Differences in β-Adrenergic Receptor Densities in Omental and Subcutaneous Adipose Tissue From Obese African American and Caucasian Women

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African American women lose less weight and at a slower rate than Caucasian women under the same weight loss conditions. This is likely due to decreased mobilization of fat, possibly involving differences in the responsiveness of adipose tissue to adrenergic stimulation. To better understand the causes behind the decreased lipolysis in African American women, this study was initiated to determine if there were differences in the numbers and affinities of β adrenoreceptors in omental and subcutaneous adipose tissue of obese African American and Caucasian women. We determined the number of β receptors using a nonselective antagonist and found the total number of receptors in both omental and subcutaneous adipose tissue preparations were higher in African American than Caucasian women. β_1, β_2, and β_3 densities were higher in omental adipose tissue ($P < .05$), but not different in the subcutaneous tissue of the African American women. No racial differences in kd values for adrenergic agents (agonists and antagonists) were found with regard to β_1, β_2, or β_3 receptors in either the omental or the subcutaneous preparations. β_1 and β_2 receptor protein (mass) was significantly increased in African American omental tissue preparations, but not subcutaneous. Our in vitro data demonstrating increased β receptor numbers in omental tissue from obese African Americans suggest that the potential for lipolysis would be higher in these women. Future studies should determine the biologic significance of the differences in the β adrenergic receptors in vivo.

AFRICAN AMERICAN women have a tendency to gain weight at an earlier age and to be heavier than Caucasians of a similar age.[1,2] In addition to their propensity for a greater weight gain, obese African American women lose less weight and lose weight at a slower rate than Caucasians across a variety of treatments including conservative interventions,[3] very low calorie diet,[4,5] and surgery.[6-9] The racial difference in weight gain and the response to weight loss treatments remains after adjustment for age and level of education and cannot be entirely attributed to socioeconomic or cultural factors.[10-13] This suggests that there are additional biologic differences that contribute significantly to the prevalence of obesity in African American women, but the precise causes that underlie these differences are not fully understood.

The mobilization of fat from adipose tissue stores is mediated by the enzyme hormone sensitive lipase (HSL). HSL catalyzes the first step of lipolysis by hydrolyzing the stored triacylglycerols and diacylglycerols to fatty acid and glycerol. In the basal state, the rate of lipolysis is low because HSL is relatively inactive, but upon HSL activation, the rate of lipolysis is enhanced. Catecholamines bind to β receptors on adipocytes and activate HSL through the G-protein signaling cascade. Insulin and other agents counter-regulate lipolysis by activation of phosphodiesterase, which leads to a decrease in the concentration of the G-protein signaling cascade intermediate, cyclic adenosine monophosphate (cAMP).

We have previously reported that the concentrations of glycerol and free fatty acids in fasting plasma of obese African American women were significantly lower than those of obese Caucasian women, consistent with other reports of lipolysis being lower in African Americans than Caucasians.[14-16] We also reported that HSL mass was decreased, but activity (expressed/milligram protein) was not different in the adipose tissue of the African American women. Thus, the decreased basal lipolysis could be partly due to the decrease in HSL mass. We followed these observations by determining the rate of lipolysis under catecholamine stimulation of adipose tissue in vitro. Our previous findings demonstrated that isoproterenol-stimulated lipolysis reached the same maximal level in both races, but because of a lower basal HSL, the African American women showed increased stimulated lipolysis when expressed as fold stimulation.[16]

The results from our previous study led us to postulate that the stimulation of lipolysis by isoproterenol in the adipose tissue of the African American women could possibly be due to differences in the individual steps of the lipolytic cascade that lead to HSL activation or an increased number or affinity of β-adrenergic receptors. Thus, this study was initiated to define the underlying mechanisms that modulate these racial differences of in vitro lipolysis. We measured β-adrenergic receptor densities and affinities, as well as protein mass in both omental and subcutaneous adipose tissue preparations of obese African American and Caucasian women. The results of these studies will help to better define the mechanisms behind the decrease in lipolysis that leads to the slower rate of weight loss in African American women.

MATERIALS AND METHODS

Subjects

Morbidly obese (body mass index [BMI] > 40) African American (n = 15) and Caucasian (n = 21) women participated in this study. The participants were free of vascular disease, diabetes, or cancer and were not taking any adrenergic medications or medications that might affect carbohydrate or lipid metabolism. The subjects were not taking hor-

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mone replacement therapy or birth control pills. The women who participated in this study were recruited consecutively over a period of 18 months from the Department of Surgery at the Brody School of Medicine, East Carolina University and were selected on the basis of BMI according to the guidelines of the World Health Organization. African American women were included in this study only if their parents and grandparents were of African American descent. Age (years) of African Americans was 42 ± 2 and 44 ± 2 for Caucasians. Body mass and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively and the BMI calculated to be 44 ± 4 and 50 ± 3, respectively, for the African American and Caucasian women. Glucose values (mg/dL) were 94 ± 3 for African American and 97 ± 5 for Caucasian women. Insulin values (μU/mL) were 7.2 ± 1.1 and 7.4 ± 2, respectively, for African American and Caucasian women. Triglycerides (mg/dL) were 89 ± 1 and 129 ± 2, respectively, for African American and Caucasian women. Free fatty acid values (μmol/L) were 522 ± 10 for African American and 847 ± 41 for Caucasian women. Adipose tissue was obtained from the volunteers during abdominal surgery for gastric bypass or total abdominal hysterectomy. Omental adipose tissue was dissected from the greater omentum and subcutaneous tissue was dissected from the epigastric region of the abdomen. Tissues were frozen in liquid nitrogen, wrapped in foil, and stored at −70°C until time of assay (1 to 4 weeks). Written consent was obtained from all of the subjects after they were informed of the nature of the study. The Institutional Review Board for human subject research approved the protocols used in this study.

Membrane Preparations

Membrane preparations were prepared by thawing approximately 1 g omental adipose tissue and homogenizing this (glass on glass tissue homogenizer) in ice cold buffer (20 mmol/L HEPES, 150 mmol/L sucrose, 1 mmol/L EDTA, pH 7.4; 1:4 vol/vol), containing protease inhibitors (2 μmol/L pepsatin, 2 μmol/L leupeptin, 0.1 mg/mL bacitracin, 100 U/mL aprotinin). The homogenate was filtered through 2 layers of cheesecloth and centrifuged for 15 minutes at 1,500 × *g*. The infranatant was collected and centrifuged at 100,000 × *g* for 1 hour. The pellet, which contained the membrane fraction of the adipocytes, was suspended in cold homogenization buffer to a protein concentration of 1 mg/mL and frozen at −70°C until time of assay. Membrane preparations were stable up to 4 months when stored in this manner.

Radioligand Binding Assay

Membrane preparations of adipose tissue from the African American and Caucasian women were rapidly thawed and maximal binding capacity (Bmax) was determined by the use of ^{125}I-labeled cyanopindolol (^{125}I-CYP), a β adrenergic antagonist, to label the receptors.[17] Briefly, ^{125}I-CYP (60 pmol/L for β_1 and β_2 receptors or 350 pmol/L for β_3 receptors) was incubated with 30 to 40 μg membrane protein in 200 μL total volume (buffer: 50 mmol/L Tris, 5 mmol/L $MgCl_2$, pH 7.4) for 30 minutes in a 37°C shaking water bath. At the end of the incubation period, the tubes were placed on ice for 10 minutes, rapidly filtered through Whatman GF/C glass fiber filters (Maidstone, UK), and washed with 12 mL ice cold incubation buffer. Nonspecific binding was determined in the presence of 10^{-7} mol/L of the β_1 antagonist, CGP20712A (Ciba Geigy, Tom's River, NJ) for β_1 receptors, the β_2 antagonist ICI 118551 (ICI) for β_2 receptors, and the β_3 antagonist L-748337 (Merck, Whitehouse Station, NJ) for β_3 receptors.[18] For the nonspecific blockade of total β receptor binding, we used 10^{-5} mol/L propranolol (Eli Lilly, Indianapolis, IN). Radioactivity remaining on the filters was quantified using a Beckman 5500 Gamma counter (Fullerton, CA). Displacement curves were generated using ^{125}I-CYP to label the receptors with increasing concentrations of β adrenergic agents (10^{-10} mol/L to 10^{-6} mol/L). For the β_3 receptor assay, the Merck compound L-748337 was dissolved in 50% dimethyl sulfoxide (DMSO). The final concentration of DMSO in the reaction mixture for both total and nonspecific binding tubes was 0.5% that has been shown to have no effect on the binding to the β_3 adrenergic receptor.[18] Total apparent numbers of receptors were calculated using both a previously determined saturating concentration of ^{125}I-CYP (60 pmol/L for β_1 and β_2, 350 pmol/L for β_3) and/or from Scatchard plot analyses.[19] Kd values were derived from Scatchard plot analyses,[19] saturation binding isotherms, and/or displacement curves. IC_{50} values, measured from competition binding assays, were converted to kd values according to the method of Cheng and Prusoff.[20] The antagonists, CGP 20712A, ICI 118551, L748337 and the agonists, dobutamine, terbutaline, CGP12177 were used to displace ^{125}I-CYP off of the β_1,β_2, and β_3 receptors, respectively.

Immunoblot Analysis

β_1, β_2, and β_3 receptor mass was determined by Western blot analysis.[21] Protein from the membrane preparations (20 μg) was mixed with sodium dodecyl sulfate (SDS) loading buffer and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) on a 10% gel. Protein content was determined as described by Bradford[21] using bovine serum albumin (BSA) as a standard. Proteins were electrotransferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA). β_1-adrenergic receptor (AR) was detected with a rabbit polyclonal antibody (IgG-A20) raised against the C-terminus of human β1-AR. β_2-AR was detected with a rabbit polyclonal antibody (IgG-H20) raised against the C-terminus of human β_2-AR. β_3-AR was detected with a goat polyclonal antibody (IgG-C20) raised against the C-terminus of human β_3-AR. All primary antibodies were used at 1.25 μg/mL, and detection of signals was performed using the SuperSignal West Pico Chemiluminescent Substrate kit (Pierce, Rockford, IL). Antirabbit horseradish peroxidase-conjugated IgG was used as secondary antibody for anti-β_1-AR and anti-β_2-AR. Antigoat horseradish peroxidase-conjugated IgG was used as secondary antibody to anti-β_3-AR. All antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A random sample was picked to serve as a control and run with each gel. After quantification on a phosphorimager, the sample was assigned a value of 1.0 and ratios were obtained by comparison with each sample.

Statistics

Comparisons of data were conducted using a Student's *t* test when data were normally distributed. For nonhomogeneous data, a Mann-Whitney Rank Sum test was used. Statistical testing was performed using SigmaStat 2.03 (SPSS, Chicago IL). The level of statistical significance for these experiments was $P < .05$. Data are expressed as the mean ± SEM.

RESULTS

To determine the underlying causes behind the difference between the African American and Caucasian women with respect to fold stimulation by isoproterenol, we measured maximal binding (Bmax) of ^{125}I-CYP to membranes of subcutaneous and omental adipose tissue from African American and Caucasian women. As seen in Fig 1, this total, nonselective β-receptor binding was significantly greater in both the subcutaneous and omental preparations from African American women ($P < .05$). To further define these changes, we measured β_1, β_2, and β_3 subtypes in the 2 types of adipose tissue from the African American and Caucasian women. As seen in Fig 2, there was a significant increase in apparent β_1, β_2, and β_3 adrenergic receptor numbers in the African American omental tissue when compared with Caucasian tissue ($P < .05$). When assessing β receptors in subcutaneous tissue, we found no significant differences in apparent β_1, β_2, or β_3 receptors in preparations from the African Americans when compared with Caucasians (Fig 3). The summation of the 3 subtypes of β

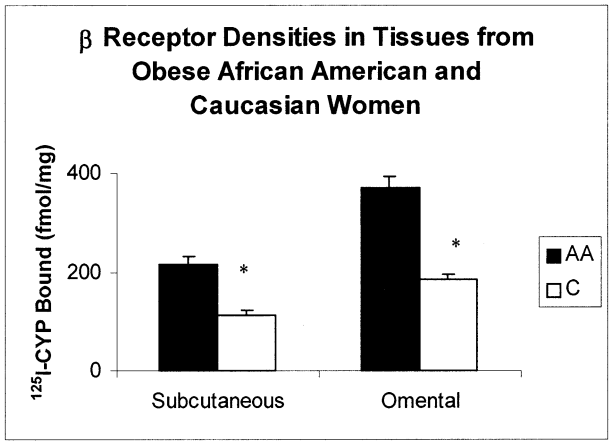

Fig 1. Total apparent β adrenergic receptor numbers (Bmax) were determined in tissue from obese African American (AA) and Caucasian (C) women using ^{125}I-CYP (350 pmol/L) to label the β receptors as described in Materials and Methods (n = 10). The nonselective antagonist, propranolol (10^{-5} mol/L) was used to determine nonspecific binding. Total apparent β receptor numbers were significantly increased in both subcutaneous and omental adipose membrane preparations from obese African American women when compared with preparations from Caucasian women. *Statistically significant ($P < .05$).

receptor densities for both omental and subcutaneous fat (Figs 2 and 3) is less than the total β receptor numbers depicted in Fig 1, in which we quantitated the total receptor density using the nonselective displacing agent, propranolol. This can possibly be explained by the fact that although ^{125}I-CYP is relatively selective for β receptors, it can label other receptors, as well, particularly at the concentration of 350 pmol/L. In addition to displacing ^{125}I-CYP from the β receptors, propranolol (10^{-5} mol/L) may be displacing the ^{125}I-CYP from other receptors, thus giving us a slightly elevated value.

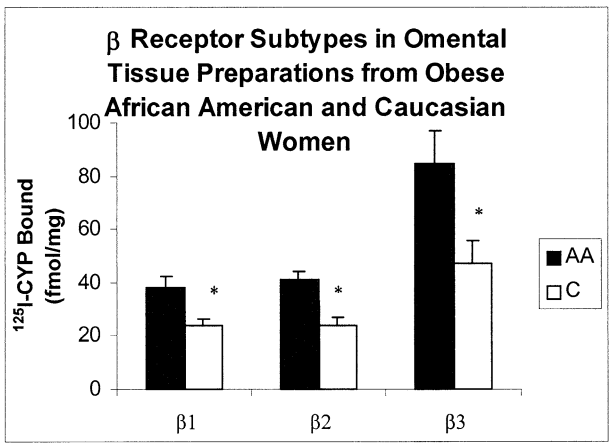

Fig 2. Total apparent β_1, β_2, and β_3 adrenergic receptors were determined in omental tissue from obese African American (AA) and Caucasian (C) women as described in Materials and Methods (β_1, β_2 n=9 (AA) and n = 12 (C); β_3 n = 9). β_1, β_2, and β_3 receptor densities were significantly increased in the African American preparations when compared with Caucasian. *Statistically significant ($P < .05$).

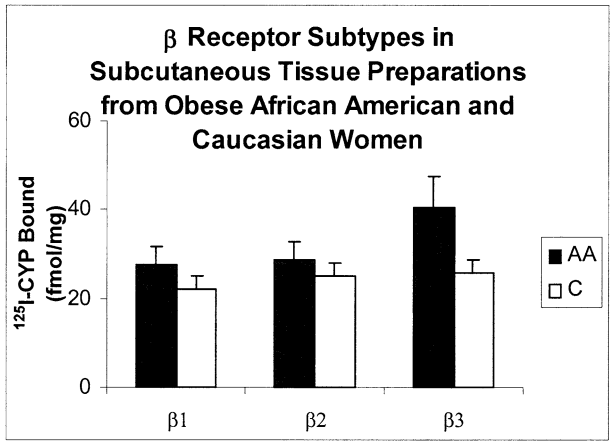

Fig 3. Total apparent β_1, β_2, and β_3 adrenergic receptors were determined in subcutaneous tissue from obese African American (AA) and Caucasian (C) women as described in Materials and Methods (β_1, β_2 n = 8; β_3 n = 12). No significant differences in β_1, β_2, or β_3 receptor densities were seen in the African American preparations when compared with Caucasian.

We found β-receptor protein mass to be significantly increased for β_1 and β_2 receptors in omental preparations from African American women as shown in Table 1. Although β_3 receptor mass appeared to be increased in omental tissues from the obese African Americans, significance was not achieved. In the subcutaneous adipose tissue preparations, we found no racial differences in the β-receptor mass for all 3 of the receptor subtypes (Table 1).

Kd values for β_1, β_2, and β_3 adrenergic agents were determined in omental adipose tissue (Table 2) and subcutaneous tissue (Table 3) from obese African American and Caucasian women. Values were determined for the 3 subtypes of receptors using saturation curves, displacement curves, and/or Scatchard analyses. In both the subcutaneous and omental tissue preparations, no racial differences in receptor affinities were found between obese African American and Caucasian women.

DISCUSSION

The results of this study produced the novel findings that β-adrenergic receptor densities and protein mass are increased

Table 1. β-Receptor Mass in Omental and Subcutaneous Adipose Tissue Preparations From Obese African American and Caucasian Women

	Omental		Subcutaneous	
Receptor Subtypes	African American	Caucasian	African American	Caucasian
β_1	0.88 ± 0.09*	0.69 ± 0.04	0.84 ± 0.06	0.93 ± 0.06
β_2	0.76 ± 0.04*	0.48 ± 0.03	0.60 ± 0.10	0.69 ± 0.07
β_3	0.80 ± 0.09	0.65 ± 0.11	0.77 ± 0.07	0.67 ± 0.08

NOTE. β-receptor mass is expressed in arbitrary units that were normalized to 1 value obtained from including the sample on all gels. Values are mean ± SEM, (n = 8 to 12). β_1-and β_2-receptor mass was significantly higher in omental tissue from obese African Americans.
*Statistically significant ($P < .01$).

in omental tissue from obese African American women when compared with Caucasian. Because kd values were not altered for either agonists or antagonists, this suggests that the receptors are functionally similar in the African Americans and Caucasians. These results are consistent with our previous findings demonstrating that in vitro isoproterenol-stimulated lipolysis reached the same maximal level in both races, despite a lower HSL mass in the adipose tissue of African American women.[21] This decrease in mass of HSL explains the lower in vitro basal lipolytic rate by adipose tissue from the African Americans. When this is taken into consideration, the fold stimulation of lipolysis in the obese African American women is higher than that of the Caucasian women. Our findings that kd values were not significantly different for any of the adrenergic agents investigated between the 2 races in either type of adipose tissue suggest that the racial differences we see in vitro with regard to enhanced fold stimulation are related primarily to increases in β-receptor densities.

There are a variety of biochemical differences between omental and subcutaneous adipose tissues.[22-27] β-receptor density and lipolysis are both higher in omental fat when compared with subcutaneous fat.[28] Also, catecholamines seem to be more lipolytic in omental fat cells than in subcutaneous cells.[28] Interestingly, our current findings showed the same trends in the tissue preparations from African American women, and the differences in all 3 β subtypes are more pronounced in the omental adipose tissue.

Collectively, our data show that the capacity of omental adipose tissue to mobilize fat in vitro is higher in African American women than Caucasian women. This is in contrast to the observation that African American women do not mobilize fat in vivo as effectively. It appears that there are endogenous biochemical differences in the capacity of adipose tissue of African American women to deposit and mobilize fat that may exacerbate their obesity. Both the differences and the underlying mechanisms need to be further investigated and defined. Studies are needed to determine the lipolytic responsiveness of individual adipose cells to selective β agonists in both omental and subcutaneous fat from African American and Caucasian women. A variety of agents are known to inhibit lipolysis in vivo. α_2 adrenoreceptor agonists, insulin, adenosine, and nitric oxide have all been reported to suppress lipolysis in vivo.[29-34] Identifying the role that any or all of these inhibitors may have in inhibiting lipolysis in vivo in the African America women would be useful. Another area that warrants further investigation deals with receptor polymorphisms. β-adrenergic receptor polymorphisms have been implicated in a variety of obesity studies,[35-38] and it is certainly possible that certain polymorphisms found in African American women could influence the lipolysis. Further studies will help to explain why obese African American women tend to be more obese and have less success losing weight than obese Caucasian women and may be helpful in designing strategies for the control and prevention of obesity not only in African American, but also in Caucasian women.

Table 2. Kd Values for Adrenergic Agents in Omental Adipose Tissue Preparations From Obese African American and Caucasian Women

Receptor Subtype	African American	Caucasian
β_1		
CGP 20712A	8.6 ± 1.3 nmol/L	10.8 ± 2.2 nmol/L
Dobutamine	7.3 ± 1.1 μmol/L	5.6 ± 2.1 μmol/L
β_2		
ICI 118551	7.1 ± 0.9 nmol/L	9.5 ± 1.6 nmol/L
Terbutaline	8.5 ± 0.5 μmol/L	8.0 ± 0.3 μmol/L
β_3		
L-748337	3.3 ± 1.2 nmol/L	2.9 ± 0.9 nmol/L
CGP 12177	1.4 ± 0.7 μmol/L	2.0 ± 1.0 μmol/L

NOTE. Kd values were determined for various adrenergic agents by Scatchard analyses, saturation curves, and/or competition binding curves in African American and Caucasian omental preparations for each of the β-receptor subtypes. Binding curves were performed on preparations of 4 pooled samples each (n = 2 to 6 (AA), n = 2 to 5 (C)). No racial differences were found with regard to kd values for any of the subtypes of receptors. Values are expressed as mean ± SEM.

Abbreviations: AA, African American; C, Caucasian.

Table 3. Kd Values for Adrenergic Agents in Subcutaneous Adipose Tissue Preparations From Obese African American and Caucasian Women

Receptor Subtype	African American	Caucasian
β_1		
CGP 20712A	6.1 ± 0.1 nmol/L	4.0 ± 2.0 nmol/L
Dobutamine	0.9 ± 0.5 μmol/L	0.9 ± 2.0 μmol/L
β_2		
ICI 118551	0.9 ± 0.1 nmol/L	1.3 ± 0.8 nmol/L
Terbutaline	1.7 ± 0.4 μmol/L	5.5 ± 1.5 μmol/L
β_3		
L-748337	2.5 ± 0.5 nmol/L	5.0 ± 3.0 nmol/L
CGP 12177	1.0 μmol/L*	1.0 μmol/L*

NOTE. Kd values were determined for various adrenergic agents by Scatchard analyses, saturation curves, and/or competition binding curves in African American and Caucasian subcutaneous preparations for each of the β-receptor subtypes. Binding curves were performed on preparations of 4 pooled samples each (n = 2 to 4 (AA), n = 2 (C)). No racial differences were found with regard to kd values for any of the subtypes of receptors. Values are expressed as mean ± SEM.

*One pooled sample comprised of 6 patient tissues.

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