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Influence of sex and β_2 adrenergic receptor haplotype on resting and terbutaline-stimulated whole body lipolysis

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Abstract

β_2 adrenergic receptors (β_2 ARs) are important mediators of lipolysis. The β_2 AR gene is highly polymorphic. To determine the contribution of β_2 AR polymorphisms to variability in whole body lipolysis, we compared basal and terbutaline-stimulated lipolytic rates (Ra) using tracer techniques in 14 healthy, non-obese males (n = 7) and females (n = 7) who were homozygous for Cys−19/Arg16/Gln27 or Arg−19/Gly16/Glu27 haplotypes. Fasting (overnight) Ra values were higher in females compared to males. Mean ± SD Ra, Ra/body weight, Ra/fat free mass, Ra/fat, and Ra/energy expenditure rates in males and females were 155 ± 46 vs 311 ± 111 μmol/min (P = .007); 2.0 ± 0.61 vs 5.2 ± 2.3 μmol/(min kg) (P = .006); 2.5 ± 0.75 vs 7.8 ± 3.4 μmol/(min kg) (P = .003); 10 ± 3.7 vs 17 ± 7.4 μmol/(min kg) (P = .09); and 144 ± 45.5 vs 392 ± 111 μmol/d (P = .0001), respectively. Mean ± SD basal glycerol concentrations were higher in females compared to males: 62 ± 5.6 vs 36 ± 17 μmol/L (P = .003). Basal glycerol concentrations and Ra values were similar by β_2 AR haplotype. Basal glucose and insulin concentrations tended to be higher in males compared to females and were similar by haplotype. Terbutaline-stimulated changes in glycerol concentrations were variable and are not related to either sex or haplotype. We conclude that compared to haplotype, sex is a more important determinant of basal lipolysis after a 12-hour fast in healthy, non-obese individuals.

1. Introduction

Fat stored in the body as triglycerides is hydrolyzed to free fatty acids and glycerol through the process of lipolysis [1]. Catecholamines are the most important stimulators of lipolysis working primarily through β_2 adrenergic receptors (β_2 ARs) expressed in adipocytes and in skeletal muscle [1-3]. Stimulation of α adrenergic receptors inhibits lipolysis [4,5] while insulin is a principal antilipolytic (lipogenic) agent [6,7].

Sex contributes importantly to the variability in lipolytic activity observed in human beings [8,9]. Whole body lipolysis stimulated by epinephrine, exercise, and fasting is higher in women compared to men [10]. Although the mechanisms underlying the sex-related differences in lipolysis are not clear, differences in adrenergic regulation of lipolysis and fatty acid mobilization have been proposed to be responsible [4,11,12].

The gene encoding the β_2 AR is located on chromosome 5q31-5q33, is intronless, and highly polymorphic [13]. Several single nucleotide polymorphisms (SNPs) in the β_2 AR gene have been identified. Three SNPs at codons −19 (Arg or Cys), 16 (Gly or Arg), and 27 (Glu or Gln) occur in high frequencies in the US population and are in tight linkage disequilibrium. Specifically, Arg at codon −19 is almost always linked with Gly and Glu at codons 16 and 27, respectively, resulting in the Arg−19/Gly16/Glu27 haplotype (RGE). Two other haplotypes are common in the human population: Cys−19/Arg16/Gln27 (CRQ) and Cys−19/Gly16/Gln27 (CGQ) [13,14]. In addition, a few studies have reported that single β_2 AR SNPs [15,16] and β_2 AR haplotypes [17] can influence catecholamine-stimulated lipolysis in adipocytes.

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The specific aim of the present study was to compare the effects of sex and β_2 AR haplotype on resting and β_2 AR–stimulated whole body lipolysis in healthy, non-obese individuals. The hypothesis underlying this aim is that sex and β_2 AR polymorphisms both contribute to the inter-subject variability in whole body lipolysis.

2. Materials and methods

2.1. Study participants

Seven healthy, non-obese, males and 7 healthy non-obese females consented to participate in the study. Subjects were selected, according to diplotype (see below), from a cohort of individuals who participated in a larger study designed to determine if obesity is associated with β AR genetic variants. All participants were between the ages of 18 and 49 years, healthy, nonsmokers, and were not taking any medications. Body composition was determined using skinfold thickness measurements and dual emission x-ray absorptiometry (Hologic QRD4500A, Waltham, Mass). Studies were performed in the Clinical Research Center, at the Wolfson's Children's Hospital (Jacksonville, Fla), and were approved by the Nemours Children's Clinic clinical research review committee and the Baptist Medical Center institutional review committee. All subjects gave informed written consent before entering the study.

2.2. Study design

Study participants were admitted into the Clinical Research Center at 9:00 PM of day 1 and were fasted for 20 hours until 5:00 PM of day 2. Water was allowed ad libitum. At 6:00 AM of day 2, an indwelling catheter was inserted into the antecubital vein of each arm. At 7:00 AM (0 time), a bolus dose of [1,1,2,3,3-$^2H_5$] glycerol (d5 glycerol; 1.3 μmol/kg body weight) was then administered into the antecubital vein, followed by a continuous infusion of d5 glycerol (0.1 μmol/[min kg of body weight]) for 600 minutes as previously described [18]. At 9:00 AM, terbutaline sulfate (0.01 mg/kg of total body weight) was infused over 1 hour through the antecubital vein used for the isotopes. Terbutaline was used to stimulate β_2 ARs because, in contrast to epinephrine, it is a selective β_2 partial agonist, is a weak agonist at α receptors, and is easier to quantify and to model because it is a xenobiotic. Venous blood samples (10 mL) were withdrawn from the other antecubital vein at 0, 30, 60, 120, 140, 160, 180, 200, 220, 240, 300, 360, and 600 minutes after the start of the study for isotopic enrichments, hormones, and substrate concentrations.

Indirect calorimetry was performed twice before and 1 hour after the start of the terbutaline infusion (Medical Graphics Corp, St Paul, Minn) using a mouth piece.

2.3. Determination of haplotype

Genomic DNA was isolated from whole blood drawn from each individual using a DNA isolation kit (Gentra Systems, Inc, Minneapolis, Minn) after the manufacturer's protocol. Final DNA was dissolved in 10 mmol/L Tris-HCl, pH 7.5, and diluted to a concentration of 100 ng/mL. The identity of the SNPs at loci −19, 16, and 27 was determined by polymerase chain reaction and restriction fragment length polymorphism, and the β_2 AR haplotype of each allele was determined in each individual by methods developed in our laboratory [19].

2.4. Analyses

Aliquots of blood were collected in chilled heparinized tubes, rapidly centrifuged at 4°C, and the resulting plasma samples were stored at −80°C until later analysis. Plasma concentrations of d5 glycerol and glycerol were quantified by gas chromatography mass spectrometry as previously described [18] using a model 5973 CI MS/6890 GC Agilent instrument (Palo Alto, Calif).

Plasma concentrations of terbutaline were quantified by high-performance liquid chromatography with electrochemical detection as previously described [20]. The within-day and day-to-day coefficients of variations were less than 3.9% and 4.4%, respectively; the limit of sensitivity was 1.0 ng/mL. Plasma concentrations of glucose were quantified by the glucose oxidase method with a Glucose Analyzer II (Beckman, Fullerton, Calif). Plasma concentrations of insulin were quantified by radioimmunoassay (INSULIN RIA, Diagnostics Systems Laboratories, Inc, Webster, Tex).

2.5. Calculations

Baseline glycerol concentrations ($C_{gly,bsl}$) were calculated as the average of values at 0, 30, 60, 90, and 120 minutes. The glycerol production rate (Ra; μmol/[min kg]) at baseline was calculated using the Steele equations [18,21], averaging the values of the d5 glycerol enrichments in plasma at isotopic steady state. The clearance of glycerol (CL_{gly}, L/[min kg]) was calculated by $CL = Rd/C_{gly,bsl}$, where Rd is the disappearance rate of glycerol, which is equal to Ra, if conditions are at steady state [21].

Substrate oxidation rates and resting energy expenditure rates (REE) were calculated from gas exchange equations as previously described [22] before and 1 hour after the start of the terbutaline infusion. Each individual recording represented 9 minutes of data collection, with average breath concentrations of oxygen and carbon dioxide reported every 30 seconds.

The effect of the terbutaline infusion on plasma concentrations of glycerol was assessed by calculating the area under the glycerol plasma concentration minus $C_{gly,bsl}$ vs time curve integrated between $t = 120$ and t', where t' is the time at which glycerol concentrations returned to baseline after the start of the terbutaline infusion. This area under the curve (AUC) value is equivalent to:

$$AUC = \int_{120}^{t'} \left(C_{gly} - C_{gly,bsl}\right)$$

Table 1
Comparison of mean ± SD baseline characteristics by sex and diplotype

Characteristic	Sex		P^a	Diplotype		P^a
	Male	Female		RGE	CRQ	
Age (y)	28 ± 3.4	37 ± 11	.08	35 ± 11	30 ± 7.4	.33
(range)	(23-33)	(22-50)		(23-50)	(22-48)	
Body weight (kg)	76 ± 7.2	62 ± 10	.02	69 ± 19	69 ± 13	.89
BMI, (kg/m^2)	24 ± 3.0	22 ± 3.4	.19	24 ± 2.9	23 ± 4.0	.62
FFM (kg)	61 ± 4.5	42 ± 5.5	4×10^{-5}	51 ± 12	52 ± 11	.81
Fat mass (kg)	15 ± 4.0	20 ± 5.8	.04	19 ± 6.2	16 ± 4.6	.41
Fat mass (% BW)	20 ± 3.7	32 ± 5.2	.0001	28 ± 8.7	24 ± 5.4	.44
REE[b] (kcal/d)	1573 ± 357	1403 ± 292	.44	1428 ± 246	1610 ± 419	.39

BMI indicates body mass index; BW, body weight.
[a] *t* Test.
[b] Pre-terbutaline REE.

Area under the curve was calculated by the trapezoidal method using the program WinNonLin Professional (ver 2.0.1, Pharsight Corporation, Palo Alto, Calif).

The pharmacokinetics of terbutaline was determined by standard techniques [23]. A 2-compartment open pharmacokinetic model with 0-order input was fitted to the terbutaline plasma concentration vs time data using the program WinNonLin Professional (ver 2.0.1, Pharmsight Corporation). The terbutaline pharmacokinetic parameters that were derived from fitting the model to the data were clearance (CL), central volume of distribution (Vc), volume of distribution at steady state (Vss), and elimination half-life.

2.6. Statistical analyses

A 2-way analysis of variance (ANOVA) with and without the interaction term haplotype × sex was used to test the significance of differences between haplotype and sex on lipolysis at rest and after terbutaline administration. A Student *t* test was used to test the significance of differences in demographic and anthropometric parameters. A *P* value of .05 or less was considered significant.

A power analysis was performed to estimate the sample size required enabling accurate and reliable statistical judgements for the 2-way ANOVA. There are no published studies of the effect on β_2 AR genetic variants on whole body lipolysis, although the results of in vitro studies in human adipocytes support a major effect [15,17]. We therefore estimated our sample size using published data of the effect of sex on β_2 AR–mediated whole body lipolysis [10,24], and a power of 0.8 and an α value of .05 [25].

3. Results

3.1. Subject characteristics

Body weight and fat free mass (FFM) were lower and fat mass was higher in females compared to males ($P < .04$; *t* test), whereas age, BMI, and REE were not different by sex (Table 1). These characteristics were similar by haplotype. Women tended to be older than men ($P = .08$). However, age did not affect resting or terbutaline-stimulated lipolysis (data not shown), which is consistent with published studies that report β_2 AR–mediated lipolysis is not affected by age [26,27].

3.2. Effect of sex and haplotype on baseline parameters

Table 2 compares baseline concentrations of glycerol, insulin, and glucose. Baseline concentrations of glycerol were 1.7-fold higher in females compared to males (Table 2, Fig. 1) ($P = .003$; ANOVA). Baseline insulin concentrations were overall higher in males compared to females (Fig. 1; Table 2, $P = .05$). Glucose concentrations at baseline tended to be higher in males compared to females ($P = .08$); however, the values were still well within reference range. No differences in baseline values for glycerol, insulin, and glucose were detected by diplotype (Table 2).

Table 3 compares resting lipolysis rates by sex and diplotype. Lipolysis rates were 2- to 3-fold higher in females

Table 2
Comparison of mean ± SD[a] baseline glycerol, insulin, and glucose concentrations by sex and diplotype

Baseline parameter	Sex		P^b	Diplotype		P^b
	Male	Female		RGE	CRQ	
Glycerol (μmol/L)	36 ± 17	62 ± 5.6	.003	47 ± 21	51 ± 16	.72
Insulin (μIU/mL)	9.1 ± 3.8	5.8 ± 1.5	.054	6.5 ± 1.8	8.3 ± 4.3	.40
Glucose (mg/dL)	93 ± 4.5	86 ± 7.8	.084	90 ± 8.5	90 ± 6.0	.87

[a] Mean ± SD of each parameter was calculated from values determined from each participant at 0, 30, 60, 90, and 120 minutes.
[b] ANOVA.

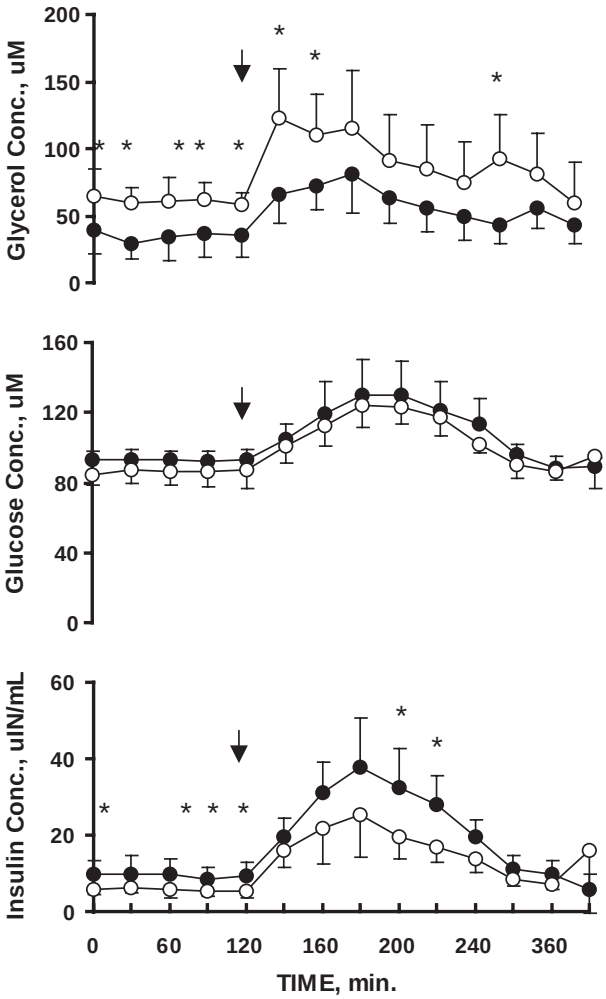

Fig. 1. Glycerol, glucose, and insulin concentrations vs time. Mean ± SD concentrations at various times before, during, and after a 1-hour infusion of terbutaline in females (open circles) and males (closed circles). The arrow indicates the time at which the terbutaline infusion was started. *$P < .05$.

compared to males ($P < .007$; ANOVA). When normalized for fat mass, Ra trended higher in females compared to males ($P = .09$; ANOVA). There was no difference in the glycerol Ra in RGE diplotypes compared to CRQ but there was substantial variability in responses (Table 3).

The baseline clearances of glycerol were similar in males and females, and were 0.063 ± 0.023 vs 0.084 ± 0.032 L/(min kg of total body weight), respectively. The clearances of glycerol did not differ in RGE and CRQ diplotypes, and

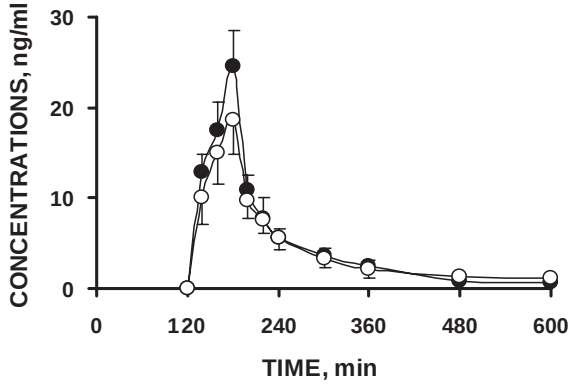

Fig. 2. Terbutaline concentrations vs time. Mean ± SD concentrations of terbutaline at various times during and after a 1-hour infusion of terbutaline sulfate (0.01 mg/kg) in females (open circles) and males (closed circles).

were 0.088 ± 0.032 vs 0.059 ± 0.017 L/(min kg of total body weight), respectively. There were no differences in glycerol clearance when normalized for FFM and fat mass by sex or diplotype (data not shown).

3.3. Terbutaline pharmacokinetics

The highest plasma concentrations of terbutaline were observed 1 hour after the start of the infusion and declined thereafter (Fig. 2). The mean ± SD peak terbutaline concentrations were higher in males compared to females: 25 ± 4.0 and 19 ± 3.7 μg/L, respectively ($P = .013$, t test). The mean ± SD peak terbutaline concentrations in RGE and CRQ diplotypes were not different, 20 ± 5.5 and 23 ± 3.5 μg/L ($P = .16$). The areas under the terbutaline plasma concentration vs time curve (AUC) did not differ by sex or diplotype: mean ± SD AUC values in males and females were 2.4 ± 0.44 and 2.2 ± 0.49 mg/(L min), respectively; in RGE and CRQ diplotypes the mean ± SD AUC values were 2.2 ± 0.52 and 2.4 ± 0.381, respectively. The clearances and elimination half-lives were also similar between groups averaging about 4 mL/(min kg of total body weight) and 2.0 hours, respectively (data not shown).

3.4. Effect of terbutaline

Fig. 1 summarizes the effects of terbutaline on glycerol, insulin, and glucose in males and females. Terbutaline administration maximally increased plasma concentration of glycerol 2.2- and 1.9-fold over baseline in males and females, respectively, 1 hour after the start of the terbutaline

Table 3
Comparison of mean ± SD baseline rates of lipolysis by sex and diplotype

Lipolytic rates	Sex		P^a	Diplotype		P^a
	Male	Female		RGE	CRQ	
Ra (μmol/min)	155 ± 46	311 ± 111	.007	273 ± 145	193 ± 64.5	.20
Ra/BW (μmol/[min kg])	2.0 ± 0.61	5.2 ± 2.3	.006	4.3 ± 2.9	3.0 ± 1.4	.38
Ra/FFM (μmol/[min kg])	2.5 ± 0.75	7.8 ± 3.4	.003	6.3 ± 4.6	4.0 ± 2.1	.28
Ra/F (μmol/[min kg])	10 ± 3.7	17 ± 7.4	.09	15 ± 8.4	12 ± 4.3	.65
Ra/REE (μmol/d)	144 ± 45.5	392 ± 111	.0001	286 ± 145	163 ± 78.7	.11

[a] ANOVA.

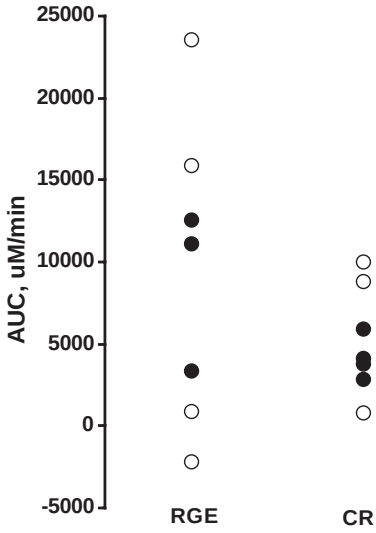

Fig. 3. Influence of sex and haplotype on glycerol AUC. Terbutaline-stimulated AUC values integrated between t = 120 minutes (start of the terbutaline infusion) and t' = 480 minutes in males (closed circles) and females (open circles) carrying RGE and CRQ haplotypes.

infusion and then declined to baseline 6 to 8 hours after the start of the terbutaline infusion. However, terbutaline-evoked changes in glycerol concentrations, although significantly higher than baseline, were not different between males and females. This is depicted in Fig. 3, which compares the areas under the plasma glycerol concentration minus baseline vs time curve integrated between t = 120 minutes (start of the terbutaline infusion) and t' = 480 minutes, in each individual. Area under the curve values varied widely among the participants, ranging between no effect evoked by terbutaline to a robust effect. As depicted in Fig. 3, although terbutaline increased glycerol concentrations above baseline, this drug effect was similar by sex and diplotype. Concentrations of d5 glycerol were relatively constant throughout the study period in each individual indicating that the terbutaline-evoked increases in glycerol were because of increases in glycerol production (Ra).

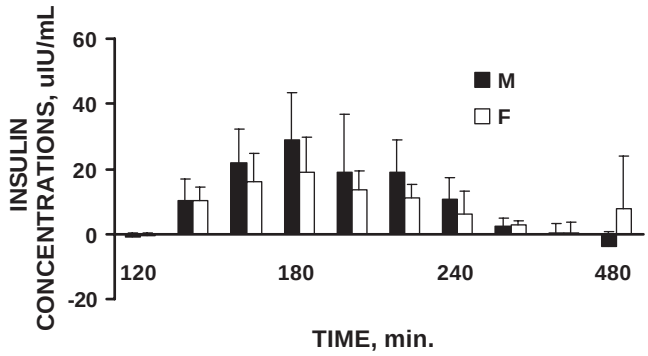

Fig. 4. Terbutaline-stimulated changes in insulin concentrations. Mean ± SD changes in insulin concentrations at various times during and after a 1-hour terbutaline infusion in males (M) and females (F). Changes in insulin concentrations were calculated as the difference between the observed insulin concentration minus baseline (pre-terbutaline).

Table 4
Comparison of mean ± SD lipid oxidation rates by sex and diplotype

	kcal/kg TBW per day	kcal/kg of FFM per day
Diplotype		
RGE	9.3 ± 4	13 ± 6
CRQ	10 ± 7	11 ± 8
Sex		
Male	7.9 ±	7.1 ± 6
Female	12 ± 3	18 ± 4[a]

[a] P = .003 vs males.

Concentrations of insulin vs time paralleled glucose and terbutaline concentrations (Figs. 1 and 2) peaking 1 hour after the start of the infusion and then declining to baseline. Terbutaline maximally increased insulin concentrations about 3-fold over baseline to 29 ± 15 and 19 ± 10 μIU/mL in males and females, respectively (P < .01; Fig. 1). However, the increases in insulin concentrations evoked by terbutaline, although significantly different compared to baseline (Fig. 1), were not different between males and females (Fig. 4). Concentrations of glucose were similar in males and females (Fig. 1). Insulin and glucose concentrations were not different by diplotype (data not shown).

3.5. Lipid oxidation rates

There were no significant differences in lipid oxidation depending on haplotype regardless of whether data are expressed per kilogram of FFM or kilogram of body weight. However, when data are analyzed by sex, there are substantial differences, with females having a higher rate of lipid oxidation as compared to males when data are expressed per kilogram of FFM (Table 4).

4. Discussion

In the present study, we compared the contributions of sex and β_2 AR haplotype to the variability in whole body lipolysis in the basal state after an overnight fast and after terbutaline administration. We observed that compared to men, basal glycerol Ra values were 2- to 3-fold higher in women. This was true for total Ra and for Ra normalized for total body weight, FFM, and REE. In addition, basal plasma concentrations of glycerol were significantly higher in females compared to males, 62 vs 36 μmol/L (Table 3), and the basal clearances of glycerol between females and males were similar. Because steady-state basal concentrations of glycerol are equal to the ratio of elimination rate (Rd) to the clearance of glycerol [21], and at steady state Rd = Ra, these data suggest that the higher resting glycerol concentrations in females were because of higher Ra rates compared to males. This was accompanied by higher rates of lipid oxidation, suggestive that females were not only mobilizing, but oxidizing higher rates of fat than males. These data are congruent with published data [24], which reported that overnight fasting increased Ra to a greater extent in women

compared to men. Whether these differences in lipolysis rates by sex may be in part mediated by the difference in sex hormone milieu will require further study.

In contrast to sex, β_2 AR genetic variants had no significant influence on short-term fasting lipolytic rates. Basal concentrations of glycerol (Table 2), basal Ra values of glycerol, and basal Ra values normalized for anthropometric indexes were similar in RGE and CRQ diplotypes (Table 3). Thus, our data clearly demonstrate that sex is a more important determinant of basal lipolysis after a 12-hour fast than β_2 AR polymorphisms in healthy, nonobese individuals.

The mechanisms that underlie sex-related differences in basal glycerol Ra and in basal concentrations of glycerol after a short-term (12 hours) fast are not clear, but may be related to differences the action of hormones that regulate lipolysis. Catecholamines and insulin are the major regulators of lipolysis. Propranolol (nonselective β AR antagonist) did not influence basal lipolytic rates significantly after an overnight fast, but phentolamine (nonselective α adrenergic receptor antagonist) markedly increased glycerol concentrations in adipocyte dialysate, suggesting that changes in lipolytic rates observed after an overnight fast are not mediated by β ARs [28]. Circulating concentrations of insulin are important inhibitors of lipolysis [29]. Basal concentrations of insulin in males were, on average, almost twice as high compared to females (Fig. 1, Table 2). Because insulin is an important inhibitor of lipolysis, the higher baseline insulin concentrations in males probably contributed to lower Ra values in males compared to females. A similar conclusion was drawn by Mittendorfer et al [24]. It is also possible that sex differences in sensitivities to α adrenergic receptor stimulation may have also contributed to differences in basal lipolytic rates.

Terbutaline administration increased glycerol plasma concentration about 2-fold over mean basal concentrations in both males and females in a concentration-dependent fashion (Fig. 1). Because terbutaline is a selective β_2 agonist, it is likely that the increased Ra was mediated through β_2 ARs. These results are consistent with numerous studies showing that agonist activation of β_2 ARs stimulate lipolysis [28,30-32].

There was marked variation in the lipolytic response to β_2 AR stimulation by terbutaline as determined by AUC values, which ranged between 0 (3 study participants) to 25 000 μmol/(L min) (Fig. 3). The marked variability was not related to sex, haplotype (Fig. 3), or the magnitude of the β_2 AR stimulus as the terbutaline plasma concentration vs time profiles among study participants was similar. In addition, the variability in terbutaline-stimulated glycerol AUC values was not related to age, anthropometric indexes, insulin, or glucose concentrations.

The mechanisms underlying this variability in the whole body lipolysis to terbutaline are not clear. It is possible that other untyped β_2 AR SNPs could have contributed to the variability in terbutaline-stimulated Ra [17]. The β_2 AR gene is highly polymorphic, and although we genotyped 3 common SNPs and selected 2 common diplotypes in our study, it is possible that our study participants differed at other loci in the β_2 AR gene, and determination of a more complex haplotype might have revealed the source of the lipolytic variability. This explanation is, however, unlikely because other β_2 AR–mediated responses observed in our study participants did not vary as dramatically. If other, untyped β_2 AR SNPs were responsible for the variability in terbutaline-stimulated lipolysis, we would expect to observe similar variability in glucose concentrations (Fig. 1), potassium concentrations, and diastolic blood pressure [33]. There was relatively little variability in these β_2 AR–mediated responses. A more likely explanation may be that variability in genes encoding downstream proteins contributed to the variability in terbutaline-stimulated whole body lipolysis. For example, polymorphisms in the hormone-sensitive lipase gene have been reported to contribute to the variability in lipolysis [16].

The effects of β_2 AR genetic variants on lipolysis in human beings have not been extensively studied. One study explored the influence of common single β_2 AR SNPs on adipocyte lipolytic function from white, overweight women, and reported that the sensitivity of adipocyte lipolytic rates to terbutaline was 5-fold higher in Gly16 homozygotes compared to Arg16 homozygotes, with no differences in basal lipolytic rates [15]. In a recent study, the sensitivities of lipolytic rates to terbutaline in adipocytes from white, overweight women were markedly dependent on β_2 AR haplotypes consisting of 10 SNPs [17]. Homozygous haplotypes carrying the Cys−19/Gly16/Gln27 alleles had the highest sensitivity to terbutaline-stimulated lipolysis, followed by carriers of the homozygous Arg−19/Gly 16/Glu27 and Arg−19/Arg16/Gln27 haplotypes. Thus, in both in vitro studies, the sensitivity of lipolytic rates to terbutaline was 5- to 12-fold higher in Gly16 homozygotes compared to Arg16 homozygotes. These data would predict that terbutaline-stimulated whole body lipolysis rates in our study would be higher in Arg−19/Gly16/Glu27 homozygotes compared to Cys−19/Arg16/Gln27 homozygotes, which clearly is not the case (Fig. 3).

Several factors may be responsible for the inconsistencies between our results and those reported by others [15,17]. Lipolytic function was compared by genotype or haplotype in adipocytes collected from obese women [15,17], whereas our study participants were not obese. Catecholamine-stimulated whole body lipolysis and lipolysis in subcutaneous adipocytes are blunted in obesity [32,34-36], which has been associated with a reduction in the number of β_2 AR in adipocytes [37]. Common β_2 AR genetic variants have different sensitivities to agonist-promoted down-regulation and can regulate β_2 AR density in vivo [13,14,38,39]. Thus, obesity may selectively down-regulate certain β_2 AR variants, thereby causing genotype- or haplotype-dependent lipolytic responses to terbutaline, which would not be evident in non-obese individuals.

Finally, in contrast to whole body lipolysis, the study of terbutaline-stimulated lipolysis in adipocytes is not limited by the pharmacokinetics of terbutaline, and is unaffected by blood flow, hormonal, or other physiological factors. Although most whole body lipolysis occurs in adipocytes, in vitro and in vivo lipolytic rates are not correlated [1,2,40]. The measurement of whole body lipolytic rates by glycerol tracer techniques reflects a reasonable estimate of the rate of adipose tissue lipolysis [1]; hence, the results of our study more accurately reflect the contribution of β_2 AR genetic variation to lipolytic rates than do the results of in vitro studies.

In summary, our study demonstrated that sex but not β_2 AR polymorphisms contributed to the variability in basal whole body lipolysis. Terbutaline increased glycerol, glucose, and insulin concentrations, but the changes in terbutaline-stimulated glycerol concentrations were not related to sex or β_2 AR polymorphisms. We conclude that compared to β_2 AR genetic variants, sex is a more important determinant of fat mobilization in vivo in healthy, non-obese individuals.

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