve was markedly shifted to the right in the low-sensitivity group as compared to the high-sensitivity one. However, the two groups differed only in lipolytic sensitivity. The amplitudes of the dose-response curve were almost the same in both subjects. The individual ED_{50} values (log mol/liter) for noradrenaline were -7.9 ± 0.2 in high-sensitivity and -6.8 ± 0.1 in low-sensitivity subjects ($P < 0.001$), which meant a 10-fold difference. The individual values for noradrenaline responsiveness (micromoles of glycerol/2 h per 10^7 cells) was 13.1 ± 1.5 in high-sensitivity and 10.8 ± 1.6 in low-sensitivity subjects, but the small difference was not statistically significant. Basal glycerol release was 2.5 ± 0.2 and 3.0 ± 0.3 µmol/2 h per 10^7 cells in the low- and high-sensitivity group, respectively. This indicates that noradrenaline, at maximum effective concentration caused about a fourfold increase in the lipolysis rate in both groups. Fig. 5 also shows the mean dose–response curves for isoprenaline in

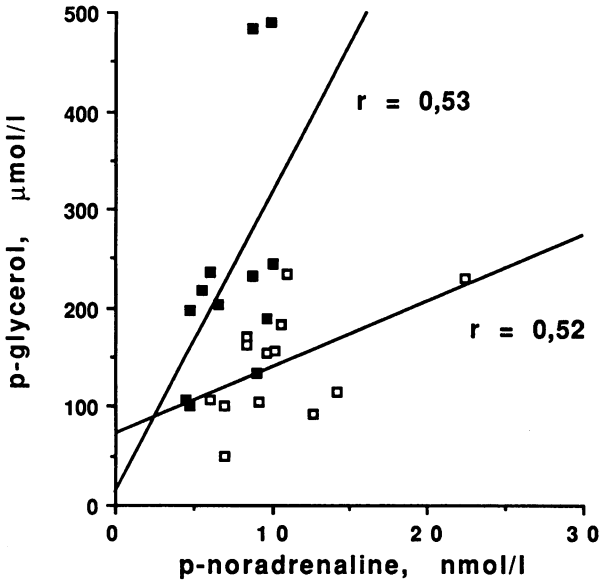


Figure 3. Comparison of plasma glycerol and noradrenaline levels at the end of exercise. The individual values at 30 min of exercise were compared using linear regression analysis. The slopes and/or position of different regression lines were compared with the aid of the *F*-distribution test. (□) Subjects with low sensitivity. (■) values for high-sensitivity subjects. See legend to Fig. 2 for further details.

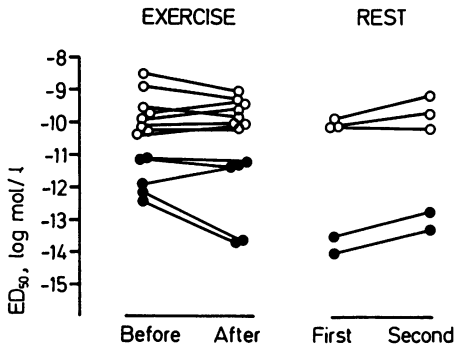


Figure 4. Interindividual variation of isoprenaline sensitivity in subjects with high (●) or low (○) isoprenaline sensitivity. Fat cells were obtained either before and immediately after a 30-min exercise period or at two different resting occasions (the interval between first and second biopsy was 11–18 mo) and were incubated with or without isoprenaline added in different concentration. Lipolysis was measured and ED_{50} for isoprenaline was calculated from the dose-response curves.

subjects with high and low isoprenaline sensitivity. As expected, there was a marked shift to the right of the curve in the low-sensitivity group. The mean difference in ED_{50} was 100-fold. However, the isoprenaline-induced maximal lipolytic response was almost identical in both groups, ~ 10-fold elevation of the basal lipolysis rate. Fig. 5 shows net lipolytic effect of catecholamines (i.e., minus basal). This manipulation of the data does not alter the conclusions.

The clinical data concerning high- and low-sensitivity subjects are shown in Table I. They were almost comparable as regards age, sex, smoking habits, body mass index, waist-to-hip ratio, fat cell weight, VO_{2max}, exercise habits, and plasma adrenaline. However, the plasma noradrenaline levels at rest were 50% higher in low-sensitivity compared to high-sensitivity subjects ($P < 0.01$). Each subject was asked for heredity for of atopic disease, cardiovascular disease, and endocrine or metabolic disorders. No difference between the two groups was observed in this respect.

In theory, the difference in lipolytic sensitivity between the two groups could be localized at any step in catecholamine-induced lipolysis from adrenoceptors to the final activation of hormone-sensitive lipase. The level at which resistance may occur was investigated by comparing the lipolytic action of various selective lipolysis agents in subjects with low and high isoprenaline sensitivity (Fig. 6, Table III). Only the lipolytic action of the selective BAR_2 agonist terbutaline differed between the groups. The mean dose–response curve was 10 times shifted to the right in low-sensitivity subjects as compared to highly sensitive ones. The individual ED_{50} values for terbutaline were ~ 15 times higher in low-sensitive than in high-sensitivity subjects ($P < 0.001$). However, terbutaline was a full agonist in both groups. The mean intrinsic activity value (maximum terbutaline effect, as compared to maximum isoprenaline effect) was about 0.9 in low- and high-sensitivity subjects, respectively. In none of the groups did this value deviate significantly from 1.0. The lipolytic action (ED_{50}, intrinsic activity) for the selective BAR_1 agonist dobutamine, the adenylate cyclase stimulating agent forskolin, and the protein kinase-activator dibuturyl cyclic AMP were almost identical in both groups. Dobutamine and forskolin were also almost full agonists in both groups. The intrinsic activity of none of these agents differed significantly from 1.0. In the whole material,

Table II. Results with Mental Stress

Sensitivity group	Adrenaline		Noradrenaline		Glycerol		Heart rate	
	Before	During	Before	During	Before	During	Before	During
	nmol/liter				*µmol/liter*		*beats/min*	
Low	0.06±0.01	0.43±0.09	1.5±0.2	2.0±0.1	54.0±7.8	73.5±5.7	63±4	80±4
High	0.04±0.01	0.12±0.03	1.1±0.1	2.0±0.2	61.1±7.5	161.0±19.8	71±3	90±8
P	NS	< 0.02	NS	NS	NS	< 0.01	NS	NS

Results of a mental stress test in six subjects with high and seven subjects with low isoprenaline sensitivity. Heart rate and the plasma levels of glycerol, adrenaline, and noradrenaline were recorded before and after 10, 20, and 30 min of mental stress. The maximum values during the mental stress were used for statistical analysis. Values with high- and low-sensitivity subjects were compared using Student's unpaired *t* test.

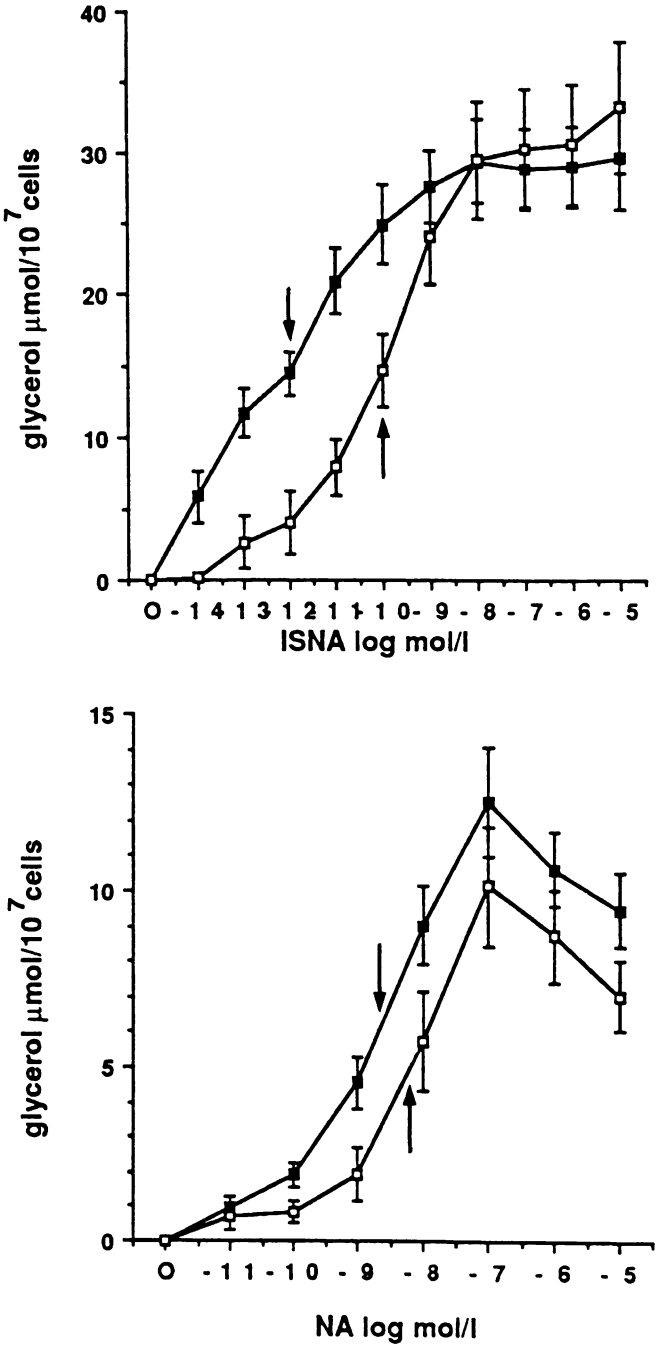


Figure 5. Mean lipolysis dose-response curves for (*top*) isoprenaline (*ISNA*) and (*bottom*) noradrenaline (*NA*) in 32 subjects with high (■) and 33 subjects with low (□) adipocyte isoprenaline sensitivity. Adipocytes were incubated with the indicated concentrations of ISNA or NA and glycerol release was determined. The glycerol value for fat cells incubated under basal conditions is substracted from the catecholamine-induced values. The concentrations causing half-maximum effect is indicated with an arrow.

ED_{50} for isoprenaline correlated strongly with ED_{50} for terbutaline ($r = 0.75$) but not with ED_{50} for dobutamine ($r = 0.15$) (Fig. 7).

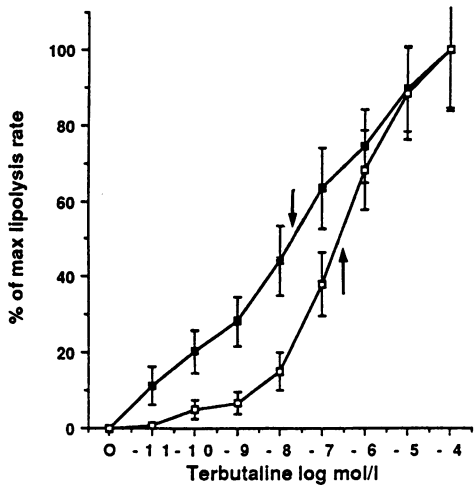
The antilipolytic action of clonidine and phenylisopropyl adenosine did not differ between the groups, as judged by mean dose-response curves (figure not shown) or by individual ED_{50} values and intrinsic activities (Table III). It could be calculated from the latter data that the basal lipolysis rate was reduced to 32±5% by clonidine and to 19±3% by the full agonist phenylisopropyl adenosine. Thus, these data indicate that clonidine, in the present type of experiments, is a partial α_2-receptor agonist. A full α_2 agonist such as noradrenaline or adrenaline in the presence of the β blocking agent propranolol can produce almost complete antilipolytic effect in human fat cells (3).

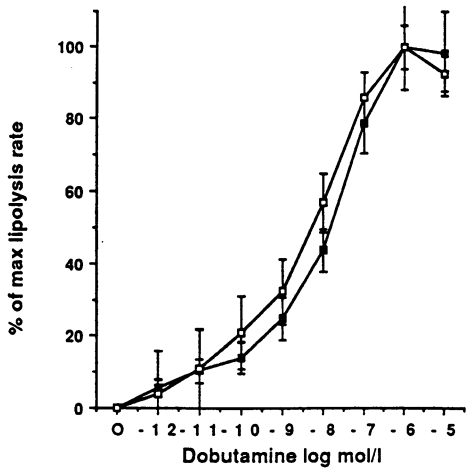
To determine whether dobutamine and terbutaline were selective BAR agonists, experiments were performed where these two agonists were combined with a selective BAR antagonist (figure not shown). The selective BAR_1 antagonist metoprolol (10^{-8} mol/liter) was able to reduce the lipolytic sensitivity of dobutamine but not of terbutaline. Contrary results were obtained with 10^{-8} mol/liter of the selective BAR_2 antagonist ICI 118,551. The findings were identical as regards selectivity in subjects with high or low isoprenaline sensitivity.

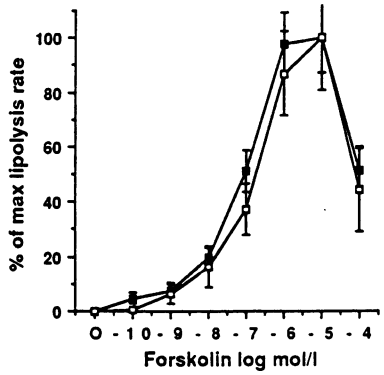
The pharmacological properties of BAR were also investigated (Table IV). Total BAR binding was twice as high in adipocytes of subjects with high as compared to low isoprenaline sensitivity ($P < 0.002$), as indicated by Scatchard analysis of ^{125}ICYP saturation binding. This was solely due to a greater number of BAR_2 in the former cells as evidenced by displacement of ^{125}ICYP binding by ICI 118,551 showing an almost threefold difference in BAR_2 number ($P < 0.002$). The fraction of BAR_2 receptors (i.e., high-affinity binding of ICI 118,551) was 54±5% in the high-sensitivity group and 32±5% in the low-sensitivity group ($P < 0.01$). However, antagonist affinity for BAR_2 was about the same in high as in low BAR-sensitive subjects. The number and K_d of BAR_1 showed no difference between the high- and low-sensitivity groups as evidenced by low-affinity binding of ICI 118,551. Neither did K_d for ^{125}ICYP binding in the saturation experiments differ between the groups (data not shown). In addition, uncharted experiments showed that ICI 118,551 displaced ^{125}ICYP binding in an almost identical fashion at 100 and 200 pmol/liter of radioligand. The latter data indicate that ^{125}ICYP binds to BAR_1 and BAR_2 with the same affinity in intact human fat cells.

Table IV also shows the values for BAR mRNA expression at steady state. The number of BAR_2 mRNA molecules was markedly increased in the high-sensitivity group. The two groups differed in BAR_2 mRNA expression by a factor of 6 ($P < 0.005$). There was also a small difference in BAR_1 mRNA between the groups but it was not statistically significant. In both groups the level of mRNA expression for BAR_1 was higher than that of BAR_2 in the same subject; the ratio was 1.5 in the high-sensitivity group and 2.5 in the low-sensitivity group. Similar differences in the ratio of BAR subtype mRNA expression has been observed previously in subcutaneous fat cells (28). The ratios of TNA/DNA in the nucleic acid extracts from fat cells were in the same order of magnitude in the high- and low-sensitivity groups: 42±7 and 49±8, respectively.

Whether or not the same variability of lipolytic β-agonist sensitivity occurred in adipocytes of different origin was investigated by comparing omental and subcutaneous fat cells obtained from the same donor (Fig. 8). ED_{50} for isoprenaline ($r = 0.81$) and terbutaline ($r = 0.95$) were strongly associated in subcutaneous fat cells as compared to omental adipocytes. This relationship was weaker for dobutamine ($r = 0.53$, graph not shown). In omental as well as in subcutaneous fat cells there was a marked interindividual variation in the lipolytic sensitivities of isoprenaline and terbutaline (~ 100,000-fold).







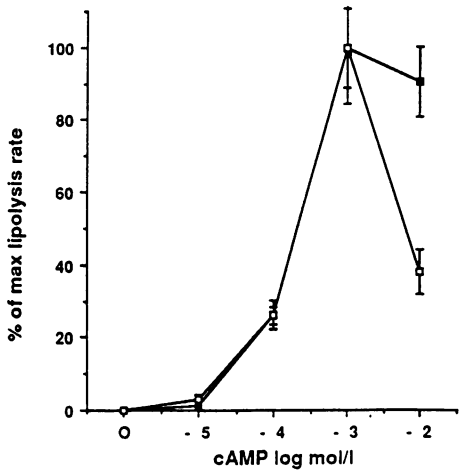


Figure 6. Sensitivity of selective lipolysis agents in adipocytes of subjects with high (■) or low (□) isoprenaline sensitivity. Fat cells were incubated in the absence or presence of the indicated concentrations of terbutaline, dobutamine, forskolin, or dibutyryl cyclic AMP (*cAMP*). Glycerol release was determined and expressed as percentage of the value at the maximum effective concentration of each lipolysis agent. The mean dose-response curves for each group are shown. Terbutaline and dobutamine were used in 18 high-sensitivity and 12 low-sensitivity subjects. Forskolin was used in 30 high-sensitivity and 30 low-sensitivity subjects. cAMP was used in 26 high-sensitivity and 29 low-sensitivity subjects.

Discussion

The present study clearly demonstrates the existence of lipolytic catecholamine resistance in the apparently normal population. This resistance is entirely different from that previously observed in catecholamine-induced lipolysis in humans (3, 4), because it is independent of age and involves BAR. There seems to be a bimodal distribution of isoprenaline ED_{50} in lipolysis experiments indicating that adipocytes of normal subjects display either high or low BAR sensitivity; the mean difference is ~ 100-fold. It can also be shown that subjects with low adipocyte BAR sensitivity, as compared to high BAR sensi-

Table III. Action on Lipolysis of Selective Agents

Sensitivity group	Intrinsic activity						ED_{50}					
	Ter	Dobu	For	cAMP	Clo	PIA	Ter	Dobu	cAMP	For	Clo	PIA
	%						*log mol/liter*					
High	90±3	95±2	95±6	81±4	32±6	17±2	−8.1±0.3	−8.0±0.2	−3.7±0.1	−7.1±0.1	−9.4±0.1	−7.1±0.1
Low	87±3	91±2	89±2	80±5	33±6	22±4	−6.6±0.2	−8.0±0.3	−3.7±0.1	−7.0±0.1	−9.2±0.2	−7.0±0.1
P	NS	NS	NS	NS	NS	NS	< 0.001	NS	NS	NS	NS	NS

The lipolytic action of terbutaline (Ter), dobutamine (Dobu), forskolin (For), dibutyryl cyclic AMP (cAMP), clondindine (Clo), and phenylisopropyl adenosine (PIA) was investigated in isolated fat cells incubated in vitro and glycerol release was measured. The concentration of each agent causing half-maximum effect (ED_{50}) on glycerol release was determined. Intrinsic activity is related to maximum isoprenaline effect for the lipolytic agents and to basal lipolysis for the antilipolytic agents. The value for glycerol release at the maximum effective concentration of each agent was used to calculate intrinsic activity. The number of experiments with Ter, Dobu, For, and cAMP are given in legend to Fig. 6. Clo was used in 29 high-sensitivity and 30 low-sensitivity subjects. PIA was used in 10 high-sensitivity and 7 low-sensitivity subjects. Values are means±SE for subjects with high or low isoprenaline sensitivity. They were statistically compared using Student's *t* test.

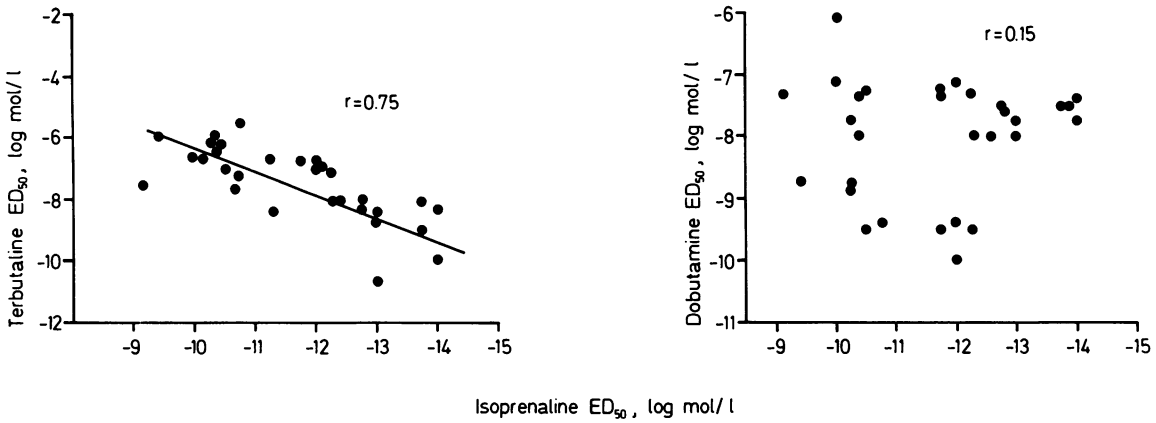


Figure 7. Relationship between lipolytic sensitivities of selective and nonselective agonists in isolated fat cells. Adipocytes of the same donor were used for lipolysis experiments with isoprenaline, terbutaline and dobutamine as described in Methods and the concentration of each agent causing half-maximum effect (ED_{50}) was determined. The relationship between ED_{50} for isoprenaline, on one hand, and terbutaline or dobutamine, on the other hand, was tested statistically using linear regression analysis.

tivity, are resistant to catecholamine-induced lipolysis in vivo and in vitro. In vivo this is reflected by a 50% increase in plasma noradrenaline response during exercise and a 350% increased plasma adrenaline response during mental stress, in spite of a 50% reduced plasma glycerol response to both forms of catecholamine challenge. At comparable noradrenaline levels during exercise the two groups differed in lipolytic responsiveness by a factor of 2.5–3. Catecholamine resistance in vitro is reflected by a 10-fold reduced sensitivity to noradrenaline stimulation. However, the in vivo and in vitro data are probably not directly comparable. The former results represent all adipose depots whereas the in vitro findings were limited to a single subcutaneous site. As discussed in detail below, however, there is strong indirect evidence for that lipolytic catecholamine resistance is present in several fat depots. No lipolytic resistance to adrenaline in vivo was observed during exercise. The same was true for noradrenaline in vivo during mental stress. This is probably due to that the two tests represent different types of catecholamine challenge. During exercise noradrenaline rather than adrenaline is the major catecholamine responsible for lipolysis activation (33), whereas the opposite is true during mental stress (11). The catecholamine resistance may be selective for lipolysis, since the cardiac response to exercise and mental stress was almost identical in subjects with high or low BAR sensitivity.

It is not likely that insulin, which is the major lipolysis regulating hormone besides catecholamines, has influenced the present finding with lipolysis in vivo. The results with circulating insulin were almost identical in subjects with high and low BAR sensitivity during exercise and mental stress. In both groups insulin started at approximately the same value and decreased to the same value during exercise. During mental stress there was no change in plasma insulin.

For several reasons we believe that the observed catecholamine resistance in vitro is representative of lipolysis regulation in vivo. First, the maximal rates of isoprenaline-induced lipolysis, as well as isoprenaline ED_{50} were almost identical in adipocytes obtained before and after exercise. Secondly, the intraindividual variation in BAR lipolysis sensitivity over time was very small at rest ($\sim$ 5%). Finally there was a 50% increase in the circulating noradrenaline level at rest in subjects with low adipocyte sensitivity. This reciprocal relationship between hormone sensitivity and circulating hormone level is typical of a resistant state. The noradrenaline level before exercise and mental stress was almost the same in high- and low-sensitivity subjects. This difference as compared to the resting state may be related to the posture and cerebral activity of the subjects who were lying and completely relaxed at rest and but sitting and slightly activated before exercise and mental stress.

The lipolytic catecholamine resistance observed in this

Table IV. BAR Number, Affinity, and mRNA Expression in Fat Cells From 9 Subjects with High and 10 Subjects with Low Isoprenaline Sensitivity

Sensitivity group	Binding capacity			K_d		mRNA	
	B_0	BAR_1	BAR_2	BAR_1	BAR_2	BAR_1	BAR_2
	pmol/10^7 cells			*log mol/liter*		*molecules/cell*	
High	1.54±0.15	0.68±0.10	0.81±0.11	−5.8±0.2	−8.2±0.2	453±141	384±103
Low	0.84±0.12	0.55±0.07	0.29±0.06	−5.8±0.2	−8.5±0.3	189±57	64±23
P	< 0.002	NS	< 0.002	NS	NS	NS	< 0.005

Total binding capacity (B_0) was obtained from Scatchard analysis of ^{125}ICYP binding. The fraction and K_d of BAR_1 and BAR_2 were obtained from competition between ^{125}ICYP and ICI 118,551 in binding experiments. Binding capacity of BAR_1 and BAR_2 was obtained by multiplying the BAR subtype fraction with B_0. mRNA was determined by solution hybridization.

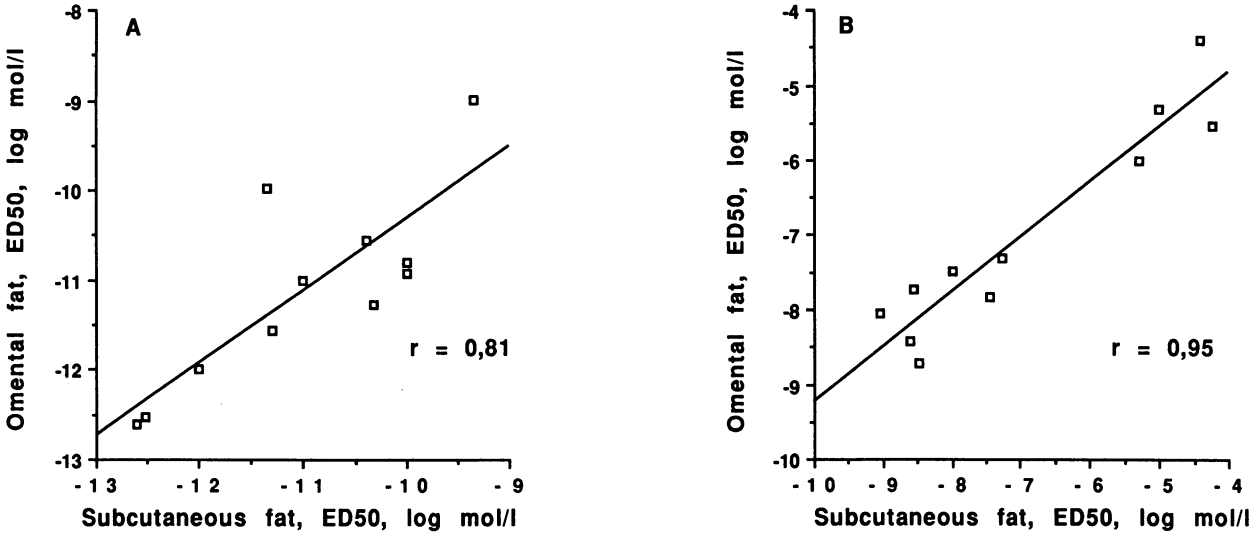


Figure 8. Lipolytic sensitivity of β-adrenergic agonists in omental and subcutaneous fat cells. Omental and subcutaneous fat cells of the same donor were incubated in the absence or presence of increasing concentrations of either isoprenaline (*A*) or terbutaline (*B*). Glycerol release was determined. Half-maximum effect (ED_{50}) was calculated from the dose–response curves and ED_{50} for subcutaneous fat cells was plotted versus ED_{50} for omental cells. A correlation was statistically tested using linear regression analysis.

study probably does not involve α_2-adrenoceptors. First, the maximum lipolytic effect of noradrenaline (i.e., the net effect of BAR stimulation and α_2 inhibition) and the maximum isoprenaline effect (BAR stimulation) were similar in subjects with high and low BAR sensitivity. Secondly, the antilipolytic effect of the partial agonist clonidine was similar in the two groups. However, the α_2 receptor was not the focus of this study. Therefore it cannot be excluded that there are variations in the function of the latter receptor in adipocytes of adult subjects, with consequences for lipolysis regulation.

In theory, the observed catecholamine resistance may be due to an alteration at any step in the activation of lipolysis from BAR to hormone sensitive lipase. With the aid of selective lipolysis-acting agents, the resistance could be localized solely to BAR_2. Thus, stimulation of lipolysis at the level of adenylate cyclase or protein kinase and the maximum lipolytic or antilipolytic action of all selective lipolysis agents used were similar in both groups. Therefore it is most unlikely that a postreceptor defect is responsible for BAR insensitivity. Likewise, the lipolytic sensitivity of the selective BAR_1 agonist dobutamine was almost identical in the two groups. However, the lipolytic sensitivity of terbutaline (BAR_2 selective agonist) was 10-fold decreased in low-sensitivity subjects, which was of the same order of magnitude as the differences in lipolytic sensitivity to noradrenaline. Furthermore, there was a close correlation ($r = 0.75$) between the lipolytic sensitivity of isoprenaline and that of terbutaline, but not between isoprenaline and dobutamine. Methodological data also show that terbutaline and dobutamine were almost full agonists with distinct BAR receptor subtype selectivity.

Our data with the selective BAR agonists differ somewhat from those previously published by other investigators, who found that dobutamine and terbutaline are partial agonists with poor selectivity (34). The difference between the results may partly be due to our use of diluted fat cell incubations (1–2% vol/vol) instead of the dense cell concentrations (10% vol/vol or higher) that have been used by previous investigators. As discussed in detail elsewhere (35), it is well known that endogenous metabolites may accumulate in dense fat cell incubations, and thus alter the lipolytic action of catecholamines. Furthermore, our subjects, unlike most of those studied previously, were not obese; the BAR function may be influenced by obesity. It may seem strange that selective agonists show almost full intrinsic activity as compared to the nonselective BAR agonist isoprenaline. This is probably due to the existence of a large reserve of functional BAR in human fat cells; only a fraction of BAR has to be occupied in order to obtain a full lipolytic response (36). Thus, the selective stimulation of BAR_1 or BAR_2 probably activates hormone-sensitive lipase entirely in human fat cells.

The mechanism underlying the observed selective BAR_2 resistance appears to be a reduced BAR_2 number. Analysis of total number of BAR and of the fraction of BAR_2, indicated a three times higher number of adipocyte BAR_2 in the sensitive group as compared to the resistant group. However, the affinity of BAR_2 was similar in the high- and low-sensitivity groups, at least for antagonists, as judged by radioligand experiments.

The decrease in adipocyte BAR_2 number in low sensitive subjects could be due to either inhibited synthesis and processing or increased internalization and degradation of the receptor. These processes cannot be investigated directly in human fat cells. However, some indirect information of receptor synthesis is obtained from the findings with mRNA.

Adrenoceptors are low abundance proteins. The small amounts of BAR_1 and BAR_2 mRNA were detected with a highly sensitive solution hybridization assay (32). The number of transcripts per cell at steady state were in accordance with the values for BAR_1 and BAR_2 mRNA previously found in abdominal subcutaneous adipocytes using the same assay method (28). Furthermore, the decrease in BAR number in adipocytes of low-sensitivity subjects occurred in parallel with a decrease in BAR_2 mRNA. This suggests that an inhibited

gene expression of BAR_2 followed by a decreased translation and thereby lowered rate of synthesis of the BAR_2 protein is the explanation behind the impaired noradrenaline function in low sensitive subjects. However, changes in BAR mRNA and BAR protein do not always run in parallel, as discussed in detail recently (37). Therefore it is possible that posttranslational modifications of BAR_2 expression also play a role for the observed low BAR sensitivity.

It is unknown at present if there is a common or specific (i.e., BAR_2) decrease in mRNA expression in adipocytes of catecholamine resistant subjects, in that there was a small but not statistically different variation in BAR_1 mRNA expression between the two groups as well. It was not possible to measure other mRNA species in the small amount of adipose tissue that was available in the present investigation. For the same reason it is unclear how the individual variations in BAR_2 mRNA expression relate to nucleic acid stability or transcriptional activity. It is, however, not likely that variability in the recovery of mRNA has influenced the results in a major way. Methodological experiments revealed that the extraction procedure was quantitative and the coefficient of variation for the assay method was only 10%. Furthermore, the amount of nucleic acids extracted per fat cell (i.e., TNA/DNA) was approximately the same in high- and low-sensitivity subjects.

Unlike many other tissues in humans, adipose tissue contains multiple BAR subtypes. In subcutaneous adipose tissue, BAR_1 and BAR_2, but not BAR_3, appear to be functionally coupled to lipolysis (7, 34). The physiological reason for the existence of different BAR subtypes in the same tissue is not yet known. It is possible that these receptors are regulated separately. BAR_1 and BAR_2 in adipocyte cell lines or adipose tissue of laboratory animals or humans have different sensitivities to corticosteroid stimulation and homologous down-regulation (8, 38, 39). Furthermore, as reviewed (2), selective BAR_1 resistance has been demonstrated previously in the myocardium of patients with various types of heart failure, contrary to the present findings. It remains to be established whether the observed difference between the heart and subcutaneous abdominal adipose tissue is due to variations in organ sensitivity of BAR_1 and BAR_2 or to the fact that the pathophysiological conditions investigated were different.

Thus, we do not know how the present findings with BAR subtypes relate to lung and heart which also express both BAR_1 and BAR_2. For obvious ethical reasons it was not possible to study BAR in these tissue in the present study. Our data suggest indirectly that BAR_2 in heart is less variable than BAR_2 in adipose tissue, because subjects with high and low BAR sensitivity had an almost identical chronotropic response to mental stress and exercise. Investigations on leukocytes or monocytes are of little value for a comparison of BAR_1 and BAR_2 in that these cells only express BAR_2. However, BAR function appears to be interrelated in different types of fat cells because cholecystectomy patients who had high BAR_2 sensitivity in subcutaneous adipocytes also had high BAR_2 sensitivity in fat cells of other origin (i.e., omental) and vice versa. There was an excellent correlation between omental and subcutaneous adipocytes as regards isoprenaline and terbutaline sensitivity and the interindividual variation of β agonist sensitivity was in the same order of magnitude in both cell types. This indicates strongly that lipolytic catecholamine resistance is present in different types of adipose tissue.

Some of the isoprenaline ED_{50} values may seem surprisingly low, since values below 10^{-12} mol/liter were found for a substantial portion of the high sensitive subjects. However, we have reexamined recently published data (4, 40) from our laboratory, where lipolytic isoprenaline sensitivity of abdominal adipocytes has been determined in a large group of healthy subjects not included in this study. Mean ED_{50} was found to be $\sim 10^{-11}$ mol/liter with a range from 10^{-14} to 10^{-9} mol/liter. In other words, previous data confirm present data on the variability of isoprenaline sensitivity. It is of importance to note that we generally report much lower ED_{50} values for catecholamine-induced lipolysis in isolated human fat cells than other laboratories (34, 41–43). This may be due to the use of different dilutions of fat cell suspensions as discussed above. When we previously used more dense human fat cell suspensions (10%, vol/vol) the adipocytes were unsensitive to isoprenaline stimulation as reported by the other investigators (44, 45). We strongly believe that high adipocyte isoprenaline sensitivity is relevant for lipolysis stimulation in situ in man. We have recently shown using microdialysis that lipolysis is maximally stimulated with 10^{-12} mol/liter of isoprenaline in situ in abdominal subcutaneous adipose tissue (46).

At present, causes of variations in BAR_2 expression in fat cells of apparently normal subjects are unknown. It is possible that there may be a feedback loop of some sort between the adipocyte and the autonomic system. The observed increase in circulating catecholamines may be a primary factor with differences in adipocyte BAR_2 function being a secondary phenomenon. As reviewed (37) a high catecholamine level can inhibit mRNA expression of BAR. It is possible that in human fat cells the BAR_2 gene is more sensitive to high catecholamine levels than the BAR_1 gene causing selective down-regulation of BAR_2. Alternatively, it is possible that the increase in circulating noradrenaline is only compensatory to a primary decrease in the BAR_2 number of adipocytes. If so, a decrease in receptor number may be caused by insulin, thyroid hormones, corticosteroids, sex hormones, products of lipolysis such as glycerol and free fatty acids, or other unknown factors that regulate BAR expression. It is also possible that there are genetic variations in the BAR_2 structure with functional consequences. In this respect it is of interest to find reports of polymorphisms of the genes encoding for BAR_1 and BAR_2 in humans (47, 48).

The clinical consequence of catecholamine resistance in apparently healthy subjects is not clear at present. Besides an increase in circulating noradrenaline, the two groups showed no differences in common clinical parameters such as age, sex distribution, body weight, fat distribution cell size, smoking habits, and exercise training. However, it is tempting to speculate that BAR_2-mediated catecholamine resistance in abdominal adipose tissue may be an early sign of disturbed peripheral sympathetic nervous activity. However, this question can only be answered by investigations on subjects with diseases involving altered catecholamine function such as obesity, hypertension, asthma, and diabetes. In this study subjects with asymptomatic endocrine, metabolic, or cardiovascular diseases were excluded from the investigation.

In summary, this study shows that lipolytic noradrenaline resistance in visceral and subcutaneous adipose tissue exists in the apparently normal adult population. Catecholamine-resistant subjects have a reduced lipolytic response to exercise and mental stress, in spite of an increased plasma catecholamine

response because of a reduced mRNA expression of BAR_2 in fat cells. In addition, the data suggest that BAR_1 and BAR_2 are independently regulated in human fat cells.

Acknowledgments

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