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β_2-Adrenergic Receptor Polymorphisms and Salbutamol-Stimulated Energy Expenditure

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The β-adrenergic system is involved in the control of energy metabolism and expenditure. The β_2-adrenergic receptor (β_2-AR) gene shows polymorphisms that have been associated with obesity in several studies. *In vitro* and *in vivo* studies suggest differences in β_2-AR-mediated function between these polymorphisms. The aim of this study was to investigate the influence of genetic variation in codon 16 of the β_2-AR gene on energy metabolism in humans.

Thirty-four subjects were recruited [Gly16Gly (n = 13), Gly16Arg (n = 16), or Arg16Arg (n = 5)]. The β_2-AR was stimulated with two doses of salbutamol (50 and 100 ng/kg fat-free mass per minute) after blockade of the β_1-adrenergic receptors with atenolol. Energy expenditure and plasma substrate and hormone concentrations were measured.

The increase in energy expenditure (ΔEE) was significantly different among groups in which the Arg16Arg group showed the lowest increase ($P < 0.05$ *vs.* Gly carriers). In a multiple regression model, variations in the increase in nonesterified fatty acid concentration during salbutamol infusion (partial r = 0.51) and the polymorphism contributed significantly to the variation in ΔEE. Thirty-five percent of the variation in ΔEE was explained by these two factors.

We conclude that subjects with the Arg16Arg polymorphism of the β_2-AR gene have a reduced thermogenic response to β_2-adrenergic stimulation. Although this relatively small study needs confirmation, the findings support a role for this polymorphism in the development and maintenance of overweight and obesity. (*J Clin Endocrinol Metab* 90: 2301–2307, 2005)

IT IS WELL DOCUMENTED that both environmental and genetic factors are involved in the onset and progression of obesity in humans. Severe obesity appears to have a particularly strong genetic component and is polygenic in nature (1). An increasing number of polymorphisms is assumed to be associated with obesity and has been described in literature (1). Despite intense effort, the pathways underlying these associations with obesity remain elusive. This is largely due to the complexities circumventing the process of developing obesity, which include age of onset; polygenic inheritance; genetic heterogeneity; incomplete penetrance; unknown mode of action of disease alleles; effect of ethnicity, age, gender, and the interaction with environmental factors, such as diet, physical activity, or smoking status.

Of all contributing factors, the components of the sympathetic nervous system are of interest because this system, in particular its β-adrenergic component, is involved in the control of energy metabolism and expenditure (2, 3).

One of the receptor subtypes of the β-adrenergic system is the β_2-adrenergic receptor (β_2-AR). It is coupled to a stimulatory G protein that promotes cAMP production, activating protein kinase A, which mediates a variety of responses, depending on the cell type. For instance, the lipolytic and glycogenolytic effects of catecholamines are mediated through members of the β_2-AR family (2, 4). One study found that the β_2-mediated thermogenic and lipolytic responses are blunted in obese, compared with lean (5). In addition, several studies (6–13) have shown an association between β_2-AR polymorphisms and weight gain, obesity, or obesity-related phenotypes. Nine polymorphisms have been identified in the coding region of the human β_2-AR, three of which are nonsynonymous (14). The two most common variants are located at codon 16 (Arg16Gly) and codon 27 (Gln27Glu) and have been studied most in relation to obesity. Both polymorphisms result in variations at the amino-terminal site of the receptor.

In vitro studies have shown differences in agonist-promoted down-regulation (15) but no differences in agonist binding or agonist-stimulated adenylyl cyclase activities among these polymorphisms of the β_2-AR gene (16). Functional consequences of these polymorphisms with respect to adipocyte lipolysis have been reported (17, 18), which may play a role in obesity. So far, the role of the β_2-AR polymorphisms in human energy expenditure (EE) has not been studied.

We investigated whether the polymorphism at codon 16 of the β_2-AR gene is of functional importance in human energy metabolism by direct stimulation of this receptor with a β_2-AR agonist in an experimental setting.

Subjects and Methods

Subjects

Thirty-four healthy, normal-weight to moderately obese volunteers [body mass index (BMI) range 19.1–37.1 kg/m²] participated in this

study (32 men and two women, aged 45 ± 0.8 yr, BMI 27.8 ± 0.8 kg/m^2). In part (n = 14) they were recruited from an existing cohort that has been described previously (13), in which the polymorphisms in the β_2-AR were known; in part they were newly recruited by advertisement (n = 20). The subjects did not use medication. Physical characteristics of the subjects are summarized in Table 1.

Experimental design

One week before the experimental day, subjects came to the laboratory after an overnight fast, and body composition was determined by hydrostatic weighing with simultaneous lung volume measurements (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the equation of Siri (19).

Subjects were asked to refrain from strenuous exercise during 24 h before the experiment. After an overnight fast, subjects arrived at the laboratory by bus or car to minimize activity before the measurements. On arrival, a cannula was inserted into a forearm vein of each arm. One cannula was used for the infusion of drugs and the other cannula for the sampling of blood. Next, indirect calorimetry measurements were started with a ventilated hood system with the subject in recumbent position and continued for the remainder of the experiment.

The study consisted of three study periods of 45 min. Before the first period, subjects received a priming dose of 50 μg/kg fat-free mass (FFM) atenolol (β_1-adrenergic receptor antagonist) (Tenormin, Zeneca, Ridderkerk, The Netherlands) in 5 min, after which a continuous infusion of 1.2 μg/kg FFM per minute atenolol was started for the remainder of the experiment. After 45 min atenolol infusion, subjects additionally received consecutive infusions of 50 and 100 ng/kg FFM per minute salbutamol (Ventolin, GlaxoWellcome, Zeist, The Netherlands). Each infusion period lasted 45 min. After 30 and 45 min of each study period, a blood sample was taken.

Measurements

Whole-body EE and respiratory exchange ratio (RER) were measured by indirect calorimetry, using an open-circuit ventilated hood system. In the study a homemade system, which is based on the analysis system for respiration chambers, which has been described previously (20), was used. The volume of air drawn through the hood was measured by a dry-gas meter (Schlumberger, Dordrecht, The Netherlands), and the composition of the in- and outflowing air was analyzed by a paramagnetic O_2 analyzer (Servomex, Crowborough, UK) and an infrared CO_2 analyzer (Hartmann and Braun, Frankfurt, Germany). The airflow rate and the O_2 and CO_2 concentrations of the in- and outflowing air were used to compute O_2 consumption and CO_2 production online through an automatic acquisition system connected to a personal computer. EE was calculated according to the formula proposed by Weir (21). EE and RER values were averaged over the last 15 min of each period. The reproducibility of the measurement was within 5% as described before (22).

TABLE 1. Subject characteristics of groups with different polymorphisms at codon 16 of the β_2-adrenergic receptor gene (mean $\pm$ SEM)

	Gly16Gly (n = 13)	Gly16Arg (n = 16)	Arg16Arg (n = 5)
Gender (M/F)	12/1	16/0	4/1
Weight (kg)	83.6 ± 4.3	87.0 ± 3.5	86.5 ± 12.9
Height (m)	1.73 ± 0.03	1.78 ± 0.02	1.74 ± 0.05
Age (yr)	46.9 ± 1.4	45.5 ± 1.3	41.5 ± 1.1
Fat percentage (%)	28.0 ± 2.6	27.4 ± 1.8	35.0 ± 1.1
Fat mass (kg)	23.9 ± 2.7	24.6 ± 2.4	30.7 ± 5.1
FFM (kg)	59.6 ± 3.0	62.4 ± 1.7	55.9 ± 7.8
BMI (kg/m^2)	27.8 ± 1.2	27.7 ± 1.3	28.1 ± 3.0
HOMA index	2.25 ± 0.52	2.15 ± 0.33	$4.90 \pm 1.84^{a,b}$

[a] Significantly different between Arg16Arg and Gly16Arg ($P < 0.05$).
[b] Tendency for difference between Arg16Arg and Gly16Gly ($P = 0.06$).

Total carbohydrate and fat oxidation were calculated using stoichiometric equations (23):

Total fat oxidation (grams per minute) = 1.695 VO$_2$ (liters per minute) − 1.701 VCO$_2$ (liters per minute).

Total carbohydrate oxidation (grams per minute) = 4.585 VCO$_2$ (liters per minute) − 3.226 VO$_2$ (liters per minute).

Substrate oxidation was expressed as percentage of the contribution to total EE (1 g fat = 37.8 kJ and 1 g carbohydrate = 16.9 kJ).

Heart rate was monitored by conventional electrocardiography. Blood pressure was measured three times by an automated blood pressure device (OMRON 705CP, Hamburg, Germany) during the last 5 min of each study period. The means of these three measurements were used for further analysis.

Analytical methods

Genomic DNA of the subjects was extracted from leukocytes by digestion with proteinase K followed by phenol/chloroform extraction. Determination of the polymorphism was performed using a PCR-restriction fragment length polymorphism analysis as described before (18).

Blood samples for the determination of nonesterified fatty acids (NE-FAs), glycerol, glucose, and insulin were preserved with sodium-EDTA and those for norepinephrine and epinephrine with heparin plus glutathione (1.5% wt/vol). Blood samples were immediately centrifuged for 10 min at $800 \times g$ at 4 C. Plasma was rapidly frozen in liquid nitrogen and stored at -70 C until further analysis. Plasma NEFA concentration was measured with the NEFA C kit (99475409, Wako, Neuss, Germany), plasma glycerol concentration was measured with a glycerol kit (148270, Boehringer, Mannheim, Germany), and plasma glucose concentration was measured with a glucose kit (UniKit III, 07367204, Roche, Basel, Switzerland), all on a Cobas Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Plasma insulin level was determined with a double-antibody RIA (Insulin RIA 100, Pharmacia, Uppsala, Sweden). The homeostasis model assessment (HOMA) index was calculated according to Matthews *et al.* (24) using baseline plasma glucose and baseline plasma insulin levels. Plasma norepinephrine and epinephrine levels were determined by HPLC with electrochemical detection according to the method of Alberts *et al.* (25) using a ClinPrep kit (Recipe, Munich, Germany).

Data analysis

All data are presented as mean $\pm$ SEM. Data for EE were adjusted for FFM for group comparison (26). The change (Δ) in EE induced by the highest dose of salbutamol (100 ng/kg FFM per minute) was calculated by subtracting baseline EE from the EE at 100 ng/kg FFM per minute salbutamol. Changes in the other variables were calculated the same way.

One-way ANOVA with repeated measurements was used to analyze the effects of salbutamol administration in the total group of subjects and the three polymorphism groups. Differences among the codon 16 polymorphism groups at baseline and in response to salbutamol administration were analyzed by one-way ANOVA. *Post hoc* pairwise comparisons were made using Bonferroni correction.

Simple regression analysis was performed with ΔEE as the dependent variable and different parameters as independent variables (see Table 3). Multiple regression analysis was conducted to estimate the independent contributions of the variables associated with ΔEE. All variables that were correlated with ΔEE in the simple regression analysis with a $P < 0.20$ were included in the analysis. In addition, the polymorphism groups were entered as dummy variables into the multiple regression model. $P < 0.05$ was considered to be statistically significant.

Results

Responses to salbutamol infusion in the whole group

Table 2 shows changes in all parameters studied at baseline and during administration of increasing doses of salbutamol for the whole group of subjects. EE, heart rate, plasma norepinephrine, and NEFA and glycerol concentrations

TABLE 2. Metabolic parameters at different doses of salbutamol for the whole group (n = 34)

Salbutamol (ng/kg FFM · min)	0	50	100	*P* value (ANOVA)
Energy expenditure (kJ/min)	4.8 ± 0.1	5.1 ± 0.1	5.3 ± 0.1	0.037
RER	0.83 ± 0.01	0.85 ± 0.01	0.82 ± 0.01	0.183
Fat oxidation (% of total energy expenditure)	53.5 ± 2.2	48.5 ± 2.8	55.6 ± 3.2	0.179
Carbohydrate oxidation (% of total energy expenditure)	47.9 ± 2.8	53.6 ± 3.2	45.7 ± 3.5	0.181
Heart rate (beats/min)	55.9 ± 1.2	62.8 ± 1.2	71.2 ± 1.4	<0.001
Diastolic blood pressure (mm Hg)	83.9 ± 1.8	81.7 ± 1.7	78.2 ± 1.7	0.075
Systolic blood pressure (mm Hg)	118.1 ± 2.0	118.9 ± 1.9	121.1 ± 1.9	0.532
[Norepinephrine] (ng/liter)	456.0 ± 20.6	513.3 ± 33.2	579 ± 32.7	0.014
[Epinephrine] (ng/liter)	33.9 ± 3.5	24.1 ± 2.5	21.8 ± 2.1	0.006
[NEFA] (μmol/liter)	366.6 ± 21.1	580.2 ± 30.8	724.9 ± 42.1	<0.001
[Glycerol] (μmol/liter)	58.5 ± 3.7	84.3 ± 5.2	106.8 ± 7.2	<0.001
[Glucose] (mmol/liter)	5.40 ± 0.12	5.36 ± 0.10	5.49 ± 0.10	0.656
[Lactate] (mmol/liter)	0.97 ± 0.06	1.00 ± 0.05	1.15 ± 0.05	0.055
[Insulin] (mU/liter)	10.1 ± 1.3	13.5 ± 1.8	15.2 ± 1.6	0.075

Data represent mean ± SEM.

showed statistically significant increases (at least *P* < 0.05). Plasma epinephrine concentrations were significantly lowered during salbutamol infusion (*P* < 0.01). No statistically significant changes in RER, blood pressure (BP), plasma glucose, lactate, and insulin were found.

Baseline values and responses to salbutamol infusion in the β₂-AR codon 16 polymorphism groups

Baseline EE adjusted for FFM was similar in all polymorphism groups (4.89 ± 0.14, 4.89 ± 0.06, and 4.92 ± 0.23 kJ/min for Gly16Gly, Gly16Arg, and Arg16Arg, respectively, NS). There was a significant difference in the increase in EE (ΔEE) among groups (ANOVA, *P* < 0.05) (Fig. 1). *Post hoc* analysis showed that the response was significantly different between the Arg16Arg and the Gly16Arg groups (*P* < 0.05) and between Arg16Arg and Gly carriers (*P* < 0.05).

At baseline RER did not differ among groups, nor did ΔRER (*P* = 0.41) (Fig. 2). Fat oxidation at baseline was not significantly different among groups (54.4 ± 3.4, 55.7 ± 3.2, and 44.6 ± 6.3% of total EE for Gly16Gly, Gly16Arg, and Arg16Arg, respectively, *P* = 0.25) nor was carbohydrate oxidation (47.0 ± 3.9, 45.6 ± 3.9, and 57.9 ± 7.0% of total EE for Gly16Gly, Gly16Arg, and Arg16Arg, respectively, *P* = 0.25). There was no difference in change of fat and carbohydrate oxidation (as percentage of total EE) in response to salbutamol among groups (ANOVA, both *P* = 0.43).

Plasma concentrations of NEFAs, glycerol, glucose, insulin, epinephrine, and norepinephrine did not differ significantly among groups at baseline nor during salbutamol infusion. The HOMA index, however, was significantly different among groups (ANOVA, *P* < 0.05), in which the Arg16Arg group showed the highest HOMA index.

During salbutamol infusion, plasma NEFA levels (Fig. 3) and norepinephrine levels (Fig. 4) increased significantly in all polymorphism groups (*P* < 0.05), with no significant difference among groups. Plasma concentrations of glycerol, glucose, insulin, and epinephrine changed significantly in the Gly16Gly and Gly16Arg groups (*P* < 0.01) but not in the Arg16Arg group; however, the responses to salbutamol infusion did not differ among groups. Plasma lactate levels were significantly higher at baseline and during salbutamol in the Arg16Arg group, compared with the Gly carriers (ANOVA, *P* < 0.05), but the lactate response to salbutamol infusion did not differ among groups (Fig. 3).
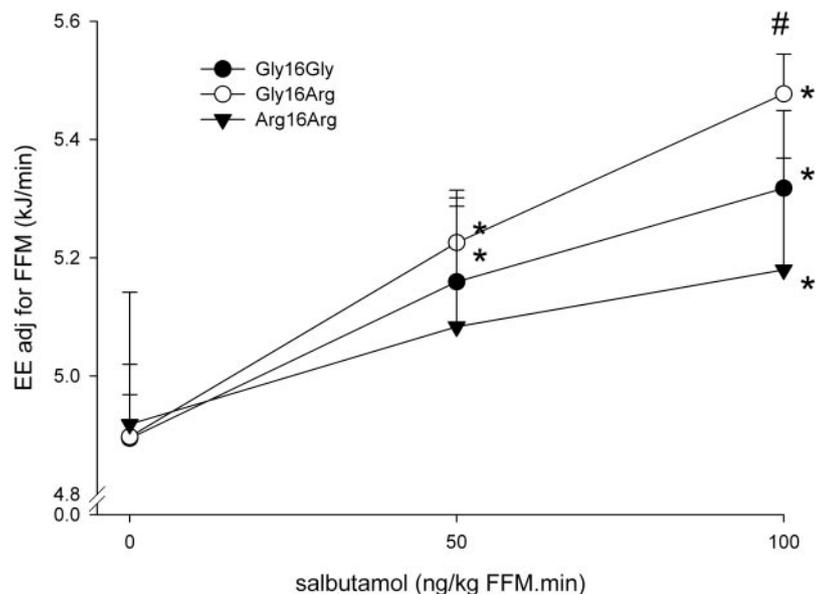

FIG. 1. EE adjusted for FFM for the codon 16 polymorphisms in the β₂-AR at baseline and during infusion of salbutamol. *, *P* < 0.05 *vs.* baseline; #, *P* < 0.05 Arg16Arg *vs.* Gly carriers.
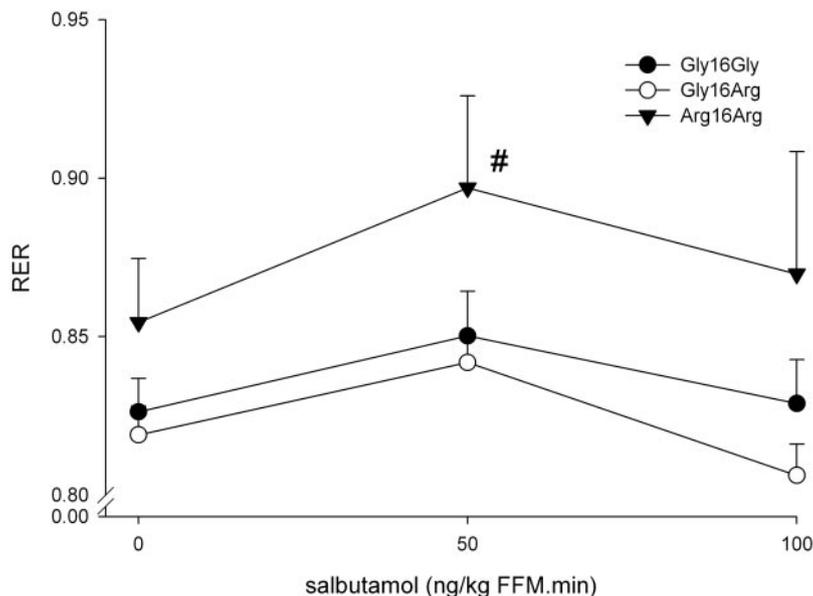
FIG. 2. RER for the codon 16 polymorphisms in the β2-AR at baseline and during infusion of salbutamol. #, $P < 0.05$ Arg16Arg *vs.* Gly carriers.

Baseline values for heart rate and systolic and diastolic BP were not significantly different among polymorphism groups. Systolic BP increased in the Gly16Gly and Arg16Arg groups ($P < 0.05$) but not in the Gly16Arg group ($P = 0.54$). Diastolic BP significantly decreased in the Gly16Gly and Gly16Arg groups ($P < 0.05$), but the decrease did not reach statistical significance in the Arg16Arg group ($P = 0.08$). However, the changes in heart rate and systolic and diastolic BP during salbutamol infusion did not differ significantly among groups (data not shown).

Factors contributing to the EE response to salbutamol infusion

Simple regression analysis with ΔEE as the dependent variable showed statistically significant correlations with basal NEFA and insulin concentration, ΔNEFA, Δglycerol, HOMA index, and ΔRER (Table 3). Multiple stepwise regression analysis with ΔEE as the dependent variable and basal plasma NEFA and insulin concentration, ΔNEFA, HOMA index, ΔRER, age, and codon 16 polymorphism as independent variables was performed subsequently. Because glycerol and NEFAs are both parameters of lipolysis and are highly correlated ($R^2 = 0.838$), only basal NEFAs and ΔNEFAs and not basal glycerol and Δglycerol were used in the multiple regression analysis. The analysis indicated statistically significant associations of ΔNEFAs and the codon 16 polymorphism with ΔEE. No significant association with the other variables was found. The partial correlation coefficient for ΔNEFAs was 0.51. The polymorphism added another 10% to the explained variance in ΔEE. The whole model therefore explained 35% of the variation in ΔEE (adjusted $R^2 = 0.354$, $P < 0.001$). Introducing basal and Δglycerol instead of basal and ΔNEFAs into the model gave similar results, ΔNEFAs being replaced by Δglycerol.

Discussion

The aim of the present study was to examine the influence of polymorphisms in codon 16 of the β2-AR gene on EE and

substrate oxidation during β2-AR stimulation by infusing the β2-AR agonist salbutamol. During salbutamol infusion subjects with the Arg16Arg variant of the β2-AR gene showed a blunted increase in EE, and their plasma lactate levels were higher. Fat oxidation as percentage of total EE and plasma NEFA and glycerol levels did not differ among groups, nor was there a difference in response to salbutamol. Multiple regression analysis showed that the polymorphism in codon 16 of the β2-AR gene and plasma NEFA or glycerol change but not RER change, percent body fat, BMI, age, and HOMA index were significantly associated with the change in EE.

The combined infusion of salbutamol and atenolol selectively stimulates the β2-AR, as shown previously (27). This selective stimulation resulted in the expected increases in EE, heart rate, plasma glycerol, NEFAs, lactate, insulin, and norepinephrine concentrations and in reductions in plasma epinephrine levels and diastolic BP (27, 28). There was no clear change of RER during salbutamol infusion in this study. However, changes in RER have been inconsistent in previous studies (27, 28).

The β2-AR gene contains the genetic code for the receptor protein. Several variants in this gene have been described in literature (14). Three of the single-nucleotide polymorphisms, at nucleotides 46, 79, and 491, lead to amino acid substitution at codons 16, 27, and 164 in the coding region of the gene and might therefore have consequences for receptor function (29). When expressed in cells, the receptors with polymorphisms at codons 16 and 27 have been associated with differences in cellular β2-AR trafficking (30). The more rare polymorphism at codon 164 is associated with depressed functional coupling to Gs and reduced β2-adrenergic receptor sequestration (31). Large *et al.* (18) showed that isolated abdominal sc fat cells from women homozygous for the Arg16 polymorphism of the β2-AR had a 5-fold lower sensitivity for β2-AR agonist-induced lipolysis than fat cells from women heterozygous or homozygous for Gly16, independent of percent body fat of the women. More recently it was shown that fat cells from subjects with different ho-
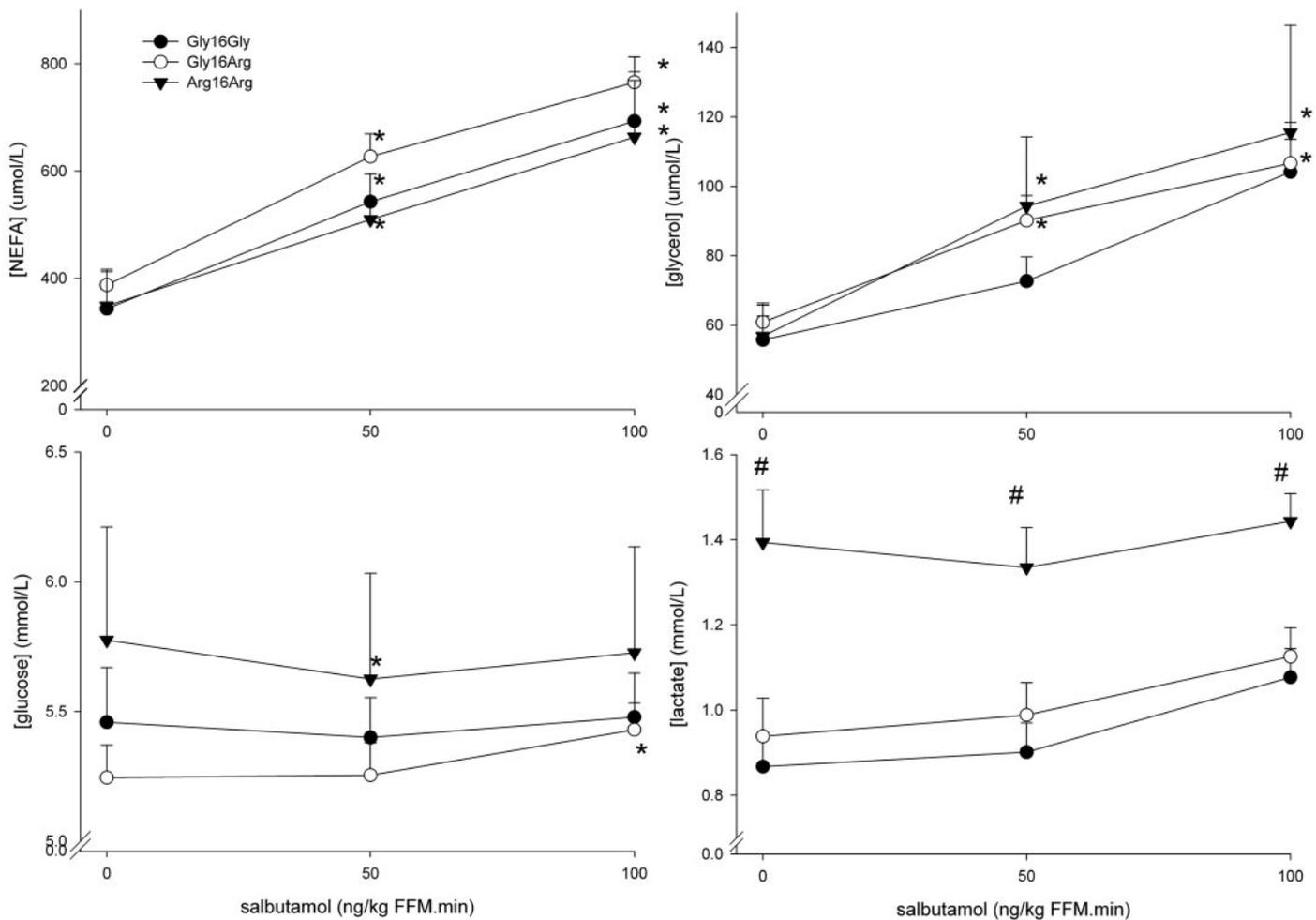
FIG. 3. Plasma NEFA concentration (micromoles per liter), plasma glycerol concentration (micromoles per liter), plasma glucose concentration (millimoles per liter), and plasma lactate concentration (millimoles per liter) for the codon 16 polymorphisms in the β_2-AR at baseline and during infusion of salbutamol. *, $P < 0.05$ *vs.* baseline; #, $P < 0.05$ Arg16Arg *vs.* Gly carriers.

mozygous haplotypes of the β_2-AR gene differed about 250-fold in sensitivity to terbutaline-induced lipolysis (17). The least sensitive homozygous haplotype group in this study contained the Arg variant at codon 16 (17).

This study extends these findings and demonstrates a reduced β_2-AR agonist-induced thermogenic response in individuals homozygous for the Arg16 polymorphism of the β_2-AR gene. On the basis of changes in plasma NEFA and glycerol concentrations, we could not confirm the reduced lipolytic response to β_2-AR stimulation in the Arg16Arg group, which has been reported by Large *et al.* (18) based on *in vitro* lipolysis. This might be related to the limitations of plasma NEFA concentration as a measure of lipolysis because not only lipolysis but also reesterification and NEFA oxidation affect plasma NEFA concentrations. The higher RER and plasma lactate concentrations at baseline as well as during salbutamol infusion in the Arg16Arg group suggest that their energy production relies more on carbohydrate oxidation, which would be compatible with a reduced lipolysis.

This study shows that the codon16 polymorphism has a significant effect on the salbutamol-induced thermogenic response, independent of the change in NEFAs. The explana-

tion for this effect is not directly apparent from our study. It could be that the β_2-AR-stimulated muscle and liver glycogen breakdown is also affected by the polymorphism, thus resulting in differences in carbohydrate substrate availability and oxidation (4).

It is not surprising that the study revealed a correlation between the change in NEFAs and the thermogenic response because we and others have previously shown an increase in EE during the infusion of a triglyceride emulsion, which elevates plasma NEFA levels (28, 32–34), although this is not found in all studies (35, 36), which may be related to differences in patient populations and study design. In addition, part of the increase in EE induced by dobutamine, a β_1-adrenergic agonist, can be blocked by inhibiting lipolysis with nicotinic acid (37).

Previous studies have suggested that the β_2-AR is involved in the reduced β-AR-mediated increase in lipolysis (5, 38, 39), lipid oxidation, and thermogenesis (5) in obese compared with lean individuals. This study suggests that variations in the lipolytic response to β_2-adrenergic stimulation are associated with variations in the thermogenic response. BMI and fat percentage were negatively correlated with ΔNEFA in our study ($P < 0.01$ and $P = 0.06$, respectively),
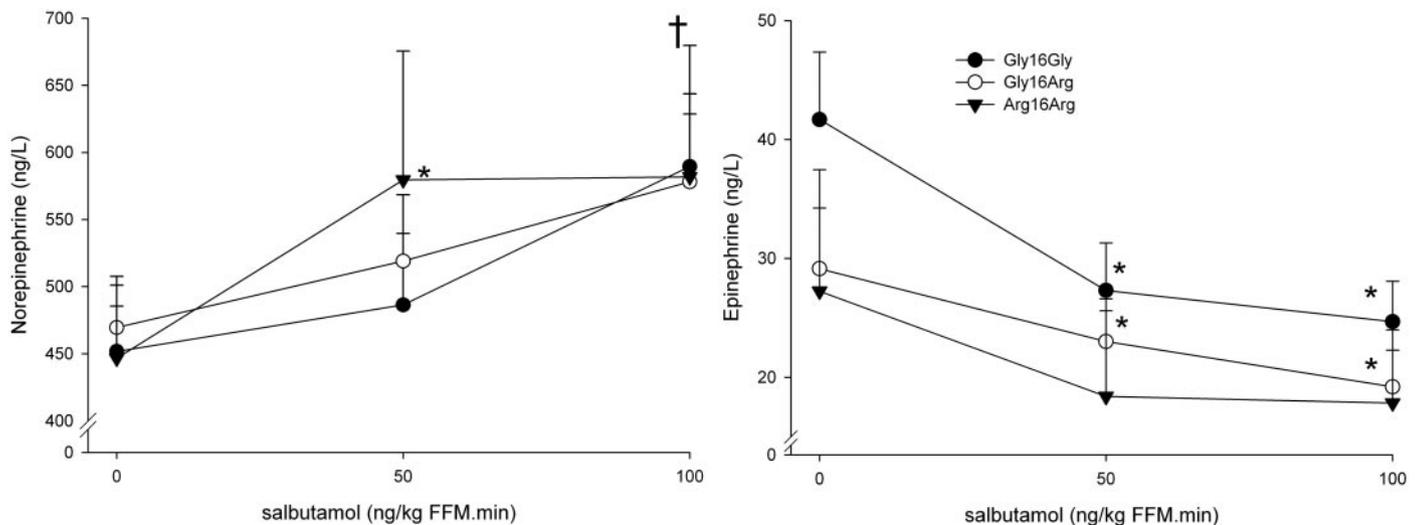
FIG. 4. Plasma norepinephrine (nanograms per liter) and epinephrine (nanograms per liter) concentrations for the codon 16 polymorphisms in the β_2-AR at baseline and during infusion of salbutamol. *, $P < 0.05$ *vs.* baseline; †, $P < 0.05$ *vs.* baseline for all three groups.

which supports these earlier findings. Whether the association between these variations in β_2-AR-mediated ΔNEFA and the variation in body fat are completely independent of the codon 16 polymorphism remains to be determined, but as discussed above, no association between the polymorphism and the response of plasma NEFAs or glycerol was found in this study. Other factors that might have influenced the lipolytic response are age, insulin sensitivity, α_2-adrenergic receptor sensitivity and other polymorphisms in the β_2-AR gene or other genes associated with lipolysis. Aging is known to reduce β-adrenergic sensitivity (40). The Arg16Arg group tended to be slightly, although not significantly ($P = 0.11$), younger than the other two groups. This difference in age would only tend to reduce the difference in responsiveness between the polymorphism groups. It is therefore unlikely that the differences in thermogenic sensitivity among groups are due to age differences. HOMA index as a measure for insulin sensitivity was different among polymorphism groups (Table 1) and correlated with ΔEE but did not significantly contribute to the multiple regression model. As to be expected, the HOMA index was also associated with fat mass.

Subjects carrying the Arg16Arg polymorphism were all

carriers of the Gln27Gln polymorphism of the β_2-AR gene. Of the Gly16Gly carriers, one subject had the Gln27Gln polymorphism and seven had the Gln27Glu polymorphism. Subjects with the Arg16Gly polymorphism were either Gln27Gln (n = 7) or Gln27Glu (n = 9). Limiting the analysis to the Gln27 homozygotes (n = 13) revealed the same difference in thermogenic response between Gly16 carriers (n = 8) and Arg16Arg carriers (n = 5) as in the total group. It is therefore unlikely that the polymorphism at codon 27 was responsible for the differences in thermogenic response between the Arg16Arg subjects and the Gly16 carriers.

We conclude that subjects with the Arg16Arg polymorphism of the β_2-AR have a reduced thermogenic response to β_2-adrenergic stimulation. Although this relatively small study needs confirmation, the findings support a role for this polymorphism in the development and maintenance of overweight and obesity, as suggested by association studies (12, 17, 18, 41).

TABLE 3. Pearson correlations with ΔEE for the whole group (n = 34)

Variables	Correlation	P value
Weight	-0.1	0.572
Fat percentage	-0.122	0.491
Fat mass	-0.117	0.512
FFM	-0.05	0.777
BMI	-0.181	0.305
Basal [NEFA]	0.397	0.022
Basal [glycerol]	0.302	0.093
Basal [insulin]	-0.408	0.025
Δ RER	-0.392	0.022
Δ [NEFA]	0.508	0.003
Δ [glycerol]	0.435	0.013
Δ [insulin]	0.224	0.234
HOMA index	-0.425	0.019
Age	0.241	0.170

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