Regional Differences in Adrenoceptor Binding and Fat Cell Lipolysis in Obese, Postmenopausal Women

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In women there is an increase in visceral obesity, subcutaneous abdominal adipocyte lipolysis, and risk of cardiovascular disease (CVD) associated with weight gain after menopause. The mechanisms underlying this increase in adrenoreceptor (AR)-agonist catecholamine-stimulated lipolysis and abdominal obesity in postmenopausal women were studied in intact adipocytes isolated from the abdominal and gluteal subcutaneous fat depots in 19 obese (48% ± 1% body fat, mean ± SE) women with a mean ± SE age of 58 ± 1 years. The fat cell size and adipose tissue lipoprotein lipase (ATLPL) activity were similar in both sites. The maximal lipolytic responsiveness and sensitivity to isoproterenol were higher ($P < .05$) in abdominal compared with gluteal adipocytes, but maximal lipolytic response to a post-AR agent was similar. Abdominal adipocytes had a higher β-AR ([^{3}H]-CGP-12177) and α_2-AR ([^{3}H]-yohimbine) affinity than gluteal cells ($P < .05$), lower α_2-AR density ($P < .05$), but similar β-AR density as gluteal cells. Both abdominal and gluteal cell size correlated with α_2-AR density ($P < .01$), but not with β-AR density. Thus, a higher β-AR affinity and lower α_2-AR relative to β-AR density may explain the higher in vitro catecholamine-mediated lipolysis in abdominal compared with gluteal adipocytes in obese, postmenopausal women.

IN POSTMENOPAUSAL WOMEN there is a tendency for weight gain to occur in the abdominal region.[1,2] The magnitude of the increase in visceral fat adiposity correlates better with obesity-related risk factors, such as hypertension, hyperlipidemia, and diabetes mellitus, for CVD and with events such as heart attack and stroke in older women than the absolute degree of obesity.[3]

The only hormones that acutely affect lipolysis in human adipocytes are catecholamines (epinephrine and norepinephrine) and insulin.[4] The regulation of lipolysis by catecholamines involves AR stimulation of adenylate cyclase (AdC) via β-AR (β_1-, β_2-, and β_3-AR) and inhibition by α_2-AR.[5,6] Functionally, there are marked regional differences in hormonal responsiveness and metabolic activity of human adipose tissue,[5,7-12] with in vitro catecholamine-stimulated lipolytic responsiveness in viscerals greater than in abdominal subcutaneous, or in peripheral, eg, gluteofemoral, subcutaneous fat cells.[13-17] Even the lipolytic response to a pure β-AR agonist (isoproterenol) is higher in subcutaneous abdominal than gluteal adipocytes.[10,18] This enhanced lipolytic reactivity to catecholamines of subcutaneous abdominal adipocytes compared with gluteofemoral adipocytes is reported in non-obese and obese men,[14,17,18] non-obese and obese premenopausal women,[10,14,18,19] as well as in obese postmenopausal women.[17,20] We recently showed that in obese postmenopausal women, regardless of fat distribution, subcutaneous abdominal adipocytes have a higher lipolytic response than gluteal cells to a mixed agonist (epinephrine), but not to a postreceptor agonist.[20] These results suggest that the mechanisms underlying the greater lipolytic reactivity in abdominal adipocytes compared with gluteal adipocytes occur at the receptor level.

Studies examining the AR-binding properties in subcutaneous abdominal and gluteal adipocytes report variable findings. A lower density of α_2 -AR relative to β-AR (α_2/β-AR)[10] as well as a greater density of β-AR are considered possible mechanisms for the increased lipolytic response to catecholamines in abdominal adipocytes compared with gluteal adipocytes from premenopausal women.[14] Little is known regarding the regional regulation of fat cell lipolysis in obese postmenopausal women. This study was designed to determine whether the increased lipolytic responsiveness of subcutaneous abdominal fat cells in obese postmenopausal women is due to an increased β-AR density and/or affinity in abdominal adipocytes compared with gluteal adipocytes. This was accomplished by measuring the β-AR and α_2-AR–binding properties and the lipolytic response to isoproterenol and a postreceptor agonist in intact subcutaneous adipocytes from abdominal and gluteal sites in healthy, obese postmenopausal women without medical conditions that might affect lipid, glucose, or adipose tissue metabolism.

SUBJECTS AND METHODS

Subjects

Nineteen healthy, obese, postmenopausal Caucasian women from the Baltimore/Washington, DC metropolitan area were selected and gave their written consent according to approved guidelines of the University of Maryland Institutional Review Board for Human Research to participate in this study. Five women had a body-mass index (BMI) less than 27.3 kg/m^2,[21] but all had percent body fat by dual-energy x-ray absorptiometry (DXA) greater than 38%. The women were considered to be postmenopausal if they had not menstruated for at least 1 year, and their plasma follicle-stimulating hormone (FSH) levels were greater than 30 mIU/mL. None of the women had been on estrogen-replacement therapy (ERT) or medications affecting blood pressure, lipid metabolism, or glucose metabolism. All women were sedentary (<20 minutes of exercise twice per week), weight stable (<2 kg weight change in 2 months), and had not smoked for at least 1 year.

Women were accepted to participate in the study after completing a medical evaluation that included a medical history, physical examination, fasting blood profile, and ECG. No subject had evidence of CVD,

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diabetes (fasting glucose level > 6.4 mmol/L), hypertension (blood pressure > 160/90 mm Hg), nor did they have evidence of hyperlipidemia, liver, renal or hematological disease. A graded exercise test (GXT) according to a modified-Bruce protocol[22] was performed to exclude subjects with an abnormal cardiovascular response to exercise.

Experimental Protocol

Women were counseled in the principles of an American Heart Association (AHA) Step 1 diet[23] for 6 weeks. Although some lost weight during this period, they were weight-stable (± 0.50 kg weight change) for at least 1 week before metabolic testing. Nutrient intake was controlled for 2 days before the fat biopsy by providing each subject with a weight-maintaining diet composed of 50% to 55% carbohydrate, 15% to 20% protein, and 30% fat with 300 to 400 mg of cholesterol and a polyunsaturated to saturated fat ratio of 0.6 to 0.8.

Body Composition, Fat Distribution, and Maximal Aerobic Capacity

Measurements of body composition and maximal aerobic capacity ($\dot{V}O_2max$) were obtained at least 1 week before adipocyte-binding and lipolysis studies. Waist-to-hip ratio (WHR) was measured as the ratio of the minimal waist circumference to the hip circumference at the maximal gluteal protuberance. Each circumference was measured by the same investigator, and the mean of three values within 2 mm of each other was used as the measurement. Percent body fat and fat-free mass were measured using DXA (Model DPX-L; Lunar Radiation Corp, Madison, WI). Intra-abdominal (IA) and subcutaneous fat areas were measured using a single-slice computed tomography (CT) scan taken midway between L4 and L5, performed on a GE Hi-Light CT scanner (Milwaukee, WI), as described previously.[20] $\dot{V}O_2max$ was measured on a motor-driven treadmill during a progressive exercise test to voluntary exhaustion.[20] A valid $\dot{V}O_2max$ was obtained when at least two of these three criteria were met: (1) maximal heart rate greater than 90% of age-predicted maximal heart rate (220 bpm-age), (2) respiratory exchange ratio of at least 1.10, and (3) plateau $\dot{V}O_2$ with increasing work rate. If this was not achieved, the test was repeated.

Glucose and Lipid Metabolism

Venous blood samples for measurement of lipoprotein, lipid, glucose, and insulin levels were taken after a 12-hour fast on 2 separate days, and the mean of the two measurements is reported. A 3-hour 75-g oral glucose tolerance test with sampling every 30 minutes was performed after a 12-hour overnight fast in all subjects to evaluate diabetes. Plasma glucose and insulin concentrations were measured in duplicate using the glucose oxidase method (Beckman Glucose Analyzer, Fullerton, CA) and radioimmunoassay with an insulin-specific antibody (cross-reactivity with proinsulin < 0.2%) (Linco, St Louis, MO), respectively. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels were measured as previously described.[24]

Adipocyte Metabolism

Isolation of Adipocytes

To eliminate the effects of physical activity on adipocyte's metabolism, subjects were asked to remain sedentary, and the fat biopsies were performed at least 1 week after the $\dot{V}O_2max$ test. After an overnight fast, 4 to 6 g of subcutaneous adipose tissue was obtained under local anesthesia (1% xylocaine) from both the abdominal and gluteal regions by aspiration with a 16-gauge needle.[25] Adipocytes were isolated by collagenase digestion of adipose tissue fragments in Krebs-Ringer Hepes (KRH) buffer containing 4% fatty acid-free bovine serum albumin (FFA-free BSA), 5 mmol/L glucose, .1 mmol/L ascorbic acid, and 200 nmol/L adenosine using a modification of the Rodbell method.[26] The isolated cells were filtered through a 250-μm nylon mesh and washed three times with the same KRH buffer to remove collagenase from the medium. The adipocytes were resuspended in the same buffer (lipolysis assays) or in the binding buffer (binding assays) so that the final cell concentration was approximately 4% to 5%. The mean fat cell lipid weight was calculated using the average mean adipocyte diameter and standard deviation,[27] and total lipid content in the suspension was determined gravimetrically after extraction.[28] Cell number was obtained by dividing total lipid weight of the suspension by the mean fat cell lipid weight, and fat cell surface area was calculated using the formula $\pi \times (d^2 + SD^2)$, where d and SD are the mean and standard deviation for fat cell diameter.[27]

Adipose Tissue Lipoprotein Lipase Activity

Heparin-releasable adipose tissue lipoprotein lipase (ATLPL) activity was measured as previously described.[29] ATLPL was eluted from 30- to 50-mg fragments of adipose tissue into 2.5 mL Krebs-Ringer-Phosphate buffer containing 5 U heparin during a 45-minute incubation at 37°C. Triplicate 0.5-mL aliquots of the eluate were incubated with 0.1 mL of substrate. The substrate was prepared by sonication of 4 μCi of 1-^{14}C-glycerol triolein, 5 mg unlabeled triolein, and 240 μg lecithin in 4 mL of .5 mol/L Tris buffer, pH 8.2, containing 2% FFA-free BSA and .25 mL fasting serum, which provides the cofactor needed for ATLPL activity, apolipoprotein (Apo) C-II. The enzyme reaction was stopped after 45 minutes at 37°C by addition of Belfrage's extraction mixture[30] to separate the product, labeled FFA, from unreacted substrate. The labeled FFA were quantitated by liquid scintillation counting and, after correction for recovery during the extraction, ATLPL activity was expressed as nanomoles FFA produced per minute by 10^6 cells.

Radioligand Binding Assay

The assays were performed under steady-state conditions, using previously described methods.[31-33] Packed cells were suspended in the binding buffer (Hanks balanced salt solution with 20 mmol/L Hepes pH 7.4 and 0.05% BSA) to a final concentration of approximately 4% and triplicate 300-μL aliquots (1 to 2 × 10^4 cells) were incubated in a final volume of 0.5 mL in the presence of radioligands with and without unlabeled adrenergic agents at 37°C. Incubations were terminated by addition of 5 mL of ice-cold NaCl (.9%) followed by rapid vacuum filtration of suspensions over Whatman GF/C glass fiber filters (Springfield Mill, Maidstone, England). The filters were washed, and the bound radioactivity determined using scintillation counting.

β-ARs. The β_1/β_2-AR hydrophilic antagonist (-)-[^{3}H]-CGP-12177 was used to quantify β_1 and β_2-AR in intact human adipocytes.[33,34] This antagonist is the ligand of choice for intact cells because it partitions to only a small extent into the cell's triacylglycerol compartment compared with the β-AR radioligand [^{125}I]-(-)iodocyanopindolol.[35] Saturation curves were generated by incubating adipocytes with increasing concentrations of [^{3}H]CGP-12177, from 0.3 to 9 nmol/L, a concentration range at which the antagonist labels only the high-affinity β_1- and β_2-AR, rather than the low-affinity β_3-AR[36] for 30 minutes at 37°C (total binding). The nonspecific binding was determined as the residual [^{3}H]-CGP-12177 bound in the presence of 1 μmol/L (-)propranolol and accounted for approximately 40% of the total binding.

α_2-ARs Saturation curves were generated by incubating adipocytes with increasing concentrations of [^{3}H]-yohimbine[37] (from 0.3 to 15 nmol/L) alone (total binding) for 15 minutes at 37°C. The nonspecific binding was calculated as the residual [^{3}H]-yohimbine remaining in the presence of 30 μmol/L phentolamine, and accounted for approximately 40% of the total binding. The specific binding (total binding minus nonspecific binding) was expressed per cell number (pmoL/10^7 cells) and per cell-surface area (amoL/mm^2). The total number of binding

sites (Bmax) and the affinity constant for the radioligands (K_D) was calculated using the specific binding. Saturation curves were analyzed according to the method of Scatchard, using the program Radlig v4.0 (Biosoft, Ferguson, MO)[38] to determine the values of Bmax and K_D.

Lipolysis Assay

Glycerol released from adipocytes was used as the index of lipolysis, since it is not reused by the fat cell.[39] Triplicate 0.75-mL aliquots of the diluted cell suspension were incubated in plastic vials with gentle shaking in a water bath at 37°C. Pharmacological agents were added just before the beginning of the incubation in 10-μL aliquots in vehicle to obtain the indicated final concentration. After 2 hours, the lipolysis reaction was stopped with 76 μL of 2.5 mol/L perchloric acid and 80 μL of the infranatant removed for the measurement glycerol concentration by an enzymatic fluorometric technique.[40] The lipolytic response of isolated adipocytes was tested with isoproterenol (nonselective β-AR agonist) and with dibutyryl 3',5'-cyclic monophosphate (db-cAMP, phosphodiesterase-resistant cAMP analog). Variations in adenosine metabolism have a major influence on the measurement of lipolysis[41,42]; therefore, in experiments measuring the stimulatory effect of isoproterenol, adenosine deaminase (ADA) was added to remove adenosine present in the medium. However, to control for the activation of lipolysis after adenosine removal, which might prevent or attenuate the response to isoproterenol, isoproterenol stimulation of lipolysis was evaluated in the presence of 1 U/mL ADA and 100 nmol/L N^6-(1-2-phenylisopropyl)-adenosine (PIA), a potent adenosine receptor agonist that is neither a substrate nor an inhibitor of ADA, as shown previously.[43] Lipolysis was expressed either per cell number in μmol glycerol/10^6 cells × 2 h or per cell-surface area in nmol glycerol/μm^2 × 10^8 × 2 h. The maximal lipolytic effect, or responsiveness, was calculated as the difference between basal glycerol released and the glycerol released at the maximally effective concentration of the lipolytic agent. The concentration of isoproterenol giving half-maximal response (EC_{50}), an index of sensitivity, was obtained by computer fitting of individual dose-response curves to isoproterenol using SigmaPlot software (Jandel Scientific, Chicago, IL).

Materials

Collagenase (type CLS-1) was purchased from Worthington Biochemical (New Jersey, NJ); fatty acid–free BSA, ADA, NAD, ATP, and enzymes for glycerol assays were purchased from Boehringer Mannheim (Mannheim, Germany); [^{3}H]-yohimbine (specific activity, 78 to 81 Ci/mmol), (-)-[^{3}H]-CGP-12177 (specific activity, 44.5 Ci/mmol) were purchased from Dupont/New England Nuclear (Boston, MA); glycerol tri(1-^{14}C)oleate (specific activity, 63 mCi/mmoL) was purchased from Amersham (Arlington Heights, IL). All other chemicals were purchased from Sigma Chemical and were of the highest purity available.

Statistics

Data are means ± SE. Student's paired *t* test was used to compare abdominal versus gluteal values in the same individual, and Pearson's product-moment correlation coefficients were calculated using Jump Software (SAS Institute, Cary, NC). Outliers were tested by studentized residuals, rejecting values that were at the $P < .05$ level; therefore, some analyses include data from only 17 subjects. Differences were considered significant when $P < .05$.

RESULTS

Patient Characteristics

These 19 sedentary women ($\dot{V}O_2$max, 16 to 27 mL/kg/min) had a wide range of body fat (% body fat, 38% to 59%), and regional fat distribution (WHR .75 to 1.01), as well as IA (103 to 386 cm^2) and subcutaneous (288 to 716 cm^2) fat areas (Table 1). IA fat area correlated significantly with waist circumference ($r = .61$, $P = .01$), but not with other indices of body composition such as BMI, percent body fat, or subcutaneous fat area. Subcutaneous fat area also was related to waist circumference ($r = .51$, $P < .05$), but correlated more strongly with indices of obesity, including BMI ($r = .85$, $P < .001$), percent body fat ($r = .92$, $P < .0001$), and hip circumference ($r = .76$, $P < .001$).

The lipid and glucose metabolic profiles in these healthy women were normal (Table 2), and there was no relationship between the metabolic variables and IA or subcutaneous fat area, percent body fat, waist circumference, or WHR.

Adipose Tissue Metabolism

Fat Cell Size

Abdominal and gluteal fat cell sizes were similar and directly related ($r = .76$, $P = .0002$) (Table 3). The size of both abdominal and gluteal adipocytes correlated with BMI (abdominal, $r = .55$, $P = .02$; gluteal, $r = .48$, $P = .05$) and percent body fat (abdominal, $r = .46$, $P = .05$; gluteal, $r = .62$, $P < .01$), but only abdominal cell size correlated with waist circumference ($r = .52$, $P = .03$) and IA fat area ($r = .59$, $P = .01$). There was no significant relationship between gluteal cell size and waist circumference ($r = .44$, $P = .1$), or IA fat area ($r = .03$, $P > .8$). Therefore, in these women, both abdominal and gluteal subcutaneous fat cells correlated with measurements of obesity (% body fat, BMI), but only abdominal fat cell size correlated with indices of visceral obesity (IA fat area, waist circumference).

ATLPL

ATLPL activity was similar in adipocytes from abdominal and gluteal fat depots (5.43 ± 0.87 *v* 5.74 ± 0.74 nmol/10^6 cells × min). There was no relationship between ATLPL activity in the abdominal or in the gluteal fat cells with any of the measurements of total or regional fat mass (IA or subcutaneous fat area, BMI, % body fat), fat cell size, insulin, or lipoprotein lipids.

Basal and Stimulated Lipolysis

Basal lipolysis in gluteal and abdominal cells was similar (Table 3) and tended to correlate positively with IA fat area, whether expressed per cell number (μmol glycerol/10^6

Table 1. Physical Characteristics and Body Composition

Characteristic	Mean ± SE	Range
Age (yr)	58 ± 1	51-67
Body weight (kg)	86.3 ± 3.8	67-113
BMI (kg/m^2)	32.1 ± 1.4	25-42
Body fat (%)	48.2 ± 1.2	38-59
Vo_2max (mL/kg/min)	20.1 ± 0.8	16-27
Regional fat distribution		
Waist circumference (cm)	99.8 ± 3.4	84-134
Hip circumference (cm)	115.8 ± 3.0	100-146
WHR	0.84 ± 0.02	0.75-1.01
Subcutaneous fat area (cm^2)	450 ± 28	288-716
IA fat area (cm^2)	185 ± 18	103-386

Table 2. Lipid and Glucose Metabolic Profiles (N = 19)

Variable	Mean ± SE	Range
Triglycerides	1.61 ± 0.12	0.7-2.66
Cholesterol	5.25 ± 0.18	3.90-6.78
LDL-C	3.26 ± 0.16	2.01-4.68
HDL-C	1.25 ± 0.25	0.93-1.76
Fasting glucose	5.44 ± 0.10	4.66-6.22
2-hour glucose	7.84 ± 0.45	4.11-10.49
Fasting insulin	76.2 ± 11.0	34.0-240.0

NOTE. All data are in mmol/L except insulin (pmol/l).

Abbreviations: LDL-C, low-density lipoprotein; HDL-C, high density lipoprotein.

cells × 2h) (abdominal lipolysis [$r = .46$, $P = .08$] and gluteal lipolysis [$r = .49$, $P = .06$] or per cell surface area (nmol glycerol/$\mu m^2 \times 10^8 \times 2$ h)(abdominal lipolysis [$r = .43$, $P = .10$] and gluteal lipolysis [$r = .53$, $P = .04$]). Basal lipolysis did not correlate with subcutaneous fat area. Addition of ADA significantly increased the basal lipolysis rate by 115% in abdominal cells and by 66% in gluteal cells. These values did not differ, and they did not correlate with IA or subcutaneous fat area.

The maximal lipolytic effect of isoproterenol in the presence of ADA and PIA was 68% greater in abdominal cells than in gluteal cells. This β-AR agonist-stimulated lipolysis in adipocytes from either site correlated with subcutaneous fat area (each $r = .52$, $P < .04$, respectively), but not IA fat area. The maximal lipolytic response of abdominal fat cells to isoproterenol correlated positively with basal lipolysis ($r = .50$, $P < .05$), and a similar tendency was observed for gluteal adipocytes ($r = .46$, $P = .06$). The sensitivity (inverse of EC_{50}) to the β-AR agonist isoproterenol was 35% greater ($P = .03$) in abdominal adipocytes compared with gluteal adipocytes. In abdominal adipocytes, there was a negative relationship between the EC_{50} for isoproterenol and both basal lipolysis ($r = .85$, $P < .001$) and maximal lipolytic response to isoproterenol ($r = .73$, $P < .001$). However, there was no relationship between β-AR sensitivity to isoproterenol in abdominal fat cells or gluteal fat cells with fat cell size, or subcutaneous fat and IA fat areas. Thus, abdominal fat cells with a higher basal lipolysis were more sensitive to the β-AR agonist isoproterenol. There was also no difference in postreceptor (db-cAMP)-stimulated lipolysis between abdominal cells and gluteal cells, suggesting that the mechanism for the regional differences in the lipolytic response to β-AR agonists is at the receptor level. There was no relationship between any of the measurements of lipolysis and ATLPL activity.

Table 3. Regional Adipocyte Metabolism (N = 19)

Variable	Abdominal	Gluteal
Regional fat cell size		
Cell weight (μg TG/cell)	0.80 ± 0.03	0.82 ± 0.03
Cell volume (pL)	827 ± 33	839 ± 36
Regional fat cell lipolysis		
Basal	0.67 ± 0.22	0.71 ± 0.17
Basal + ADA (1 U/mL)	1.44 ± 0.34	1.18 ± 0.23
Basal + ADA (1 U/mL) + PIA (100 nmol/L)	0.27 ± 0.06	0.34 ± 0.07
Isoproterenol max (Δ)	2.50 ± 0.32*	1.49 ± 0.22
EC_{50} isoproterenol (nmol/L)	54.9 ± 9.9*	84.6 ± 21.8
db-cAMP (Δ) (2 mmol/L)	3.65 ± 0.40	3.41 ± 0.37

NOTE. Values are means ± SE. Lipolysis is expressed in μmol glycerol/10^6 cells × 2 h. Maximal response (Δ) and sensitivity (EC_{50}) to isoproterenol were calculated from dose-response curves (10^{-10} to 10^{-5} mol/L) in the presence of ADA and PIA.

Abbreviation: TG, triglyceride.

*$P < .05$ *v* gluteal adipocytes.

Binding Characteristics of β-and α_2-AR Radioligand Antagonists

Measurements of binding to intact abdominal adipocytes and gluteal adipocytes showed that the affinity (inverse of K_D) for both β- and α_2 -AR antagonists was higher (65% and 27%, respectively; $P < .05$) in abdominal fat cells compared with gluteal fat cells. There was no difference in the density of β-AR between abdominal cells and gluteal cells (when expressed either per cell surface area or per cell number), but the density of α_2-AR was 32% lower in abdominal adipocytes compared with gluteal adipocytes ($P < .05$) (Fig 1). Therefore, the functional balance of α_2-AR to β-AR density (ie, the ratio of α_2-AR/β-AR), considered an index of the differential in vitro reactivity to epinephrine in adipocytes from different fat depots,[10] was 49% lower in abdominal cells ($P < .05$) (Fig 2). There was no relationship between isoproterenol-stimulated lipolysis and β-AR density in either abdominal ($r = .08$, $P > .7$) or gluteal ($r = .20$, $P = .4$) adipocytes.

There was a positive correlation between the density of α_2-AR (expressed per cell number or per cell surface area) and cell size in abdominal ($r = .64$, $P < .01$) and in gluteal adipocytes ($r = .53$, $P = .01$) (Fig 3). However, there was no relationship between the remaining binding parameters and cell size in adipocytes from either site.

DISCUSSION

The results of this study show that regional differences in the AR binding properties may underlie the enhanced catecholamine- and pure β-AR agonist-stimulated lipolysis in subcutaneous abdominal versus gluteal adipocytes in obese postmenopausal women. The finding of a lower ratio of α_2-AR/β-AR in abdominal adipocytes compared with gluteal adipocytes may account for some of the enhanced responsiveness of abdominal fat cells versus gluteal fat cells to mixed AR agonists (epinephrine and norepinephrine) previously shown in obese postmenopausal women.[17,20] Furthermore, the increased affinity of abdominal β-AR is consistent with an increased sensitivity of abdominal adipocytes compared with gluteal adipocytes to stimulation of lipolysis by a pure β-AR agonist, such as isoproterenol, as reported by others[10,18] and confirmed in our study.

The significant relationship found between IA (but not subcutaneous) fat area and the size of subcutaneous abdominal fat cells (from ~ 420 to 1,065 PL) shown in this study, suggests that in obese postmenopausal women, there may be a relationship between visceral obesity and subcutaneous abdominal fat cell size. In this study, there were no differences in the ATLPL activity or the size of subcutaneous abdominal adipocytes versus gluteal adipocytes, similar to data reported previously in obese postmenopausal women by our group[20] and others.[17] This is contrary to what is seen in premenopausal women, whose gluteal fat cells are larger and have a higher ATLPL activity than that in abdominal adipocytes.[19] The increase in ATLPL activity in gluteal adipocytes in premenopausal women is considered

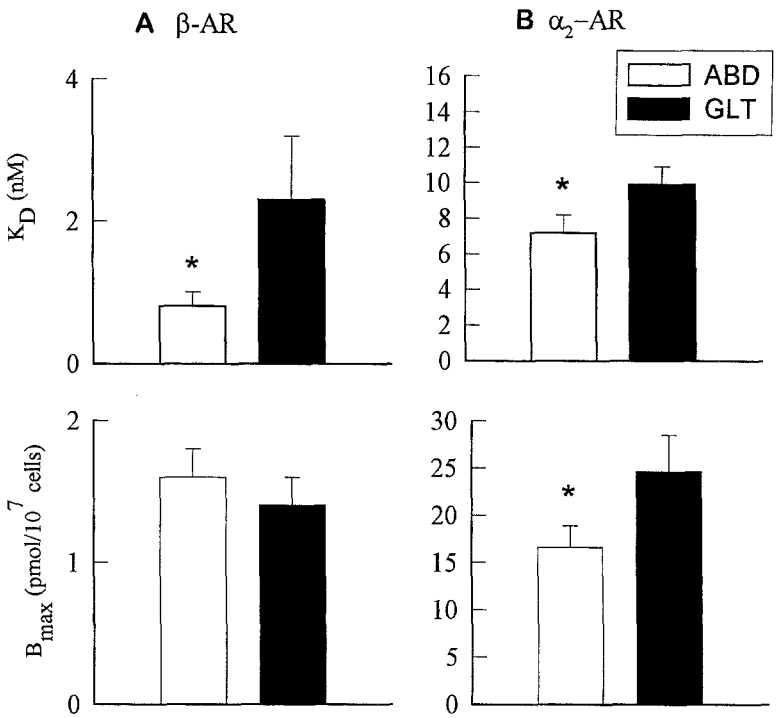

Fig 1. Regional affinities (K_D) and densities (B_{max}) of β-AR (A) and α_2-AR (B) in adipocytes isolated from abdominal (ABD) and gluteal (GLT) depots. (A) β-AR dissociation constant, K_D, and maximum number of binding sites, B_{max}, were obtained from saturation-binding curves with the β-antagonist [^{3}H]CGP-12177. (B) α_2-AR K_D and B_{max}, were obtained from saturation-binding curves with the α_2-antagonist [^{3}H]-yohimbine. Values are means ± SE. n = 19, *$P < .05$ *v* GLT adipocytes.

one mechanism for their enlarged gluteal fat cells compared with abdominal fat cells, as well as increased fat deposition in the gluteofemoral depots.[19] Thus, the similarity in ATLPL activity in abdominal adipocytes and gluteal adipocytes suggests that ATLPL activity may contribute to the increased abdominal fat deposition associated with obesity in postmenopausal women.[1] Collectively, these results suggest that an increase in ATLPL activity and β-AR affinity, as well as a decrease in the α_2-AR/β-AR density in abdominal fat cells, are possible mechanisms for the increased abdominal fat cell lipolysis in obese postmenopausal women.

In agreement with Arner et al,[44] we found no relationship between basal and maximal lipolytic response or sensitivity to a β-AR agonist and cell size. Nevertheless, our results suggest a role for α_2-AR density in regulation of the size of subcutaneous abdominal adipocytes and gluteal adipocytes of these obese women. Unfortunately, we did not measure antilipolytic responses, and, therefore, cannot directly compare our results with those of Arner et al,[44] who found a negative relationship between the α_2-inhibitory effect of noradrenaline and fat cell size, ie, that an increase in fat cell size is associated with a diminished α_2-AR antilipolytic effect of the hormone. Variations in adenosine metabolism strongly affect lipolysis.[41,42] Therefore, we chose to study the stimulatory effect of isoproterenol on lipolysis under uniformly controlled conditions by removing the endogenous adenosine in the medium with ADA, and maintaining a constant activation of adenosine receptors by addition of a nonhydrolizable analog of adenosine, PIA. The enhanced lipolytic reactivity of abdominal cells compared with gluteal cells observed under these experimental conditions is similar to that reported by other investigators in the absence of ADA and PIA in the medium.[10,18]

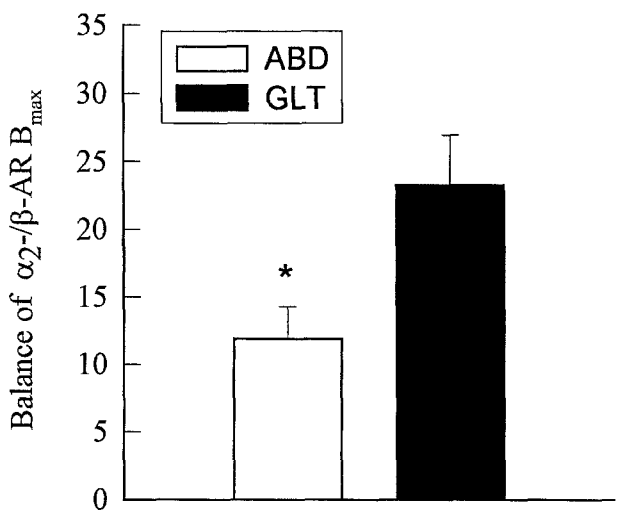

Fig 2. Regional balance of α_2/β-AR density (B_{max}) in adipocytes isolated from abdominal (ABD) and gluteal (GLT) depots. Data are expressed as the ratio of [^{3}H]-yohimbine to [^{3}H]CGP-12177 binding sites, which was calculated from individual saturation-binding curves. Values are means ± SE. n = 19, *$P < .05$ *v* GLT adipocytes.

The increased density (almost twofold) of β-AR and a lower α_2-AR affinity reported by Wahrenberg et al[14] in abdominal adipocytes compared with gluteal adipocytes in non-obese premenopausal and postmenopausal women, using intact cells and the same radioligands we used, could explain the higher response to β-Ar agonists in abdominal adipocytes compared with gluteal adipocytes. However, in that study,[14] the subjects included non-obese premenopausal and postmenopausal women with a wide range (24 to 55 years); hence, the absence of an increase in β-AR number in abdominal adipocytes compared with gluteal adipocytes in the women in our study could reflect their obesity, as well as their uniform postmenopausal, estrogen-deficient status. The recent report of a selective 70% reduction in the number of β_2-AR (but not β_1-AR or total β-AR) in abdominal fat cells from obese compared with non-obese

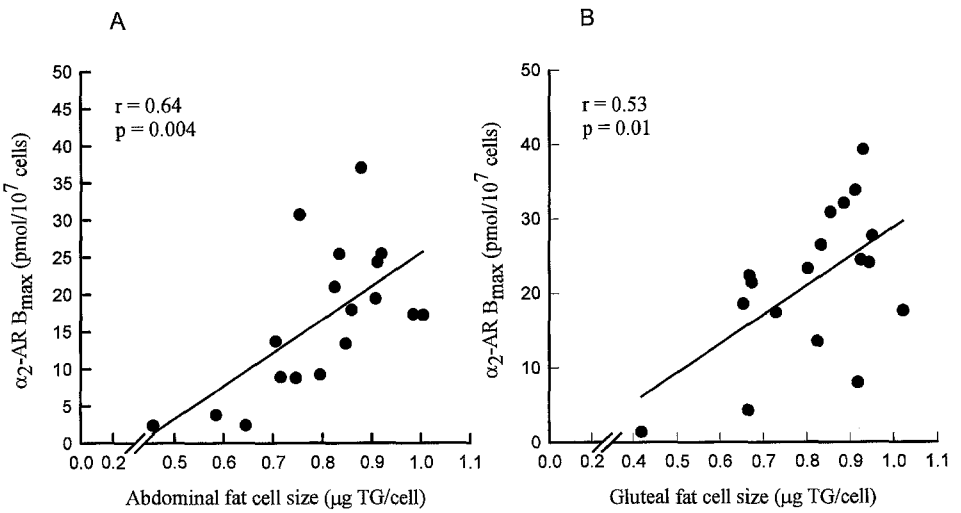

Fig 3. Relationship of density of α_2-AR (B_{max}) and fat cell weight (μg triglyceride/cell) in abdominal (A) and gluteal (B) adipocytes of 18 obese, postmenopausal women.

premenopausal women, supports an effect of obesity in the regulation of regional fat cell metabolism.[45] The re-evaluation of these women after weight loss will provide insight into the mechanisms by which obesity and weight loss affect fat cell metabolism in postmenopausal women.

Healthy, obese postmenopausal women with a wide range of body fat distribution, indexed as WHR, were studied to determine the regional regulation of subcutaneous adipocyte metabolism and its relationship to visceral obesity. We excluded women on ERT because of its effects on the distribution and metabolism of body fat.[2] There was an increase in ATLPL activity in the femoral adipocytes compared with the abdominal adipocytes in postmenopausal women receiving ERT,[17] similar to the regional difference in ATLPL activity observed in premenopausal women.[19,46] In this context, our data showing similar ATLPL activities in abdominal adipocytes and gluteal adipocytes from obese postmenopausal women not on ERT agree with those of Rebuffe-Scrive et al[17] and suggest that changes in regional ATLPL activity (ie, equal levels in abdominal sites and gluteal sites) may partially account for the increased deposition of fat in the abdomen with weight gain in the postmenopausal estrogen-deficient state.[1,2] Little is known about the effects of estrogen deficiency on lipolysis in adipose tissue. There was an increase in plasma free fatty acid levels in postmenopausal women off ERT compared with those receiving estrogen[47]; however, in vitro studies showed norepinephrine-stimulated lipolysis in abdominal adipocytes and gluteal adipocytes was similar in postmenopausal women receiving ERT.[17] In a study by Mauriege et al,[10] there were no regional differences in the affinity or density of β-AR in membranes isolated from adipocytes of obese premenopausal women, despite a larger density of α_2-AR in femoral adipocytes compared with abdominal adipocytes. The differences between our results and theirs could be related to the postmenopausal status of the women in our study, but could also be due to the fact that we used [^{3}H]CGP-12177 as radioligand and measured its binding to intact cells, while they used [^{3}H]dihidroalprenolol, a highly lipophilic radioligand, and measured its binding to broken cell membranes. To determine the independent effects of obesity and estrogen on the AR-binding properties of subcutaneous adipocytes, it will be necessary to study normal-weight and obese postmenopausal women before and during estrogen therapy.

In summary, these findings suggest that the enhanced lipolytic reactivity to β-AR agonists and catecholamines observed in abdominal subcutaneous adipocytes compared with gluteal subcutaneous adipocytes of obese, postmenopausal women involves both an increased affinity of the β-AR receptor, and a decreased density of α_2-AR. The relative density of α_2-AR to β-AR in subcutaneous adipocytes also may affect the size of abdominal cells and gluteal cells in these women. Further studies are needed to clarify how a higher β-AR affinity, a lower functional balance of α_2-AR to β-AR, and comparable activity of ATLPL in subcutaneous abdominal adipocytes and gluteal adipocytes are related to the development of abdominal obesity and metabolic dysfunction in postmenopausal women.

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