Two novel prevalent polymorphisms in the hormone-sensitive lipase gene have no effect on insulin sensitivity of lipolysis and glucose disposal

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Abstract **Free fatty acids released during triglyceride lipolysis play an important role in obesity-associated insulin resistance of glucose disposal. Individual sensitivity of lipolysis to the suppressive effect of insulin varies greatly among healthy subjects. It is possible that genetic factors contribute to this variation. Among the many proteins involved in the regulation of lipolysis, hormone-sensitive lipase (HSL) represents a prime candidate for genetic variants contributing to the biological variation of insulin sensitivity of lipolysis. We determined the insulin sensitivity of lipolysis (suppression of isotopically [primed-continuous infusion of d_5 glycerol] measured glycerol rate of appearance) and of glucose disposal, using a three-step (n = 20) or standard (n = 53) hyperinsulinemic euglycemic clamp in 73 healthy, unrelated subjects. To assess the possible role of genetic polymorphisms, we directly sequenced the coding region of the HSL gene and the noncoding exon B from these subjects. We identified two silent mutations and three amino acid polymorphisms: Arg262Met (prevalence, 5%), Glu620Asp (prevalence, 31%) and Ser681Ile (prevalence, 22%). The latter two are located in the regulatory domain of HSL but neither had a significant impact on insulin sensitivity of lipolysis or glucose disposal (with and without adjustment for obesity and age as covariates; all *P* values > 0.20).** We conclude that a number of genetic polymorphisms in HSL exist, some of which are highly prevalent. Neither of the polymorphisms we identified in the coding region, however, contributed measurably to the biological variation of insulin sensitivity in our lean, healthy population.—Stumvoll, M., H. G. Wahl, S. Jacob, A. Rettig, F. Machicao, and H. Häring. **Two novel prevalent polymorphisms in the hormone-sensitive lipase gene have no effect on insulin sensitivity of lipolysis and glucose disposal.** ***J. Lipid Res.*** **2001.** 42: **1782–1788.**

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Insulin resistance is a key factor in the development of type 2 diabetes. Although the complex pathogenesis of insulin resistance is incompletely understood it is generally accepted that adipose tissue, particularly obesity, is of pivotal importance (1, 2). It is still unclear, however, whether and which genetic factors are involved (3, 4). Among the mediators of the cross-talk between adipose tissue and insulin sensitivity, FFA are best characterized. They have been demonstrated to influence glucose homeostasis through inhibition of peripheral glucose disposal and stimulation of endogenous glucose production (5). FFA are stored as triglycerides and released via the process of lipolysis.

Lipolysis is highly sensitive to the suppressive effects of insulin (6). In both type 2 diabetes and simple obesity the suppression of lipolysis is insulin resistant, resulting in excessive release of FFA into plasma (7–9). These conditions are invariably associated with insulin resistance also of glucose disposal. It is thus possible that insulin resistance of glucose disposal, which ultimately favors the development of type 2 diabetes, is a result of insulin resistance of lipolysis. To fathom the range and identify extremes in insulin sensitivity of lipolysis among healthy individuals we performed a series of studies using a combination of euglycemic clamp and isotope dilution techniques (10, 11). Even after adjusting for obesity, extremes in insulin sensitivity varied by >400%. It is well possible that genetic factors contribute to this variation.

Many intracellular proteins are involved in the hormonal regulation of lipolysis (12, 13). Any of these could have altered functionality as a result of genetic variants. Because of its rate-limiting properties, however, hormone-sensitive lipase (HSL) represents a prime candidate for genetic polymorphisms that, if sufficiently prevalent and functionally relevant, could modify insulin sensitivity of lipolysis. Conceivably, such a polymorphism could also affect insulin sensitivity of glucose disposal via availability of FFA.

The human adipocyte isoform of HSL is a protein containing 775 amino acids (88 kDa) and the encoding gene

Abbreviations: BMI, body mass index; GIR, mean infusion rate of exogenous glucose; HSL, hormone-sensitive lipase; ISI, insulin sensitivity index; MCR, metabolic clearance rate of glucose; R_a, rate of appearance.

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is located on chromosome 19q13.1-13.2 (14). The HSL gene comprises 9 exons (15), of which exons 1 to 4 largely encode for the N-terminal domain (codons 1 to 315) and exons 5 to 9 encode the C-terminal catalytic plus regulatory domains (codons 336 to 775) [reviewed in ref. (16)]. Three polymorphic sites in the HSL gene have been described (17–19). Evidence of association with type 2 diabetes, however, was either not reported (17) or was reported only in the context of obesity (20, 21). Interestingly, active HSL is also present in beta cells and might contribute to the regulation of glucose-stimulated insulin secretion (22). In the pathogenesis of familial combined hyperlipidemia variations in the HSL gene do not seem to play a role (23). What is not known, however, is *1*) the prevalence and distribution of functionally relevant polymorphisms of HSL in a healthy, Caucasian population and *2*) whether and how such polymorphisms affect insulin sensitivity of lipolysis and (possibly secondarily) of glucose disposal. We therefore sequenced the nine exons of the HSL gene and the noncoding exon B (1.5 kb upstream of the first coding exon) in 73 healthy subjects in whom insulin sensitivity of lipolysis in vivo was determined using the rate of appearance (R_a) of glycerol in plasma. This was directly measured during a three-step hyperinsulinemic euglycemic clamp (n = 20) or estimated from the standard 2-h hyperinsulinemic clamp (n = 53) using an index derived from the glycerol R_a at 60 min (24).

MATERIALS AND METHODS

Subjects

In the Tübingen Family Study for type 2 diabetes, to date more than 500 subjects of Caucasian origin living in southwest Germany have been metabolically characterized. In this study primarily normal glucose-tolerant subjects with (also without) a family history of type 2 diabetes were recruited. As part of a more recent add-on protocol lipolysis was determined by stable isotope methodology. At the time of recruitment the HSL genotype was unknown and subjects were included in an unselected fashion. We studied 73 healthy, unrelated subjects, of whom 53 underwent a standard hyperinsulinemic clamp and 20 a stepwise hyperinsulinemic euglycemic clamp. The subject characteristics are shown in Tables 3 and 4. All subjects underwent the standard preparatory procedures and investigations of the protocol of the Tübingen Family Study (medical history, physical examination, routine blood test, oral glucose tolerance test). All participants showed normal glucose tolerance according to World Health Organization (WHO, Geneva, Switzerland) criteria. The protocols were approved by the local ethics committee and after explaining the nature of the study all subjects gave informed written consent.

Experimental protocol

Subjects were studied after an overnight fast. At approximately 6:00 AM an antecubital vein was cannulated for infusion of insulin, glucose, and isotopes. A dorsal hand vein on the contralateral arm was cannulated retrogradely and placed under a heating device to permit sampling of arterial blood. A primed continuous infusion of [$^2H_5$]glycerol (1 μmol/kg, 0.4 μmol/min; Cambridge Isotope Laboratories, Andover, MA) was immediately started. At 8:00 AM one of the following clamp protocols was started.

Three-step hyperinsulinemic-euglycemic clamp

After the baseline period subjects received sequential insulin infusions at rates of 0.1, 0.25, and 1.0 mU·kg^{-1}·min^{-1} for 2 h each rate, respectively. Blood was drawn every 5 to 10 min for determination of blood glucose and glucose infusion was adjusted appropriately to maintain the baseline glucose level. Arterial blood samples were obtained −20, −10, and 0 min before the start of the insulin infusion and at 100, 110, and 120 min during each 2-h insulin infusion for determination of plasma [$^2H_5$]glycerol enrichment, plasma glycerol concentration, and serum insulin and FFA concentrations.

Standard hyperinsulinemic-euglycemic clamp

After the baseline period subjects received a primed insulin infusion at a rate of 1.0 mU·kg^{-1}·min^{-1} for 2 h (25). Blood was drawn every 5 to 10 min for determination of blood glucose and the glucose infusion was adjusted appropriately to maintain the baseline glucose level. Arterial blood samples were obtained −20, −10, and 0 min before the start of the insulin infusion and at 30, 60, 90, 100, 110, and 120 min for determination of plasma [$^2H_5$]glycerol enrichment, plasma glycerol concentration, and serum insulin and FFA concentrations.

Analytical procedures

Blood glucose was determined with a bedside glucose analyzer (glucose oxidase method; Yellow Springs Instruments, Yellow Springs, CO). Serum insulin was measured by a microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany), serum FFA by an enzymatic method (Wako Chemicals, Neuss, Germany), and plasma glycerol by an enzymatic method (Sigma Diagnostics, Deisenhofen, Germany). Plasma [$^2H_5$]glycerol enrichment was determined by gas chromatography-mass spectrometry, using the trimethylsilyl derivative of glycerol. Electron impact ionization was applied and the mass-to-charge ratios of 205 and 208 were monitored (26).

Genotyping

Fasting blood samples were drawn into 10-ml vacuum tubes containing EDTA. Plasma was separated by centrifugation and stored at 4°C until analysis. Genomic DNA was isolated from whole blood with a commercial DNA isolation kit (Nucleospin; Macharey-Nagel, Düren, Germany). PCR was performed with intronic primers for amplification of the coding HSL exons and the noncoding exon located 1.5 kb upstream of the first coding exon. Because exons were too large to be analyzed in one fragment, overlapping sets of primers were used. For exons 1, 8, and 9 overlapping sets of two primers (exons 1/1, 1/2, 9/1, and 9/2) or 3 (exon 8, 8/1, 8/2, and 8/3) were used. The nine exons and the noncoding regions were amplified by PCR and specific primers (**Table 1**). The PCR conditions were as follows: denaturation at 96°C for 3 min, followed by 30 cycles of denaturation (96°C for 30 s), annealing for 30 s (see Table 1 for various annealing temperatures), and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. The reactions were done in a total volume of 25 μl with 67 mM Tris (pH 8.8), 0.13 mM dNTP, 1.5 mM $MgCl_2$, a 0.2 mM concentration of both primers, and 0.5 U of *Taq* polymerase, using 21 ng of genomic DNA as template. Either 3% DMSO, 1.5% formamide, or 5% glycerol (Table 1) was used to increase PCR sensitivity.

Mutation screening of the human HSL gene was done by direct sequencing. PCR products were sequenced bidirectionally, using an ABI Prism dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, Foster City, CA) and analyzed on an automated sequencer (ABI model 310; Perkin-Elmer). In the 73 subjects, all coding exons and the noncoding exon B 1.5 kb upstream of the first coding exon were screened. The nucleotide and codon

TABLE 1. Sequences of primer pairs and conditions for PCR-SSCP analysis of the HSL gene

Exon	Primer Sequence	Fragment Length	Annealing Temperature	Enhancer of PCR Specificity
		bp	*°C*	
Noncod f	5′-TCTGTTTACAGCACGTGGTCC-3′	301	62	1.5% Formamide
Noncod r	5′-TGCTCCAGTGACTTCCCTCC-3′			
Exon 1/1 f	5′-AACAGGCCTCCCCACCTGCC-3	314	63	5% Glycerol
Exon 1/1 r	5′-CGGGATTTGTGCAGGAGGTG-3′			
Exon 1/2 f	5′-TACCGCAGCCTAGTGCACAC-3′	347	63	5% Glycerol
Exon 1/2 r	5′-GCCTTCATTGTGGGGCCCAG-3′			
Exon 2 f	5′-CATCCCTCTCTTGAGCGGTG-3′	217	63	1.5% Formamide
Exon 2 r	5′-CCAGTGGGTCAGGCTGCTTG-3′			
Exon 3 f	5′-CAAGCAGCCTGACCCACTGG-3′	246	63	3% DMSO
Exon 3 r	5′-CCTCAGATGATGCTCTGGGC-3′			
Exon 4 f	5′-ACCCCTGCAGGCAGACCTTC-3′	285	64	1.5% Formamide
Exon 4 r	5′-CCACGCTCCTCGGCTCTGTC-3			
Exon 5 f	5′-AGCTCTCCCCAACCTCACAC-3′	348	63	1.5% Formamide
Exon 5 r	5′-GGCTCACCAAGGAGGGCGCA-3′			
Exon 6 f	5′-TCTGCCCCTGCCAGGTTGTC-3′	328	65	1.5% Formamide
Exon 6 r	5′-GCAGGAGTCAGACATCCATGCA-3′			
Exon 7 f	5′-GGAAAACAACTCAGCTACCCAC-3′	267	65	1.5% Formamide
Exon 7 r	5′-AGGCTGTCCCTCCTGCCCAC-3′			
Exon 8/1 f	5′-ACCAAACTAACGGAGCCAGG-3′	285	58	1.5% Formamide
Exon 8/1 r	5′-CAGCTCATTTTTGGCCTCAG-3′			
Exon 8/2 f	5′-GACACTTAGCCCCTCCACAC-3′	239	62	1.5% Formamide
Exon 8/2 r	5′-TGCTGTCGGGTGCCAGCAGC-3′			
Exon 8/3 f	5′-AGAGGCCTGGGCGTCCGTGC-3	256	66	3% DMSO
Exon 8/3 r	5′-AGGTGTACCGTGCCCGGTCC-3′			
Exon 9/1 f	5′-ACCCTCTCTCCACGTCCCTC-3′	237	64	1.5% Formamide
Exon 9/1r	5′-ACGAGGCGGATGCGCTCCAC-3′			
Exon 9/2 f	5′-CGGCGCTGTGCCGCGAGAC-3′	283	63	3% DMSO
Exon 9/2 r	5′-TGGCGAGGGTCTCAGCTTTC-3′			

Abbreviations: noncod, noncoding; f, forward; r, reverse; SSCP, single-strand conformation polymorphism.

numbering is based on GI 896474 (GenBank), accession number L11706 HUMHSLA 3,255 bp for DNA, and accession number AAA 69,810 version 1 (GenPept) 775 amino acids for protein.

Calculations

The plasma rate of appearance (R_a) of glycerol was used as index for systemic lipolysis. In the steady state, that is, at baseline and during the last 20 min glycerol R_a was assumed to equal the rate of disappearance (R_d) and was calculated according to the steady state equation: $R_a = (ENR_{inf}/ENR_{pl} - 1)F$, where ENR_{inf} is the isotopic enrichment of the infusate, ENR_{pl} is the isotopic enrichment of plasma (both in atom% excess), and F is the rate of the isotope infusion (in μmol/min). The means of the −20, −10, and 0 min and the 100, 110, and 120 min values of each step were used as steady state values. During the standard hyperinsulinemic-euglycemic clamp at 30, 60, 90, 100, 110, and 120 min the DeBodo (27) modification of Steele's non-steady state equations were used to calculate R_a. A fractional pool size of 0.5 and a volume of distribution of 650 ml/kg were assumed (28).

Insulin sensitivity of systemic lipolysis was assessed as the serum insulin concentration that effectively suppressed plasma glycerol R_a by 50% of the maximal suppression (EC_{50}). The EC_{50} for suppression of glycerol R_a was estimated by fitting a mono-exponential function (glycerol $R_a = max + a \cdot e^{-Ins \cdot k}$) to the mean basal value and the three mean steady state values, where max (i.e., maximal suppression), a and k represent the fitted parameters and Ins represents the serum insulin concentration. The EC_{50} was calculated as $EC_{50} = Ins_0 + \ln 2/k$, where Ins_0 represents the serum insulin concentration at baseline. In the subjects undergoing the standard hyperinsulinemic clamp the insulin EC_{50} for the suppression of lipolysis was estimated by using the following equation: $EC_{50} = 90 -$ glycerol $R_{a,60min}$ (% decrease from baseline) $\times$ 0.79 (24).

The insulin sensitivity index (ISI, in $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$) for systemic glucose uptake was calculated as mean infusion rate of exogenous glucose (GIR, in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) necessary to maintain euglycemia during the last 60 min of the standard clamp (third step of the three-step clamp) divided by the steady state serum insulin concentration during step 3. The metabolic clearance rate of glucose (MCR) was calculated as the GIR divided by the steady state glucose concentration.

Statistical analysis

Unless otherwise stated data are given as means ± SEM. Statistical comparisons between genotype groups were made by using the unpaired Student's *t*-test for normally distributed parameters (only age) and the nonparametric Wilcoxon rank test for non-normally distributed parameters. Distribution was tested for normality by using the Shapiro-Wilk W test. For the purpose of statistical comparison, the insulin EC_{50} was adjusted for body mass index (BMI) and age. A *P* value of less than 0.05 was considered to be statistically significant. The statistical software package JMP (SAS Institute, Cary, NC) was used.

TABLE 2. Identified polymorphisms in the HSL gene

Exon	Codon	Base Exchange	Amino Acid Exchange	Rare Allele Frequency	Carrier Prevalence[a]	Structural Location[b]
				%	%	
4	262	G → T	Arginine → methionine	4.8	9.6	N-Terminal domain
8	605	C → A	Proline → proline	1.3	2.6	Regulatory module
8	606	C → A	Serine → serine	1.3	2.6	Regulatory module
8	620	G → T	Glutamate → aspartate	15.5	31.0	Regulatory module
8	681	G → T	Serine → isoleucine	11.0	22.0	Regulatory module

[a] Heterozygous carriers only were detected.
[b] See ref. 31.

RESULTS

Polymorphisms in the HSL gene

In the 73 subjects 4 amino acid polymorphisms were identified in the HSL gene, of which 2 had an allelic frequency greater than 10% (**Table 2**). The Arg309Cys polymorphism in exon 4, which was previously described in a Japanese population, could not be detected in this Caucasian population. Comparisons of metabolic parameters were performed only between carriers of the Glu620Asp and Ser681Ile polymorphisms and their respective control subjects because the Arg262Met polymorphism was of inadequate prevalence. As it turned out, the different genotypes included in the comparative analysis were reasonably well matched for BMI, waist-hip ratio, age, and gender (**Tables 3** and **4**). The frequency of subjects with a family history of type 2 diabetes was not significantly different in the two genotype groups of HSL codon 620 (68% Glu/Glu; 47% Glu/Asp; $P = 0.11$, χ^2 test) or HSL codon 681 (76% Ser/Ser; 55% Ser/Ile $P = 0.08$, χ^2 test). The allelic combinations for the polymorphisms in codons 620 and 681 were as follows: 620 Glu/Glu + 681 Ser/Ser, n = 32; 620 Glu/Asp + 681 Ser/Ser, n = 23; 620 Glu/Asp + 681 Ser/Ile, n = 6; 620 Glu/Glu + 681 Ser/Ile, n = 6.

Insulin sensitivity of glucose disposal

Combining both clamp protocols, the ISI was not different between carriers of the Glu620Asp and Ser681Ile polymorphisms and their respective control subjects (0.11 ± 0.01 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$ in Glu/Glu vs. 0.10 ± 0.01 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$ in Glu/Asp, $P = 0.6$ and 0.10 ± 0.01 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$ in Ser/Ser vs. 0.10 ± 0.01 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pM^{-1}$ in Ser/Ile, $P = 0.6$). The glucose MCR was also not different (7.6 ± 0.4 $ml \cdot kg^{-1} \cdot min^{-1}$ in Glu/Glu vs. 7.4 ± 0.5 $ml \cdot kg^{-1} \cdot min^{-1}$ in Glu/Asp, $P = 0.8$ and 7.6 ± 0.3 $ml \cdot kg^{-1} \cdot min^{-1}$ in Ser/Ser vs. 7.6 ± 0.7 $ml \cdot kg^{-1} \cdot min^{-1}$ in Ser/Ile, $P = 0.9$). No significant differences were observed on division of subjects into male and female subcategories (**Table 5**).

Insulin sensitivity of lipolysis

In the standard hyperinsulinemic euglycemic clamp protocol the glycerol R_a in the Glu/Glu group decreased from 1.74 ± 0.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at baseline to 0.57 ± 0.08 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 120 min. In the Glu/Asp group the glycerol R_a decreased from 1.79 ± 0.24 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at baseline to 0.67 ± 0.07 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 120 min. The estimated insulin EC_{50} for the suppression of lipolysis was 48 ± 3 pM in the Glu/Glu group and 52 ± 2 pM in the Glu/Asp group ($P = 0.3$). No significant differences were observed on division of subjects into male and female subcategories (Table 5).

Glycerol R_a in the Ser/Ser group decreased from 1.79 ± 0.18 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at baseline to 0.64 ± 0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 120 min. In the Ser/Ile group the glycerol R_a decreased from 1.47 ± 0.13 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at baseline to 0.60 ± 0.11 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 120 min. The estimated insulin EC_{50} for the suppression of lipolysis was 53 ± 2 pM in the Ser/Ser group and 49 ± 4 pM in the Ser/Ile group ($P = 0.3$). In the subjects undergoing the three-step hy-

TABLE 3. Characteristics of subjects: Glu620Asp polymorphism[a]

	All Subjects			Female Subjects			Male Subjects		
	Glu/Glu	Glu/Asp	*P*	Glu/Glu	Glu/Asp	*P*	Glu/Glu	Glu/Asp	*P*
Number (M/F)	42 (25/17)	29 (14/15)	0.05[b]	17	15		25	14	
Age (years)	29 ± 1	27 ± 1	0.15	32 ± 2	28 ± 2	0.13	27 ± 1	26 ± 0.9	0.42
Weight (kg)	69.1 ± 1.8	72.6 ± 2.3	0.23	69.2 ± 3.5	69.3 ± 4.0	1.0	69.1 ± 1.7	76.1 ± 1.6	0.01
BMI $(kg/m)^2$	23.0 ± 0.5	23.7 ± 0.7	0.43	24.3 ± 1.1	24.0 ± 1.2	0.86	22.1 ± 0.5	23.3 ± 0.5	0.11
Waist-hip ratio	0.83 ± 0.01	0.80 ± 0.01	0.08	0.81 ± 0.02	0.76 ± 0.02	0.14	0.85 ± 0.01	0.84 ± 0.01	0.73
Fasting serum glucose (mg/dl)	83 ± 3	88 ± 1	0.22	76 ± 7	87 ± 2	0.16	88 ± 1	88 ± 1	0.79
Fasting serum insulin (pM)	38 ± 3	41 ± 3	0.55	47 ± 7	47 ± 6	0.96	32 ± 2	34 ± 3	0.56
Serum cholesterol (mg/dl)	184 ± 6	190 ± 7	0.52	190 ± 7	183 ± 7	0.48	180 ± 8	198 ± 13	0.23
Serum triglycerides (mg/dl)	83 ± 6	111 ± 17	0.10	81 ± 8	95 ± 11	0.33	84 ± 8	127 ± 33	0.13
LDL cholesterol (mg/dl)	118 ± 5	118 ± 6	0.98	121 ± 6	105 ± 5	0.07	116 ± 6	132 ± 11	0.22
HDL cholesterol (mg/dl)	57 ± 2	58 ± 3	0.73	62 ± 3	66 ± 4	0.33	54 ± 2	49 ± 3	0.10

[a] Genotyping possible only in 71 subjects.
[b] χ^2 test.

TABLE 4. Characteristics of subjects: Ser681Ile polymorphism

	All Subjects			Female Subjects			Male Subjects		
	Ser/Ser	Ser/Ile	*P*	Ser/Ser	Ser/Ile	*P*	Ser/Ser	Ser/Ile	*P*
Number (M/F)	57 (29/28)	16 (9/7)	0.44[a]	28	7		29	9	
Age (years)	29 ± 1	28 ± 1	0.71	30 ± 2	29 ± 3	0.70	28 ± 1	27 ± 1	0.98
Weight (kg)	70.8 ± 1.6	74.5 ± 3.1	0.28	67.2 ± 2.5	78.6 ± 5.9	0.07	74.3 ± 1.6	71.3 ± 1.6	0.38
BMI (kg/m^2)	23.5 ± 0.5	24.3 ± 0.9	0.41	23.8 ± 0.8	26.9 ± 1.5	0.11	23.2 ± 0.4	22.4 ± 0.4	0.33
Waist-hip ratio	0.83 ± 0.01	0.84 ± 0.02	0.61	0.79 ± 0.02	0.85 ± 0.04	0.12	0.86 ± 0.01	0.82 ± 0.01	0.07
Fasting serum glucose (mg/dl)	84 ± 2	83 ± 5	0.78	81 ± 4	86 ± 3	0.60	88 ± 1	81 ± 1	0.23
Fasting serum insulin (pM)	39 ± 3	50 ± 6	0.09	43 ± 5	70 ± 8	0.01	35 ± 3	34 ± 3	0.77
Serum cholesterol (mg/dl)	187 ± 5	197 ± 10	0.39	185 ± 6	204 ± 17	0.19	189 ± 9	191 ± 9	0.90
Serum triglycerides (mg/dl)	95 ± 5	117 ± 25	0.28	92 ± 8	100 ± 24	0.70	98 ± 13	130 ± 13	0.34
LDL cholesterol (mg/dl)	119 ± 4	124 ± 9	0.61	113 ± 5	124 ± 15	0.39	125 ± 7	124 ± 7	0.96
HDL cholesterol (mg/dl)	57 ± 2	60 ± 4	0.44	62 ± 2	70 ± 7	0.17	52 ± 2	52 ± 2	0.92

[a] χ^2 test.

perinsulinemic euglycemic clamp protocol the directly determined insulin EC_{50} for the suppression of lipolysis was 57 ± 6 pM in the Glu/Glu group and 57 ± 9 pM in the Glu/Asp group ($P = 0.9$) for the Glu620Asp polymorphism. For the Ser681Ile polymorphism the insulin EC_{50} was 53 ± 5 pM in the Ser/Ser group and 70 ± 20 pM in the Ser/Ile group ($P = 0.3$). In both protocols combined, the EC_{50} was not different between carriers of the Glu620Asp and Ser681Ile polymorphisms and their respective control subjects (52 ± 3 pM in Glu/Glu vs. 53 ± 3 pM in Glu/Asp, $P = 0.8$ and 53 ± 2 pM in Ser/Ser vs. 53 ± 5 pM in Ser/Ile, $P = 0.9$) (**Figs. 1** and **2**). Among the females alone there was also no difference in antilipolysis. However, among Ser/Ile males the insulin EC_{50} was significantly lower ($P = 0.01$), indicating greater insulin sensitivity of lipolysis. The significance disappeared after adjusting for BMI and age ($P = 0.06$) (Table 5). Because there was no significant difference in the area under the curve over time during the euglycemic clamp ($P = 0.48$, data not shown) we believe that the above-described significance is without meaning.

We also assessed combinations of the rare alleles of the two polymorphisms. There was no significant difference in ISI or EC_{50} between Glu620Asp plus Ser681Ile and wild type in both codons (all $P > 0.46$).

FFA decreased from 463 ± 31 μM at baseline to 40 ± 4 μM at the end of the clamp in the Glu/Glu group and from 438 ± 29 to 50 ± 5 μM in the Glu/Asp group (*P* for difference = 0.4). In the Ser/Ser group FFA decreased from 464 ± 27 μM at baseline to 44 ± 3 μM at the end of the clamp; in the Ser/Ile group it decreased from 451 ± 43 to 56 ± 8 μM (*P* for difference = 0.9).

DISCUSSION

The main aim of the present study was to test the hypothesis that HSL represents a candidate gene for insulin resistance and thus type 2 diabetes. Our approach was to assess whether any functionally relevant amino acid polymorphisms exist and contribute to the biological variation of insulin sensitivity of lipolysis in healthy subjects. The insulin EC_{50} for suppression of lipolysis in our subjects ranged from 20 to 120 pM. A mean EC_{50} of 132 pM was previously reported for insulin-resistant, obese subjects (9). Thus, the insulin sensitivity of lipolysis in our population covered an adequate range for subsequent association studies.

On sequencing the nine exons of the HSL gene we identified four polymorphisms, of which two (Glu620Asp and Ser681Ile) were not only highly prevalent but also located in the regulatory domain of the protein. One resulted in replacement of serine by isoleucine. The serine phosphorylation sites involved in the regulation of the catalytic activity, however, have been identified at positions 552, 554, 660, and 661 (16). We could not detect any genetic variations in the catalytic (codons 336 to 544) or lipid-binding (codons 682 to 775) domains. Neither the

TABLE 5. Insulin sensitivity: Glu620Asp and Ser681Ile polymorphism

	All Subjects			Female Subjects			Male Subjects		
	Glu/Glu	Glu/Asp	*P*	Glu/Glu	Glu/Asp	*P*	Glu/Glu	Glu/Asp	*P*
ISI (μmol·kg^{-1}·min^{-1}·pM^{-1})	0.11 ± 0.01	0.10 ± 0.01	0.54	0.09 ± 0.01	0.09 ± 0.01	0.98	0.12 ± 0.01	0.11 ± 0.01	0.60
ISI (adjusted BMI)	0.11 ± 0.01	0.11 ± 0.01	0.88	0.10 ± 0.01	0.10 ± 0.01	0.92	0.12 ± 0.01	0.12 ± 0.01	0.86
EC_{50} (pM)	52 ± 3	53 ± 3	0.83	56 ± 4	56 ± 4	0.97	50 ± 3	51 ± 3	0.90
EC_{50} (adjusted BMI and age)	64 ± 3	62 ± 2	0.63	66 ± 4	64 ± 4	0.72	63 ± 4	60 ± 3	0.63
	Ser/Ser	Ser/Ile		Ser/Ser	Ser/Ile		Ser/Ser	Ser/Ile	
ISI (μmol·kg^{-1}·min^{-1}·pM^{-1})	0.10 ± 0.01	0.10 ± 0.01	0.62	0.09 ± 0.01	0.07 ± 0.02	0.25	0.12 ± 0.01	0.12 ± 0.01	0.79
ISI (adjusted BMI)	0.11 ± 0.01	0.11 ± 0.01	0.89	0.10 ± 0.01	0.09 ± 0.01	0.78	0.12 ± 0.01	0.12 ± 0.01	0.89
EC_{50} (pM)	53 ± 2	53 ± 5	0.93	54 ± 3	69 ± 8	0.06	52 ± 2	40 ± 2	0.01
EC_{50} (adjusted BMI and age)	64 ± 2	61 ± 4	0.54	65 ± 3	72 ± 6	0.22	62 ± 3	52 ± 3	0.06

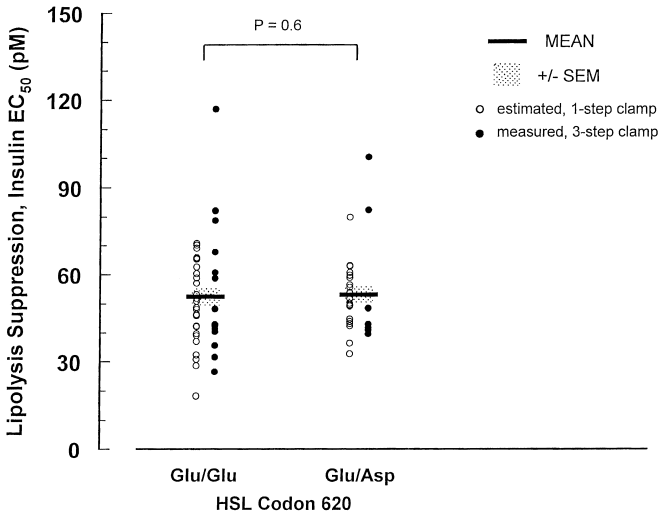

Fig. 1. Insulin sensitivity of suppression of lipolysis (insulin EC_{50}) in subjects with and without the Glu620Asp polymorphism in the HSL gene (Glu/Glu, N = 42; Glu/Asp, N = 29).

Glu620Asp nor Ser681Ile polymorphism was associated with differences in insulin sensitivity of glucose disposal or lipolysis in this healthy population. It is therefore unlikely that these polymorphisms contribute to the biological variation of insulin sensitivity and thus, at least directly, to the pathogenesis of insulin resistance. However, only transfection studies using recombinant HSL, which was beyond the scope of the present work, will permit a definitive statement on functionality.

Previously, a polymorphism in exon 4 of the HSL gene resulting in an amino acid exchange (Arg309Cys) was detected in a Japanese population with a prevalence of 11%. Cholesterol levels were higher in carriers but no association with type 2 diabetes was found (17). We did not detect this polymorphism in our population. The first polymorphism at the HSL locus was detected in intron 7 (18) and used for subsequent linkage and association studies. In Scandinavian and French populations this polymorphism was found with a greater frequency in patients with type 2 diabetes than in healthy control subjects (20, 21). However, because this polymorphism was primarily associated with obesity, in particular abdominal obesity, it was speculated that this locus is in linkage disequilibrium with a gene increasing susceptibility to abdominal obesity and possibly secondarily to type 2 diabetes (20). It is of note that in almost 50 DNA samples (from the Northwick Park Heart Study) the Glu620Asp and Ser681Ile polymorphisms were not identified (19). Differences in ethnic background (British Isles vs. southwest Germany) and methodology may explain this discrepancy. Single-strand conformational polymorphism analysis has a lower ability to detect mutations compared with direct sequencing. In that study a silent G-to-C exchange in codon Ala-729 (allele frequency based on 860 samples, 1.2%) and a T-to-C exchange in intron 4 (allelic frequency, 5.2%) were observed. In summary, the available evidence argues largely against a direct involvement of variants of the HSL gene in the development of type 2 diabetes, which is consistent with our observations.

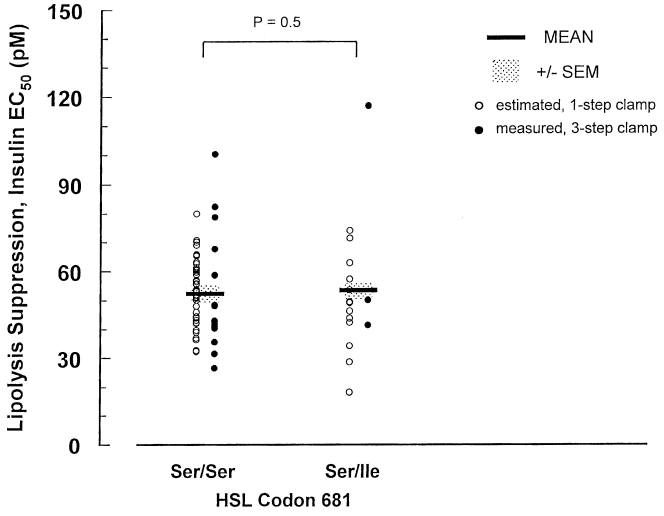

Fig. 2. Insulin sensitivity of suppression of lipolysis (insulin EC_{50}) in subjects with and without the Ser681Ile polymorphism in the HSL gene (Ser/Ser, N = 57; Ser/Ile, N = 16).

As with all negative finding the question of a type 2 error arises, in our case the probability to erroneously assume no difference between two genotypes when there is in fact a difference. On the basis of a similar population and using identical methodology, we reported an effect of the Pro12Ala polymorphism in peroxisome proliferator activated receptor γ2 on insulin sensitivity of lipolysis (10). The carriers of this polymorphism had an approximately 25% percent lower insulin EC_{50} compared with the wild-type group. In the present study the statistical power to detect a similar difference was >99%. This minimizes the risk for a major statistical type 2 error. Nevertheless, the smallest detectable difference we could have picked up in this population was 15% (power of 80%).

Our findings and the data from the literature, however, do not preclude the possibility that genetic factors play a role in the biological variation of insulin sensitivity of lipolysis. Conceivably, polymorphisms with functional relevance in other genes encoding for proteins upstream of HSL (e.g., phosphatidylinositol 3-kinase, phosphodiesterase isoforms) could be involved. It is also possible that polymorphisms in HSL contribute to the development of obesity and indirectly to insulin resistance via mechanisms other than FFA as previously suggested (20).

A number of novel aspects regarding the role of HSL were introduced with the HSL knockout mouse (29). It came as a surprise, for example, that triglyceride lipase activity in contrast to cholesterol esterase activity was unchanged in brown adipose tissue and reduced by only 50% in white adipose tissue by deletion of the HSL gene. It was consequently suggested that a second catecholamine-sensitive lipase may exist that is responsible for triglyceride hydrolysis and uncoupling protein activation, whereas the "classic" HSL is primarily responsible for cholesterol ester hydrolysis (30). These observations are, in a way, in

agreement with our data, arguing against the fact that genetically (or otherwise) determined alterations in HSL activity would affect insulin sensitivity via mechanism involving FFA availability.

In summary, we identified two previously unknown, highly prevalent amino acid polymorphisms in the coding region of the HSL gene. In our lean healthy population, however, neither the Glu620Asp polymorphism nor the Ser681Ile polymorphism contributed to the interindividual variation in insulin sensitivity of lipolysis or glucose disposal. It is possible, nevertheless, that in different populations (including obese or diabetic subjects) polymorphisms in HSL are important or that mutations in other genes encoding proteins upstream of HSL contribute to the biological variation of insulin sensitivity of lipolysis.

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