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Blunted β-adrenoceptor–mediated fat oxidation in overweight subjects: a role for the hormone-sensitive lipase gene

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Abstract

Obesity is associated with blunted β-adrenoceptor–mediated lipolysis and fat oxidation, which persist after weight reduction. We investigated whether dinucleotide $(CA)_n$ repeat polymorphisms in intron 6 (i6) or 7 (i7) and a C-60G promoter substitution of the hormone-sensitive lipase (HSL) gene are associated with a blunted in vivo β-adrenoceptor–mediated increase in circulating fatty acids and glycerol (estimation of lipolytic response) and fat oxidation in overweight-obese subjects. A total of 103 overweight (25 kg/m^2 ≤ body mass index < 30 kg/m^2) and obese (body mass index ≥30 kg/m^2) subjects (62 men, 41 women) were included. Energy expenditure, respiratory quotient (RQ), and circulating fatty acid and glycerol were determined after stepwise infusion of increasing doses of the nonselective β-agonist isoprenaline. The i6, i7 $(CA)_n$ repeat polymorphisms were determined by size-resolved capillary electrophoresis; and a C-60G promoter substitution was determined by restriction enzyme digestion assay. Female noncarriers of allele 184 i7 (n = 18) and female carriers of allele 240 i6 (n = 12) showed an overall reduced fat oxidation (as indicated by changes in RQ) after β-adrenoceptor–mediated stimulation, explaining, respectively, 6.9% and 20.8% of the variance in RQ. These effects were not seen in male subjects. In conclusion, our results suggest that variation in i7 and i6 of the HSL gene might be associated with a physiological effect on in vivo β-adrenoceptor–mediated fat oxidation, at least in overweight-obese female subjects.

1. Introduction

Obesity is characterized by excess fat accumulation, mainly in adipose tissue. Studies in our laboratory showed a blunted lipolytic response and blunted fat oxidation after β-adrenergic stimulation or exercise in obese and obese type 2 diabetes mellitus subjects [1-4]. These blunted β-adrenoceptor–mediated lipolysis and fat oxidation persisted even after weight reduction, indicating that these disturbances may be a primary factor in the development or maintenance of increased adipose stores [1,5]. There are indications that the blunted β-adrenoceptor–mediated lipolysis in adipose tissue may be related to a decreased function or number of β_2-adrenoceptors [6,7]. In addition, in vitro studies in adipocytes from first-degree relatives of obese subjects and adipocytes from elderly male subjects with several manifestations of the metabolic syndrome also indicate alterations at the level of the protein kinase A/hormone-sensitive lipase (HSL) complex [7,8]. Hormone-sensitive lipase catalyzes the hydrolysis of triglycerides and diglycerides. Recently, a new lipase (adipose triglyceride lipase) was identified preferentially hydrolyzing triglycerides [9]. Nevertheless, HSL seems to be the major lipase for catecholamine-stimulated lipolysis in humans [10]. Although environmental factors contribute, it is obvious that genetic factors play an important role in the etiology of obesity [8]. On the basis of the findings described above, the hormone-sensitive lipase (HSL/LIPE) gene may be of particular interest.

The human gene encoding HSL is located on the long arm of chromosome 19 (q13.1→13.2) [11,12]. The exon-intron organization comprises 9 main coding exons, spanning approximately 11 kilobases. A C-60G substitution in the HSL gene promoter region is associated with a 40% reduced

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promoter activity [13], an increased insulin sensitivity in women, and a decreased level of plasma free fatty acids (FFA) in men [14]. Moreover, this polymorphism is protective against insulin resistance in healthy young male subjects [13,15]. In addition, a dinucleotide $(CA)_n$ repeat polymorphism located in intron 7 (i7) of the HSL gene has shown to be in linkage disequilibrium (LD) with a gene increasing susceptibility to abdominal obesity and thereby possibly to type 2 diabetes mellitus [16]. Furthermore, a $(CA)_n$ repeat polymorphism in intron 6 (i6) of the HSL gene is associated with a decreased in vitro lipolytic rate in abdominal subcutaneous fat cells, being more pronounced in men than in women [17]. Moreover, the i6 polymorphism has shown to be a risk factor for body fat accumulation in women [18]. Magré et al [19] indicated an increased allelic frequency of this polymorphism in obese, impaired glucose-tolerant, or type 2 diabetes mellitus subjects compared with controls. So far, no data are available on the relationship between these polymorphic markers and in vivo lipolysis and fat oxidation in overweight and obese subjects.

The aim of this study was to determine if variation in dinucleotide $(CA)_n$ repeats in i6 and i7 or a C-60G substitution in the promoter region of the HSL gene is associated with a blunted in vivo β-adrenoceptor–mediated increase in circulating fatty acid and glycerol concentrations and fat oxidation during stimulation with the nonselective β-agonist isoprenaline (ISO) in overweight and obese subjects.

2. Subjects and methods

2.1. Subjects

The study group consisted of 103 overweight-obese subjects, 62 men and 41 women. *Overweight* and *obesity* were defined according to the following criteria: 25 kg/m^2 ≤ body mass index (BMI) < 30 kg/m^2 for overweight and BMI ≥30 kg/m^2 for obese subjects. Clinical characteristics of the subjects are shown in Table 1. All subjects were recruited by means of an advertisement in a local newspaper. All subjects were in good health as assessed by medical history and physical examination and were not taking any medication. Women were premenopausal, and some were taking oral anticonceptives. Normal resting electrocardiogram (ECG) result and normal blood pressure were prerequisites for participation. The study protocol was reviewed and approved by the Medical Ethical Review Committee of Maastricht University. The subjects were informed in detail about the investigation, and their consent was obtained before participating in the study.

2.2. Body composition

Body weight was determined on an electronic scale accurate to 0.1 kg. Waist and hip circumference measurements to the nearest 1 cm were made with subjects standing upright. Waist-to-hip ratio (WHR) was calculated as waist divided by hip circumference. Body mass index was calculated as body weight in kilograms divided by squared height in meters. Body density obtained by underwater weighing with residual pulmonary volume measurement by the helium dilution method (Volugraph 2000; Mijnhardt, Bunnik, The Netherlands) was converted to percentage body fat (%BF) using the equation of Siri [20]. Fat mass and fat-free mass (FFM) were calculated from the %BF and body weight.

2.3. Experimental design

Three days before participating in the study, subjects were asked to maintain a standardized diet and physical activity level. At the day of the experiment, subjects arrived at the laboratory at 8 AM by car or public transport after an overnight fast (of at least 12 hours). Subjects were studied while resting supine on a comfortable bed in a room kept at 23°C to 25°C. At the beginning of the experiment, a catheter was inserted into a forearm vein for blood sampling. A second catheter was inserted into the contralateral arm for infusion of the nonselective β-adrenergic agonist ISO. After placement of the catheters, the subjects were placed under an

Table 1
Clinical characteristics of the subjects

	Female (n = 41)		Male (n = 62)	
	25 ≤ BMI <30	BMI ≥30	25 ≤ BMI <30	BMI ≥30
n	11	30	13	49
Age (y)	37 ± 3	38 ± 1	47 ± 2	42 ± 1 †
BMI (kg/m^2)	28.6 ± 0.4	33.9 ± 0.6 *	28.9 ± 0.2	33.8 ± 0.5 *
%BF	38.2 ± 1.0	44.1 ± 0.8 *	28.3 ± 1.4	32.8 ± 0.8 *,†
WHR	0.84 ± 0.01	0.88 ± 0.02	1.04 ± 0.02	1.04 ± 0.01 †
EE rest [a] (kJ/min)	4.82 ± 0.16	4.94 ± 0.09	6.00 ± 0.19	6.21 ± 0.09
RQ rest	0.83 ± 0.2	0.82 ± 0.01	0.81 ± 0.01	0.81 ± 0.01
FFA rest (μmol/L)	669 ± 71	584 ± 35	475 ± 52	480 ± 33 †
Glycerol rest (μmol/L)	63 ± 14	79 ± 8	69 ± 9	76 ± 3

[a] Adjusted mean for FFM.
* $P < .05$, overweight vs obese.
† $P < .05$, male vs female.

open-circuit ventilated hood system. Energy expenditure (EE) was calculated using the equation of Weir [21], and respiratory quotient (RQ) was calculated as CO_2 production divided by O_2 consumption. After a 30-minute resting period, blood was sampled for baseline measurements and genotyping. After the 30-minute baseline period, ISO infusion started at increasing concentrations of 6, 12, and 24 ng per kilogram of FFM per minute, each dose for 30 minutes. At the end of each infusion period, blood samples were taken and immediately put into liquid nitrogen until further analysis. During ISO infusion, heart rate was kept under close observation by means of an ECG. The ISO infusion was stopped for some subjects, at different concentrations, based on an increase in heart rate by more than 30 beats per minute or in case of an irregular ECG result.

2.4. Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by digestion with protein K followed by purification with QIAamp DNA blood mini kit (Qiagen, Benelux BV, Venlo, The Netherlands). Isolated DNA was examined for variable lengths of $(CA)_n$ repeats in i6 and i7 of the HSL gene, and a C-60G polymorphism in the promoter region was investigated.

Polymerase chain reaction (PCR) amplification of the DNA segment containing i6 or i7 of the HSL gene was carried out as described previously [16,19]. The i6 fragment was amplified using the forward (sense) primer 5′-CTCAGCAGGGAAACAGGACTG-3′ and backward (antisense) primer 5′-GTTTGAGCCACTGCACTCAGC-3′. The i7 fragment was amplified using the forward (sense) primer 5′-CAAAACTGCACCTAATCTTCCC-3′ and reverse (antisense) primer 5′-GTAGGCTGTGTTTCCCCAGACT-3′. A negative control without DNA was performed every amplification run. Amplified products were size resolved by capillary electrophoresis on an ABI-310 PRISM genetic analyzer (Applied Biosystems, Foster City, CA) using performance-optimized polymer 4 (Applied Biosystems). Alleles were designated according to the size of the PCR product (Genescan Analysis 2.0, Applied Biosystems).

The PCR amplification of the C-60G polymorphism of the HSL gene promoter was carried out as described previously [14]. The forward (sense) primer was 5′-GAGGGAGGAGGGGCTATGGGT-3′, and the reverse (antisense) primer was 5′-TCCCTGGGCTGGGACTACTGG-3′.

2.5. Biochemical analysis

Whole blood was collected in tubes containing EDTA and centrifuged for 10 minutes at 3000 rpm (4°C). Plasma was removed for the enzymatic calorimetric quantization of FFA (WAKO Chemicals, Nuess, Germany) and glycerol (Boehringer Mannheim, Mannheim, Germany) on a COBAS FARA centrifugal spectrometer (Roche Diagnostica, Basel, Switzerland).

2.6. Statistical analysis

All statistical calculations were performed with SPSS for Macintosh (version 10.0; SPSS, Chicago, IL). The distribution of each variable was tested using the 1-sample Kolmogorov-Smirnov test. Because of the relatively small sample size and high standard deviation (SD) of variables, nonparametric statistics were used to analyze the data set. Sex differences in clinical parameters were tested by Mann-Whitney U nonparametric statistics. A χ^2 test was performed to test sex difference in allele frequency distribution after pooling frequencies less than 5%. The goodness of fit between observed and expected (under Hardy-Weinberg equilibrium) genotype frequency was statistically tested using the χ^2 test [22]. Energy expenditure was adjusted for FFM using analysis of covariance. The Kruskal-Wallis test, the nonparametric version of the 2-way analysis of variance, was used to observe the overall trend in fat oxidation and circulating fatty acid and glycerol concentrations between allele carriers and noncarriers during β-adrenergic stimulation. A P value less than 0.05 was considered statistically significant. All data are presented as mean ± standard error of the mean (SEM).

2.7. Power calculation

There are little or no published studies regarding the effect of the HSL gene on whole-body lipolysis and fat oxidation, although the results of in vitro studies in human adipocytes support a major effect. We therefore estimated our sample size using published data of the effect of β-adrenergic stimulation on whole-body lipolysis and fat oxidation [4,23,24]. Power calculation indicated that to detect a difference in circulating fatty acids of 100 μmol/L (SD of 50 μmol/L), circulating glycerol of 50 μmol/L (SD of 25 μmol/L), or RQ of 0.01 (SD of 0.005) and a power of 0.80 ($\alpha = .05$ and $\beta = .20$), the number of subjects in each group (2-tailed) should be 16.

3. Results

Clinical characteristics of the subjects are shown in Table 1. Body mass index, fasting RQ, and fasting glycerol were not significantly different between sexes. Because regression analysis with sex as a covariate showed significant allele × sex interaction, we stratified our sample by sex for subsequent analysis. Women (n = 41) had significantly higher %BF and fat mass and lower WHR compared with men (n = 62).

3.1. Frequency distribution

Frequency distributions, shown in Table 2, were comparable with other obese white populations [14,16,19]. We identified 12 alleles for the $(CA)_n$ repeat in i7 of the HSL gene, ranging in size from 166 to 190 base pairs (bp). Alleles 180, 182, and 184 were found to be the most common in both

Table 2
Allele frequency distribution of the different dinucleotide repeat polymorphisms in i7 or i6 of the HSL gene and the genotype frequency distribution of the C-60G substitution in the HSL gene promoter region

	Female	Male
CA repeat i7 (bp)	n = 40	n = 61
166	–	1.6
170	1.3	0.8
172	7.5	8.2
174	1.3	0.8
176	1.3	1.6
178	1.3	2.5
180	8.8	18.9
182	31.3	29.5
184	35.0	30.3
186	7.5	4.1
188	3.8	1.6
190	1.3	–
CA repeat i6 (bp)	n = 38	n = 55
222	1.3	0.9
230	1.3	4.5
232	–	0.9
234	6.6	11.8
236	6.6	7.3
238	52.6	50.9
240	19.7	17.3
242	10.5	3.6
244	1.3	2.7
C-60G	n = 40	n = 59
CC	95.0	88.1
CG	5.0	11.9
GG	0.0	0.0

All values are expressed as a percentage. Alleles with frequency less than 5% were pooled for further statistical analysis.

men and women. For both sexes, alleles with frequencies greater than 5% were further analyzed for differences in fatty acid and glycerol concentrations and fat oxidation (alleles 172, 180, 182, 184, and 186). Alleles with a frequency less than 5% were pooled into one group for further statistical analysis. For the $(CA)_n$ repeat in i6 of the HSL gene, we identified 9 alleles, ranging in size from 222 to 244 bp. Allele 232 was not observed in female subjects. Allele 238 was found to be the most common (>50%) in both male and female subjects, with allele 240 being the second most common (>16%). For both sexes, alleles with frequencies greater than 5% were further analyzed for differences in fatty acid and glycerol concentrations and fat oxidation (alleles 234, 236, 238, 240, and 242). Again, alleles with a frequency less than 5% were pooled into one group for further statistical analysis. The allele frequency for the CA repeats in i6 ($\chi^2 = 6.123$, 5 *df*, $P = .294$) and in i7 ($\chi^2 = 4.803$, 5 *df*, $P = .440$) was not significantly different in male vs female subjects. Genotype frequencies for the C-60G polymorphism are also shown in Table 2. The G-60G genotype was not observed in this population. The C-60G genotype frequency distribution was not significantly different for male vs female subjects ($\chi^2 = 1.383$, 1 *df*, $P = .240$). Both male and female genotype and allele frequency distributions were in Hardy-Weinberg equilibrium.

3.2. Dinucleotide $(CA)_n$ repeat polymorphisms in i7

Female noncarriers of allele 184 i7 (clinical characteristics in Table 3) showed an overall blunted decrease in RQ after β-adrenergic stimulation (Kruskal-Wallis $P = .013$, Fig. 1C) and comparable thermogenic response. This indicates a diminished ability to stimulate fat oxidation in female noncarriers of allele 184 i7. Of the variability in ΔRQ after β-adrenergic stimulation, 6.9% could be explained by allele 184 in female obese subjects. No associations were found in male noncarriers of allele 184. Furthermore, no associations were found for all other alleles in i7 of the HSL gene (ie, alleles 172, 180, 182, and 186) with circulating fatty acid or glycerol concentrations and fat oxidation after β-adrenergic stimulation.

3.3. Dinucleotide $(CA)_n$ repeat polymorphisms in i6

Female carriers of allele 240 i6 (clinical characteristics in Table 4) showed a significant increase in RQ (Kruskal-Wallis $P < .001$, Fig. 2C) and comparable thermogenic response after β-adrenergic stimulation, suggesting a diminished

Table 3
Clinical characteristics of allele 184 i7 carriers and noncarriers

	Female (n = 40)		Male (n = 61)	
	Carrier	Noncarrier	Carrier	Noncarrier
n	22	18	33	28
Age (y)	39 ± 2	37 ± 2	42 ± 1	44 ± 2
Weight (kg)	89.5 ± 2.2	90.6 ± 2.8	104.4 ± 2.9	105.0 ± 2.1
BMI (kg/m^2)	33 ± 0.7	33 ± 1.0	32.4 ± 0.8	33.4 ± 0.6
%BF	42 ± 1.2	43 ± 0.9	31.1 ± 1.1	32.8 ± 1.1
FFM (kg)	51.7 ± 1.0	51.0 ± 1.3	71.2 ± 1.4	70.2 ± 1.2
WHR	0.84 ± 0.02	0.89 ± 0.03	1.04 ± 0.01	1.04 ± 0.01
EE rest[a] (kJ/min)	4.83 ± 0.11	5.04 ± 012	6.07 ± 0.15	6.49 ± 0.15
RQ rest	0.83 ± 0.01	0.82 ± 0.009	0.81 ± 0.008	0.81 ± 0.007
FFA rest (μmol/L)	560 ± 26	670 ± 60	495 ± 41	461 ± 39
Glycerol rest (μmol/L)	69 ± 9	81 ± 12	72 ± 5	78 ± 4

All values are expressed as mean ± SEM.
[a] Adjusted mean for FFM.

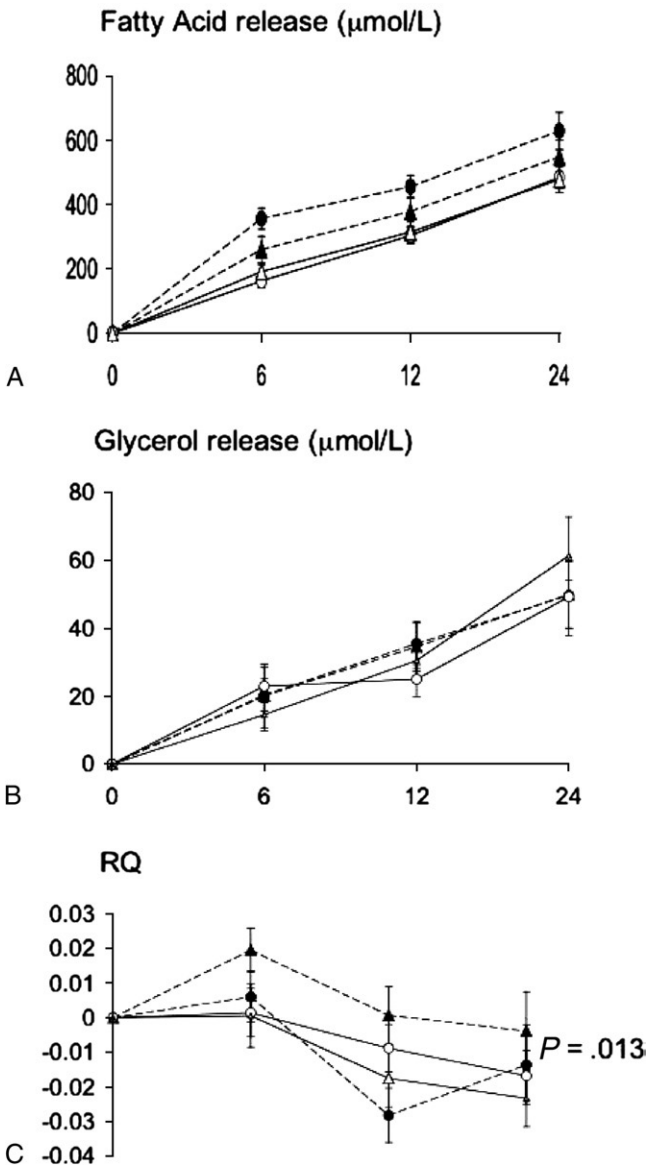

Fig. 1. Change in circulating fatty acids (A) and glycerol (B) and fat oxidation (RQ) (C) for male (solid lines) and female (dashed lines) carriers (circles) vs noncarriers (triangles) of allele 184 i7 of the HSL gene. Values are mean ± SEM. P = .013, female carrier vs noncarrier using Kruskal-Wallis nonparametric statistics.

ability to stimulate fat oxidation in female carriers of allele 240 i6. Moreover, 20.8% (P = .098) of the variability in ΔRQ could be explained by allele 240 in female obese subjects. No associations were found in male carriers of allele 240. Furthermore, no associations were found for alleles 234, 236, 238, and 242 in i6 of the HSL gene with circulating fatty acid or glycerol concentrations and fat oxidation after β-adrenergic stimulation.

3.4. C-60G substitution in the HSL promoter region

In our population, the G-60G genotype was not apparent. The C-60G genotype was apparent in 2 of 40 female subjects. In male subjects, the C-60G genotype was apparent in 7 of 59 subjects. Within these small groups, no significant associations were found between genotypes and circulating fatty acid or glycerol concentrations and fat oxidation.

4. Discussion

In obese subjects, it has been shown that β-adrenoceptor–mediated lipolysis and fat oxidation are blunted [3,4,23]. These blunted lipolysis and fat oxidation persisted even after weight reduction, indicating that they may be an early, or even primary, factor in the development of increased fat stores in obesity [1,5]. For this reason, we investigated, for the first time, whether genetic variation in the HSL gene is associated with a blunted in vivo β-adrenoceptor–mediated increase in circulating fatty acid and glycerol concentration (as estimation of lipolysis) and fat oxidation in overweight and obese subjects. The major finding of the present study is that a blunted β-adrenoceptor–mediated increase in fat oxidation might be associated with genetic variation in the HSL gene (allele 184 i7 and allele 240 i6), at least in overweight-obese women.

4.1. Limitations of the study

The primary objective of this study was to investigate the effect of genetic variation in the HSL gene on β-adrenoceptor–mediated lipolysis and fat oxidation within a group of overweight-obese subjects. Because of the extensive phenotyping, it was for practical reasons not possible to study a larger group. However, power analysis indicated that the number of subjects was adequate to detect differences in the primary outcomes of our study. An overall significant effect of allele 184 i7 and allele 240 i6 on fat oxidation (indicated as RQ) was observed in our population. Moreover, both alleles explained, respectively, 6.9% and 20.8% of the variance in the change in RQ, indicating that these polymorphisms might have a physiological effect. Nevertheless, our results need to be confirmed in a larger population.

An in vitro experiment by Hoffstedt et al [17] showed that overweight male individuals with allele 238 (HSL i6 A5) had a 50% lower lipolytic response in isolated abdominal subcutaneous adipocytes after stimulation with noradrenaline, ISO, forskolin, and dibutyryl cyclic adenosine monophosphate. Our study found no associations between allele 238 and a blunted increase in circulating fatty acid or glycerol concentrations during ISO stimulation. The reason for this discrepancy is not entirely clear, but may be related to the use of in vitro adipose tissue biopsies vs our in vivo approach. In vivo local lipolysis is influenced by the neuroendocrine environment and blood flow. The latter determines the supply of different hormones (like insulin and catecholamines) that have an effect on local adipocyte lipolysis. In the in vitro situation, these factors are not taken into account. In addition, most in vitro studies are performed on adipocytes derived from the subcutaneous region in both sexes. It should be

Table 4
Clinical characteristics of allele 240 i6 carriers and noncarriers

	Female (n = 38)		Male (n = 55)	
	Carrier	Noncarrier	Carrier	Noncarrier
n	12	26	19	36
Age (y)	37 ± 2	38 ± 1	42 ± 2	44 ± 1
Weight (kg)	86.4 ± 3.4	90.5 ± 2.1	107.6 ± 2.1	104.7 ± 2.8
BMI (kg/m^2)	32.8 ± 1.1	32.6 ± 0.8	33.6 ± 0.8	32.9 ± 0.7
%BF	41.6 ± 1.5	42.7 ± 0.9	33.1 ± 1.3	31.9 ± 1.0
FFM (kg)	50.1 ± 1.6	51.5 ± 0.9	71.8 ± 1.6	70.6 ± 1.3
WHR	0.84 ± 0.03	0.88 ± 0.02	1.04 ± 0.02	1.04 ± 0.01
EE rest[a] (kJ/min)	4.73 ± 0.15	4.99 ± 0.10	6.37 ± 0.17	6.21 ± 0.13
RQ rest	0.83 ± 0.01	0.82 ± 0.009	0.81 ± 0.009	0.81 ± 0.006
FFA rest (μmol/L)	604 ± 65	618 ± 39	518 ± 57	472 ± 34
Glycerol rest (μmol/L)	73 ± 15	73 ± 9	78 ± 7	75 ± 4

All values are expressed as mean ± SEM.
[a] Adjusted mean for FFM.

mentioned that there are major differences in catecholamine-induced lipolysis between depots (subcutaneous vs visceral) and body fat distribution between sexes [25-27].

In contrast to men, an association with fat oxidation was observed in female noncarriers of allele 184 (i7) and female carriers of allele 240 (i6) (Figs. 1C and 2C). Both alleles explained, respectively, 6.9% and 20.8% of the variance in the change in RQ, suggesting that genetic variability in the HSL gene might contribute to the β-adrenoceptor–mediated fatty acid handling in women. An in vivo study by Qi et al [28] found sex-specific associations between genetic variation in the HSL gene (promoter region and intron 2) and plasma lipid and glucose concentrations, being more pronounced in women. On this basis, it may be speculated that, in women, fatty acid handling during β-adrenergic stimulation is more influenced by genetic factors. In men, gene-environment interactions like eating habits, physical activity, or metabolic factors might override genetic predisposition. As shown by Meirhaeghe et al [29], physical activity or other behavioral factors may counterbalance the effect of genetic predisposition to increase body weight, body fat, and obesity in men. We only can hypothesize that, in men, behavior might counterbalance genetic predisposition; and further research is necessary to elucidate this interaction.

As already suggested by Klannemark et al [16], the polymorphic marker in i7 is in LD with an allele and/or gene that increases susceptibility to abdominal obesity and thereby possibly to type 2 diabetes mellitus. The distance between i6 and i7 is only approximately 2800 bp. Therefore, it is possible that there is LD between allele 184 (i7) and allele 240 (i6). However, the frequency distribution of both alleles does not suggest a strong LD (eg, for women D' = 0.22, r^2 = 0.02) in our population [30]. It would be worthwhile to confirm these results in a larger population. Because of the fact that the investigated polymorphic markers are situated in noncoding intron structures of the HSL gene, it seems unlikely that these markers result in functional conformational changes of the HSL protein or act alone. However, it is unknown whether the polymorphic

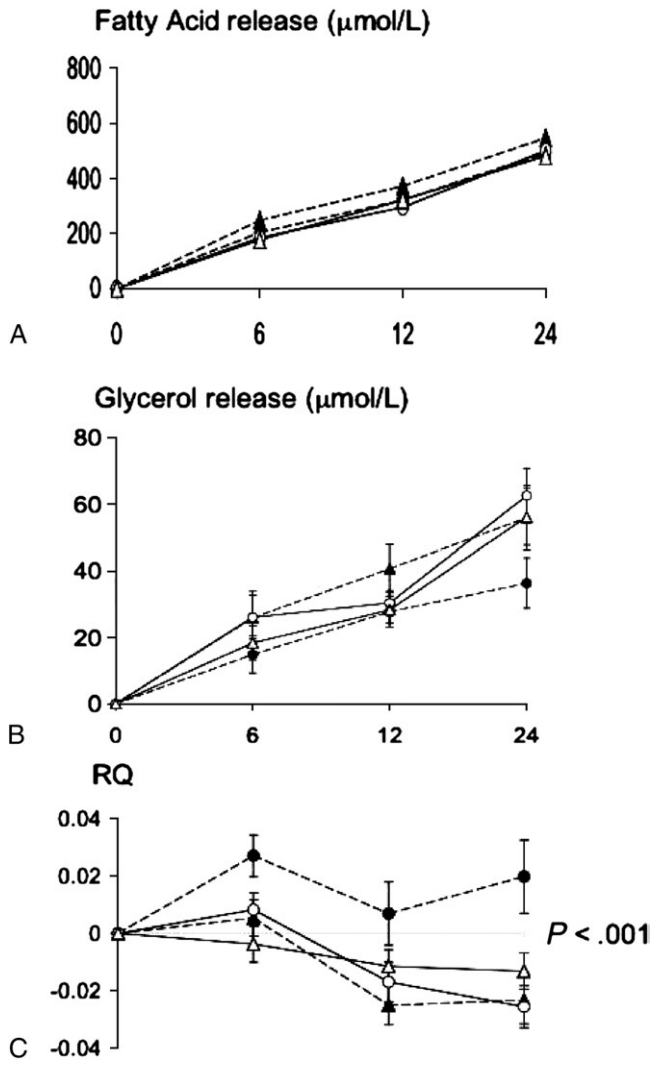

Fig. 2. Change in circulating fatty acids (A) and glycerol (B) and fat oxidation (RQ) (C) for male (solid lines) and female (dashed lines) carriers (circles) vs noncarriers (triangles) of allele 240 i6 of the HSL gene. Values are mean ± SEM. $P < .001$, female carrier vs noncarrier using Kruskal-Wallis nonparametric statistics.

markers in i6 and i7 of the HSL gene are associated with, for example, cryptic splice junctions or alternative promoter creations, which could have major effects on function and expression of HSL, or are in LD with another functional polymorphism in the HSL gene. Furthermore, we cannot exclude that a polymorphism in another gene, located nearby the HSL gene, is in LD with (240) i6 or (184) i7.

In conclusion, the present results suggest that variation in the polymorphic DNA markers i7 and i6 of the HSL gene might be associated with a physiological effect on in vivo β-adrenoceptor–mediated fat oxidation in overweight-obese female subjects. Further studies in larger populations are needed to confirm our results and to see whether another polymorphism in the HSL gene is in LD with i6 or i7, which might partly explain the current findings.

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