

Polymorphisms in the *SCD1* Gene: Associations With Body Fat Distribution and Insulin Sensitivity

Eva Warensjö,* Erik Ingelsson,† Per Lundmark,‡ Lars Lannfelt,† Ann-Christine Syvänen,‡ Bengt Vessby,* and Ulf Risérus*

Abstract

WARENSJÖ, EVA, ERIK INGELSSON, PER LUNDMARK, LARS LANNFELT, ANN-CHRISTINE SYVÄNEN, BENGT VESSBY, AND ULF RISÉRUS. Polymorphisms in the *SCD1* gene: associations with body fat distribution and insulin sensitivity. *Obesity*. 2007;15:1732–1740.

Objective: Obesity and insulin resistance are major risk factors for metabolic diseases and are influenced by lifestyle and genetics. The lipogenic enzyme, stearoyl-coenzyme A-desaturase (SCD), is related to obesity. Further, *SCD1*-deficient mice are protected against obesity and insulin resistance. We hypothesized that genetic polymorphisms in the *SCD1* gene would be associated with obesity, insulin sensitivity, and estimated SCD activity in humans.

Research Methods and Procedures: The study population was 1143 elderly Swedish men taking part of a population-based cohort study, the Uppsala Longitudinal Study of Adult Men. Associations between single nucleotide polymorphisms and obesity (waist circumference and BMI), insulin sensitivity (assessed by hyperinsulinemic euglycemic clamp), and estimated SCD activity (fatty acid ratios) were analyzed using linear regression analysis.

Results: Subjects homozygous for the rare alleles of *rs10883463*, *rs7849*, *rs2167444*, and *rs508384* had decreased BMI and waist circumference and improved insulin sensitivity. The rare allele of *rs7849* demonstrated the stron-

gest effect on both insulin sensitivity [regression coefficient (β) = 1.19, p = 0.007] and waist circumference (β = -4.4, p = 0.028), corresponding to 23% higher insulin sensitivity and 4 cm less waist circumference.

Conclusion: This study indicates that genetic variations in the *SCD1* gene are associated with body fat distribution and insulin sensitivity, results that accord well with animal data. These results need confirmation in other populations with a larger sample size.

Key words: stearoyl-coenzyme A-desaturase, waist circumference, insulin sensitivity, single-nucleotide polymorphism

Introduction

Development of metabolic diseases, including type 2 diabetes and cardiovascular disease, is influenced by both lifestyle and genetic factors. Obesity (mainly abdominal obesity) and insulin resistance are risk factors for metabolic diseases. They are highly correlated, but a link between these two factors does not necessarily mean that one factor causes the other but could imply that they are caused by a common factor (1). Metabolic diseases are associated with aberrations in intracellular lipid metabolism, frequently leading to ectopic fat accumulation (e.g., liver and muscle). This involves partitioning between de novo lipid synthesis and oxidation of lipids. The enzyme stearoyl-coenzyme A-desaturase (SCD)¹ has been proposed as a key regulator (2) of both these processes.

Epidemiological studies have suggested that a disturbed fatty acid composition pattern in body tissues and high estimated activity of SCD are related to metabolic diseases (3–5). Fatty acid composition is influenced by dietary fat, as well as endogenous fatty acid metabolism, in which Δ -de-

Received for review October 27, 2006

Accepted in final form December 20, 2006.

The costs of publication of this article were defrayed, in part, by the payment of page charges. This article must, therefore, be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*Clinical Nutrition and Metabolism, Department of Public Health and Caring Sciences, †Geriatrics, Department of Public Health and Caring Sciences, and ‡Molecular Medicine, Department of Medical Sciences, Uppsala University, Sweden.

Address correspondence to Eva Warensjö, Clinical Nutrition and Metabolism, Department of Public Health and Caring Sciences, Uppsala University, Uppsala Science Park, 751 85 Uppsala, Sweden.

E-mail: eva.warensjo@pubcare.uu.se

Copyright © 2007 NAASO

¹ Nonstandard abbreviations: SCD, stearoyl-coenzyme A-desaturase; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; SNP, single-nucleotide polymorphism; WC, waist circumference; LD, linkage disequilibrium; HWE, Hardy-Weinberg equilibrium.

saturases, e.g., SCD, are involved (3). SCD catalyzes the synthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acids (SFAs). MUFAs are important for maintaining lipid structures (e.g., phospholipids, cholesterol esters), and as mediators of signal transduction, cellular differentiation, and apoptosis (6). Further, MUFAs improve insulin sensitivity in humans (7). *SCD1*-deficient mice have higher energy metabolism and improved insulin sensitivity and are resistant to diet-induced obesity (8), and studies in rodents have revealed that SCD enzyme activity is high in conditions including obesity (9), diabetes, and insulin resistance (10). Two human *SCD* genes, *SCD1* and *SCD5*, exist and *SCD1* shares 85% homology with murine *SCD* genes (11). Recently, it was observed that the relative mRNA expression level of *SCD1* was 3-fold in skeletal muscle from extremely obese compared with lean subjects (12). The increased expression corresponded to changes in fatty acid composition as measured by estimated SCD activity and suggested a possible genetic predisposition for the association between *SCD1* expression and obesity (12). SCD has been proposed as a possible site for a future therapeutic agent in weight control (13). Using a population-based cohort of elderly men, including a large sample of insulin sensitivity assessments (euglycemic clamp), we hypothesized that genetic polymorphisms (single-nucleotide polymorphisms, SNPs) in the *SCD1* gene would be associated with obesity, insulin sensitivity, and estimated SCD-activity, all known risk factors for type 2 diabetes and cardiovascular disease.

Research Methods and Procedures

Subjects

The participants in the present study took part in a population-based cohort study, the Uppsala Longitudinal Study of Adult Men (<http://www.pubcare.uu.SE/ULSAM/>). For the present analyses, data from 1143 men at age 70 with a valid DNA sample were used. The study was approved by the Ethics Committee at Uppsala University, Sweden, and all participants gave their informed consent.

Investigations

All clinical and biochemical investigations have been described previously (14,15). BMI was calculated as weight (kg) divided by height (m) squared. Waist circumference (WC) was measured midway between the lowest rib and the iliac crest in the supine position. SCD activity was estimated as the product to precursor fatty acid ratio according to the following: 16:1 [n-7]/16:0 and 18:1 [n-9]/18:0 as previously described (3,4). Fatty acid analysis was carried out as previously described (16). Insulin sensitivity was determined by the golden standard euglycemic hyperinsulinemic clamp technique according to DeFronzo et al. (17), slightly modified. Briefly, insulin was infused in a primary dose during

the initial 10 minutes and then as a continuous infusion for 110 minutes. The infusion rate was 56 mU (instead of 40 mU) per minute per body surface area (meters squared) to achieve nearly complete suppression of hepatic glucose output (18). The level of plasma glucose was maintained at 5.1 mM/L by glucose infusion. The total amount of glucose infused serves as the sensitivity to the existing plasma insulin concentrations. The glucose disposal [M ($\text{mg} \times \text{kg}^{-1} \times \text{min}^{-1}$)] was calculated as the amount of glucose taken up by during the last 60 minutes of the study. Tissue insulin sensitivity (amount of glucose metabolized per unit of plasma insulin) was also calculated as the insulin sensitivity index [MI ($\text{mg} \times \text{kg}^{-1} \times \text{min}^{-1} / 100 \text{ mgU/L}$)], where M is the glucose disposal and I is the mean insulin concentration during the clamp.

SNP Selection and Genotyping

Eight SNPs were selected from the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) to be evenly distributed over a region, including the *SCD1* gene and ~10 kb upstream and 2 kb downstream of the gene. Most SNPs in this area are in high pairwise linkage disequilibrium (LD) with each other. Judging by the HapMap data, the markers genotyped represent the gene variation satisfactory for SNPs with a minor allele frequency below 44%. Using the HapMap data as a reference, the genotyped SNPs (except

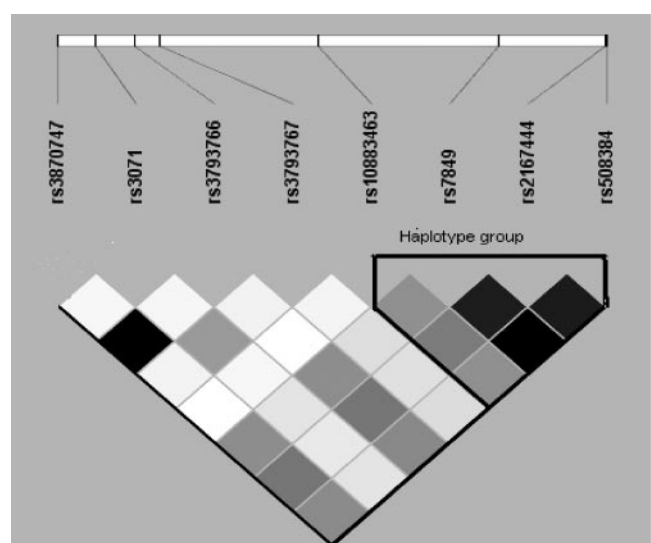


Figure 1: The pairwise linkage disequilibrium (LD) expressed as r^2 between the investigated stearoyl-coenzyme A-desaturase 1 single nucleotide polymorphisms (SNPs) in the Haploview software setting. Shading represents magnitude and significance of the pairwise r^2 . Black, r^2 0.8 (high r^2); dark grey, r^2 between 0.8 and 0.6; moderate grey, r^2 between 0.4 and 0.6; and white, r^2 < 0.2 (low r^2). The haplotype group was constructed of *rs10883463*, *rs7849*, *rs2167444*, and *rs508384*.

Table 1. Clinical characteristics of study population

Variable	n	Mean	SD
BMI (kg/m ²)	1215	26.3	3.4
Waist circumference (cm)	1192	94.8	9.7
Systolic BP (mmHg)	1216	146.8	18.5
Diastolic BP (mmHg)	1216	83.8	9.5
Fasting insulin (mU/L)*	1206	11.1	(10.8; 11.5)
Fasting glucose (mM/L)*	1219	5.6	(5.57; 5.70)
M value (mg × kg ⁻¹ × min ⁻¹)†	1160	5.2	2.5
SCD (16:1[n-7]/16:0)*‡	611	0.31	(0.30; 0.31)
SCD (18:1[n-9]/18:0)*‡	611	22.3	4.9

SD, standard deviation; BP, blood pressure; M value, insulin sensitivity; SCD, stearoyl-coenzyme A-desaturase; CI, confidence interval.

* Skewed variable presented with geometric mean and 95% CI.

† Insulin sensitivity measured by hyperinsulinemic euglycemic clamp.

‡ Estimated as the product to precursor fatty acid ratio.

rs2167444, which has not been genotyped in the HapMap project), could tag 22 of 29 SNPs in the region.

The SNPs were genotyped at the SNP technology platform at Uppsala University (<http://www.genotyping.SE/>) using the 12-plex GenomeLab SNPStream system (Beckman Coulter, Inc., Fullerton, CA) (19). Primer sequences are available from the authors on request. The quality of the genotype data were assessed by testing for Hardy-Weinberg equilibrium (HWE) using the χ^2 distribution for each assay. All SNPs conferred to HWE. The overall genotype call rate was 96.7%, and the accuracy was 99.96% according to duplicate analysis of, on average, 40% (4723 of 11,870) of the genotypes.

Haplotype Estimation

Haplotypes were estimated using the PHASE software (20). One group of haplotypes was estimated by selecting four SNPs in strong LD based on pairwise marker comparisons (r^2) in the Haploview software, version 3.2 (21) (Figure 1). To account for uncertain phase in the haplotype estimations, only haplotypes with the probability of >0.98 were included in the statistical analysis.

Statistical Analysis

Continuous, normally distributed variables are presented as mean \pm standard deviation. Skewed variables (fasting insulin, fasting glucose, and SCD-16) are presented with geometric

Table 2. SCD SNPs studied in the ULSAM population

db SNP ID	Relative to ATG (+1)*	Role	MAF	Genotypes†		
<i>rs3870747</i>	6417	Intron	0.069	CC 921 (86.6)	TC 139 (13)	TT 4 (0.4)
<i>rs3071</i>	7202	Intron, Exon/intron boundaries	0.342	AA 463 (42.7)	AC 501 (46.2)	CC 120 (11.1)
<i>rs3793766</i>	7981	Intron	0.072	CC 963 (85.9)	CT 150 (13.8)	TT 4 (0.3)
<i>rs3793767</i>	8484	Intron	0.393	AA 367 (36.4)	AG 490 (48.6)	GG 151 (15)
<i>rs10883463</i>	11692	Intron	0.068	TT 976 (86.5)	CT 148 (13.1)	CC 5 (0.4)
<i>rs7849</i>	15341	Exon 6	0.165	TT 758 (69.5)	CT 305 (28)	CC 28 (2.5)
<i>rs2167444</i>	17482	3'UTR	0.141	TT818 (72.9)	AT 283 (25.2)	AA 21 (1.9)
<i>rs508384</i>	17499	3'UTR	0.166	CC 727 (69)	AC 299 (28)	AA 31 (3.0)

SCD1, stearoyl-coenzyme A-desaturase 1; SNP, single-nucleotide polymorphism; ULSAM, Uppsala Longitudinal Study of Adult Men; MAF, minor allele frequency.

* From the translation initiation site (ATG = +1).

† Data for genotypes are the number (%) of subjects.

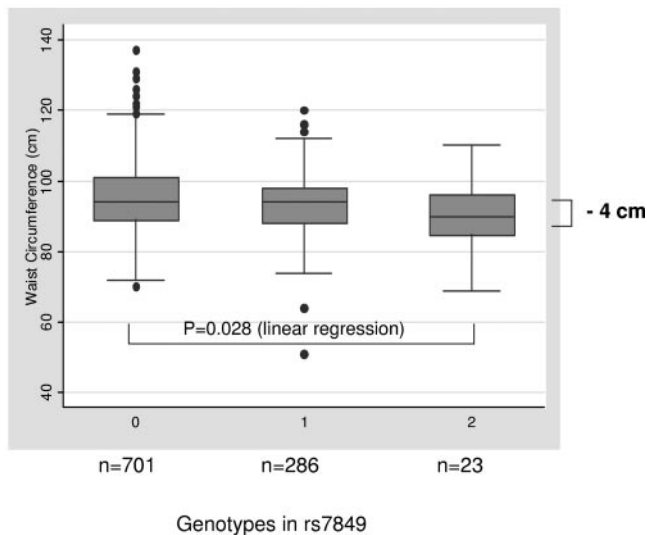


Figure 2: The waist circumference (WC, cm) in subjects who were homozygous for the common allele, heterozygous, or homozygous for the rare allele of *rs7849*. The box represents the minimum, 25th percentile, the median, 75th percentile, and the maximum of WC within each group of subjects. Subjects homozygous for the rare allele had, on average, 4 cm less WC compared with subjects homozygous for the common allele. 0, homozygous for the common allele, TT; 1, heterozygous allele, CT; 2, homozygous for rare allele, CC.

mean and 95% confidence intervals and were log-transformed before analysis. Associations between genotypes, SNPs and haplotypes, and phenotypes were analyzed in a linear regression model. General (co-dominant, stepwise test) and additive (trend test) genetic models, using the most common homozygous allele (for each SNP) and the non-carrier of a specific haplotype as reference level, were carried out.

We performed multivariate linear models adjusting the SNP (*rs7849*) that showed the strongest association with insulin sensitivity for BMI and WC, as well as for concomitant comorbidity and drug use (hypertension treatment, lipid medication, diabetes prevalence, and variables based on questionnaire questions). Furthermore, to investigate whether *rs7849* and insulin sensitivity was modified by the level of obesity, the study population was stratified into tertiles of BMI and WC and a linear regression model was carried out on these strata, separately. The association between *rs7849* and WC was also adjusted for insulin sensitivity.

To account for possible false positive tests due to multiple testing, an adjusted significance level using Meff correction (22) was calculated. *p* Values were evaluated by comparing them with the conventional significance level ($p < 0.05$) and the adjusted α level = 0.0076. The statistical analysis was carried out with STATA, version 8.2 (StataCorp LP., College Station, TX), and the adjusted α level was calculated with a macro in SAS, version 8.0 (SAS Institute, Inc., Cary, NC).

Results

Clinical Characteristics and SNPs

The clinical characteristics of the study sample are presented in Table 1. The number of observations was much lower ($n = 611$) for the estimated SCD activity than for other variables since the fatty acid analyses were only carried out in a randomly selected subsample. Information regarding the investigated SNPs is presented in Table 2. None of the SNPs caused an amino acid change in the protein.

SNPs and Phenotypes

Obesity (WC and BMI) and insulin sensitivity (M value, $n = 986$) were associated with the same SNPs, but with effects in opposite directions. *rs10883463*, *rs7849*, *rs2167444*, and *rs508384* were all negatively associated with obesity and positively associated with insulin sensitivity. All associations between obesity, insulin sensitivity, and the above mentioned SNPs indicated an additive effect of each copy of the allele. Subjects homozygous for the rare allele had both lower BMI and WC and higher insulin sensitivity. The strongest effect on WC was observed for the homozygous C allele in *rs7849* (β (regression coefficient) = -4.4 , $p = 0.028$, Figure 2). The same alleles were also associated with improved insulin sensitivity ($\beta = 1.19$, $p = 0.007$, Figure 3). As illustrated in Figures 2 and 3, the rare allele (CC) of *rs7849* was associated with 4 cm less

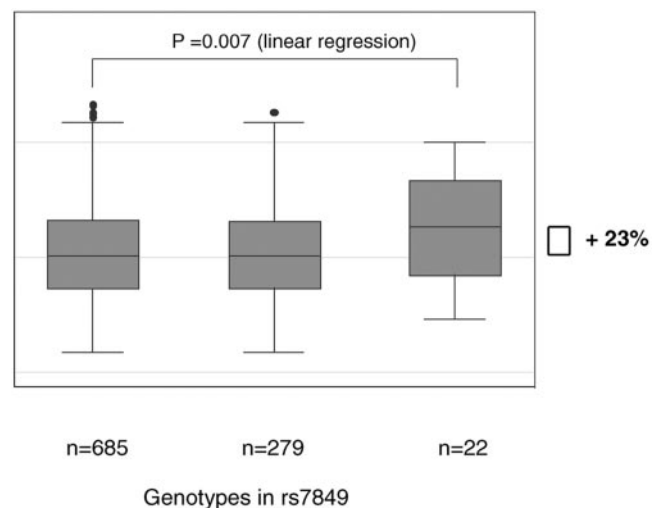


Figure 3: The insulin sensitivity ($\text{mg} \times \text{kg}^{-1} \times \text{min}^{-1}$) in subjects who were homozygous for the common allele, heterozygous, or homozygous for the rare allele of *rs7849*. The box represents the minimum, 25th percentile, the median, 75th percentile, and maximum of insulin sensitivity within each group of subjects. Subjects homozygous for the rare allele had, on average, 23% higher insulin sensitivity compared with subjects homozygous for the common allele. 0, homozygous for the common allele, TT; 1, heterozygous allele, CT; 2, homozygous for rare allele, CC.

Table 3. Associations between SNPs and phenotypes

	WC (cm) [β (<i>p</i>)]	BMI (kg/m ²) [β (<i>p</i>)]	M value (mg × kg ⁻¹ × min ⁻¹) [β (<i>p</i>)]	SCD ratio (16:1[n-7]/16:0) [β (<i>p</i>)]
<i>rs3870747</i>				
0→1	-0.85 (0.34)	-0.24 (0.44)	0.05 (0.79)	-0.025 (0.48)
0→2	-5.65 (0.23)	-1.38 (0.41)	0.27 (0.79)	—
Trend	-1.08 (0.20)	-0.29 (0.32)	0.06 (0.74)	-0.025 (0.49)
<i>rs3071</i>				
0→1	0.98 (0.12)	0.32 (0.14)	-0.30 (0.032)	0.001 (0.95)
0→2	-0.66 (0.52)	-0.11 (0.36)	0.24 (0.3)	-0.077 (0.048)
Trend	0.13 (0.77)	0.08 (0.61)	-0.27 (0.79)	-0.025 (0.15)
<i>rs3793766</i>				
0→1	-0.70 (0.41)	-0.14 (0.64)	0.04 (0.84)	0.009 (0.80)
0→2	-5.60 (0.23)	-1.38 (0.41)	0.27 (0.79)	—
Trend	-0.93 (0.25)	-0.20 (0.48)	0.05 (0.79)	0.009 (0.80)
<i>rs3793767</i>				
0→1	0.84 (0.21)	0.16 (0.50)	-0.14 (0.36)	-0.001 (0.96)
0→2	0.62 (0.51)	0.06 (0.85)	0.015 (0.94)	0.073 (0.051)
Trend	0.42 (0.34)	0.06 (0.71)	-0.024 (0.81)	0.29 (0.10)
<i>rs10883463</i>				
0→1	-1.9 (0.034)	-0.4 (0.18)	0.16 (0.41)	-0.042 (0.23)
0→2	-5.7 (0.18)	-2.3 (0.12)	1.6 (0.081)	0.24 (0.37)
Trend	-2.0 (0.015)	-0.5 (0.071)	0.25 (0.16)	-0.032 (0.35)
<i>rs7849</i>				
0→1	-1.1 (0.094)	-0.26 (0.26)	-0.006 (0.97)	-0.20 (0.45)
0→2	-4.4 (0.028)	-1.3 (0.062)	1.19 (0.007)	0.15 (0.16)
Trend	-1.4 (0.014)	-0.4 (0.063)	0.16 (0.20)	-0.003 (0.91)
<i>rs2167444</i>				
0→1	-1.2 (0.065)	-0.28 (0.23)	0.024 (0.87)	-0.03 (0.26)
0→2	-3.7 (0.1)	-1.3 (0.11)	1.11 (0.022)	0.017 (0.86)
Trend	-1.4 (0.020)	-0.4 (0.079)	0.16 (0.23)	-0.02 (0.37)
<i>Rs508384</i>				
0→1	-1.2 (0.063)	-0.32 (0.18)	0.006 (0.97)	-0.02 (0.40)
0→2	-4.0 (0.036)	-1.1 (0.10)	0.89 (0.029)	0.10 (0.25)
Trend	-1.5 (0.010)	-0.4 (0.052)	0.15 (0.23)	-0.004 (0.86)

SNP, single-nucleotide polymorphism; WC, waist circumference; SCD, stearoyl-coenzyme A-desaturase.

0, most common allele was used as reference in the linear regression model; 0→1, heterozygote allele vs. 0; 0→2, homozygote for rare allele vs. 0; —, dropped because no observations available; β , beta regression coefficient obtained in linear regression analysis; M value, insulin sensitivity as assessed with hyperinsulinemic euglycemic clamp technique.

WC and 23% higher insulin sensitivity. The associations between each SNP and BMI followed the same pattern as between WC and SNPs but did not reach significance. If the M/I value was used instead of the M value in the analyses, the β coefficients and *p* values were in the same range (data not shown). The estimated SCD activity (16:1 [n-7]/16:0,

n = 489) was associated with CC in *rs3071* and to GG in *rs3793767* but the effects were minor (Table 3).

When the association between the M value and *rs7849* was adjusted for BMI, the association of the homozygous rare allele was still significant when the conventional α level was considered (β coefficient = 0.75, *p* = 0.034, data

Table 4. Effect of *rs7849* on insulin sensitivity (M value, $\text{mg} \times \text{min}^{-1} \times \text{kg}^{-1}$) when the study population was stratified into tertiles of WC

Tertiles of WC (cm) (mean; range)	TT reference	CT β (p)	CC β (p)	p for trend	n
I (85; 51–90)	—	−0.25 (0.24)	1.21 (0.023)	0.027	333
II (95; 91–98)	—	−0.07 (0.72)	−0.43 (0.53)	0.78	360
III (106; 99–138)	—	−0.045 (0.83)	0.68 (0.42)	0.70	283

WC, waist circumference; β , beta regression coefficient obtained in linear regression analysis.

not shown). However, after adjustment for WC, the significance was lost (β coefficient = 0.62, $p = 0.084$, data not shown). Concomitant comorbidity and drug use did not influence this association. Further, when the study population was stratified into tertiles of BMI and WC, the stepwise test demonstrated that the association between *rs7849* and insulin sensitivity was not associated with BMI (data not shown). However, subjects in the lowest tertile of WC (85 ± 5 cm, mean \pm standard deviation) homozygous for the rare allele of *rs7849* had higher insulin sensitivity (β coefficient = 1.21, $p = 0.023$) (Table 4). When the associations between *rs7849* and WC were adjusted for insulin sensitivity, the associations were attenuated compared with the univariable analysis (β coefficient = -1.12 , $p = 0.036$ for heterozygotes of the rare allele and β coefficient = -1.10 , $p = 0.51$ for homozygotes, as compared with homozygotes of the common allele, data not shown).

Haplotypes and Phenotypes

Three haplotypes (I, II, and III) with a frequency of >0.05 were estimated (Table 5), where haplotype I was the most common haplotype. Subjects heterozygous (one copy) for I and III were associated with higher obesity and lower obesity, respectively. The effect was strongest between the haplotypes and WC, but the effect on BMI followed the same pattern and did not reach significance. The direction of the association between the M value and I and III was opposite of that observed between obesity and haplotypes. The effect of an extra copy of the haplotypes was additive (Table 6).

Discussion

This study investigated the effect of polymorphisms in the *SCD1* gene on obesity and directly measured insulin sensitivity, and the results add new information regarding intermediate traits of metabolic disease. These results accord well with animal data; mice with a disrupted *SCD1* gene have reduced adiposity and increased insulin sensitivity and are resistant to diet induced weight gain (8). Each added copy of the rare allele of *rs10883463*, *rs7849*, *rs2167444*, and *rs508384* was associated with lower BMI and WC and improved insulin sensitivity. Being homozygous for the rare allele (C) of *rs7849* demonstrated the strongest effect on both insulin sensitivity ($\beta = 1.19$, $p = 0.007$) and abdominal obesity ($\beta = -4.4$, $p = 0.028$). Subjects with this gene variant had, on average, 23% higher insulin sensitivity and 4 cm reduced waist circumference, which might be clinically significant differences. The association between the rare gene variant of *rs7849* and insulin sensitivity was still significant when the multiple comparison α level was considered. Given the location of *rs7849*, in exon 6, this might indicate that the SNP is located in an important functional part of the gene. However, an association between a SNP and a phenotype does not necessarily mean a casual link between that variation and the phenotype, because the association might be caused by LD (23) to another functional polymorphism or to a nearby gene. In the present study, the haplotype-phenotype associations indicated that haplotype I (consisting of the common alleles)

Table 5. Haplotypes estimated in the *SCD1* gene

Haplotype	<i>rs3870747</i>	<i>rs3071</i>	<i>rs3793766</i>	<i>rs3793767</i>	<i>rs10883463</i>	<i>rs7849</i>	<i>rs2167444</i>	<i>rs508384</i>	Hap f
I	—	—	—	—	T	T	T	C	0.82
II	—	—	—	—	T	C	T	A	0.077
III	—	—	—	—	C	C	A	A	0.071

SCD1, stearoyl-coenzyme A-desaturase 1; Hap f, haplotype frequencies in the studied population.

Table 6. Associations between haplotypes and phenotypes

Haplotype	WC (cm) [β (<i>p</i>)]	BMI (kg/m ²) [β (<i>p</i>)]	M value (mg × kg ⁻¹ × min ⁻¹) [β (<i>p</i>)]	SCD ratio (16:1[n-7]/16:0) [β (<i>p</i>)]
I				
0→1	2.75 (0.17)	1.0 (0.15)	-0.96 (0.032)	-0.04 (0.66)
0→2	3.8 (0.057)	1.26 (0.066)	-0.95 (0.029)	-0.17 (0.86)
Trend	1.26 (0.027)	0.37 (0.068)	-0.13 (0.30)	0.02 (0.39)
II				
0→1	-0.75 (0.38)	-0.22 (0.48)	0.06 (0.73)	-0.004 (0.91)
0→2	-1.2 (0.75)	-0.09 (0.94)	-0.24 (0.76)	0.016 (0.91)
Trend	1.26 (0.36)	-0.19 (0.50)	0.32 (0.85)	-0.002 (0.96)
III				
0→1	-1.9 (0.035)	-0.45 (0.15)	0.16 (0.40)	-0.6 (0.096)
0→2	-5.7 (0.18)	-2.3 (0.13)	1.6 (0.082)	0.23 (0.36)
Trend	-2.0 (0.015)	-0.55 (0.059)	0.26 (0.16)	-0.05 (0.17)

WC, waist circumference; SCD, stearoyl-coenzyme A-desaturase; β , beta regression coefficient obtained in linear regression analysis; 0, non-carriers of the specific haplotype were used as reference in the linear regression model; 0→1, heterozygote for the specific haplotype vs. 0; 0→2, homozygote for the specific haplotype vs. 0; M value, insulin sensitivity as assessed with hyperinsulinemic euglycemic clamp technique.

was significantly associated with an increased WC and decreased insulin sensitivity, while haplotype III (consisting of the rare alleles) was significantly associated with decreased WC. The frequency of homozygous carriers for the uncommon haplotype (III) was low (only 4 in the entire sample), rendering non-significant associations with WC when compared with non-carriers.

It is possible that the effect of the rare allele of *rs7849* and insulin sensitivity might be mediated by the effect of *rs7849* on adiposity. The relationship between *rs7849* and insulin sensitivity remained after adjustment for BMI but was lost after adjustment for WC, indicating that abdominal obesity, rather than general obesity, might mediate this association. The same pattern was observed when the study population was stratified into tertiles of BMI and WC. The stratification on BMI did not indicate any effect on the association between *rs7849* and insulin sensitivity. Only subjects homozygous for the rare allele of *rs7849* in the lowest tertile of WC had improved insulin sensitivity. Moreover, the haplotype-phenotype analysis indicated that the rare alleles were more strongly related to WC than to insulin sensitivity. Thus, it is possible that the effect of *rs7849* on insulin sensitivity is secondary to abdominal obesity (WC), although this is difficult to disentangle in this study. Conversely, if the association between *rs7849* and WC was adjusted for insulin sensitivity, the effect remained significant in heterozygous individuals. Taken together, these data suggest

a possible direct relationship between *rs7849* and WC but an indirect relationship between *rs7849* and insulin sensitivity. In line with our observations, earlier studies have suggested that abdominal obesity is more genetically determined than general obesity (24). Also, we have previously reported estimated activity of SCD to be significantly associated with the development of metabolic syndrome, but the association was abolished when adjusted for obesity (4), which support the possibility that the effect of *rs7849* (and other SNPs) would primarily be on obesity. Also, the key function of SCD is to regulate lipogenesis, whereas effects on glucose metabolism are thought to be secondary.

A previous study by Liew et al. (25) investigated the association between SCD variation and type 2 diabetes but failed to detect any significant associations. However, the authors pointed out that failure to detect significant associations in their study did not exclude the possibility that the investigated SCD variants (or other) influence intermediate traits relevant in the etiology of type 2 diabetes. Indeed, we found significant associations between SCD polymorphism and metabolic traits. Only one SNP (*rs3071*) investigated in the present study was also studied by Liew et al. (25), and it demonstrated only a weak association to insulin sensitivity, which might explain the different results.

The effects of genotypes on the estimated SCD ratio (16:1 [n-7]/16:0) were small, uncertain, borderline significant, and somewhat disappointing, because the estimated

SCD activity is regarded as a surrogate measure of SCD activity (3). These results should, however, be interpreted cautiously. First, fatty acid composition was only assessed in a subsample of the study population suggesting decreased power. Second, SCD activity is estimated and is not a true enzyme activity. Third, the activity was estimated in serum cholesterol esters and not in target tissues, i.e., adipose tissue, liver, or skeletal muscle. The estimated SCD activity can also be estimated from 18:1 (n-9)/18:0. If this ratio was used in the analysis, no significant associations were detected. This might seem surprising because the preferential substrate for SCD is 18:0 (6). However, the lack of associations between SNPs in *SCD* and 18:1 (n-9)/18:0 might be explained by the higher dietary content of 18:1, compared with 16:1. This would lead to a “dilution” of the index by exogenous 18:1 and, thus, result in a worse reflection of the desaturation by SCD.

To be able to detect effects of a genetic polymorphism, the sample size needs to be sufficient (26). The present study population was not very large but homogeneous (sex, age, and geographically matched) and of the same ethnic background, decreasing the risk for population stratification. The consistency in the results indicates that the associations are probably true findings and not generated by chance. Also, the haplotype analyses indicated a possible positive effect of the rare alleles. Further, all analyses were specified a priori and hypothesis-driven, and adjustments for multiple testing were made. A major strength was our detailed phenotype assessment, i.e., fatty acid analyses and the large sample of golden standard measurement of insulin sensitivity support our results. The mechanisms behind the associations remain to be elucidated, and the results and clinical importance need to be verified in future studies.

There are several limitations of this study. First, the cohort consisted of only men, and the data are observational. Second, even though the 8 SNPs examined in this study adequately capture most of the genetic variability in the *SCD1* gene, it should be noted that we might lack possible genetic variation. Also, replication of the results in another cohort would be ideal but unfeasible at the present stage because we are unaware of any cohort with both clamp and fatty acid data.

This study suggests that variations in the *SCD1* gene are associated with obesity (especially abdominal obesity) and insulin sensitivity, in line with previous data in *SCD* deficient mice. In summary, genetic polymorphisms in the *SCD1* gene are associated with decreased WC and improved insulin sensitivity that in theory could protect against obesity-related diseases. However, this possibility should be further investigated to determine the role of *SCD* as a possible candidate gene for obesity control and obesity-related diseases.

Acknowledgments

The authors thank Marie Lindersson for technical assistance in SNP genotyping. The SNP platform was supported by the K&A Wallenberg Foundation via Wallenberg Consortium North. The study was funded by Wallenberg Consortium North, the Swedish Society for Medical Research, and the Swedish Nutrition Foundation.

References

1. **Frayn KN.** Visceral fat and insulin resistance: causative or correlative? *Br J Nutr.* 2000;83(Suppl 1):71–7.
2. **Sampath H, Ntambi JM.** Stearoyl-coenzyme A desaturase 1, sterol regulatory element binding protein-1c and peroxisome proliferator-activated receptor- α : independent and interactive roles in the regulation of lipid metabolism. *Curr Opin Clin Nutr Metab Care.* 2006;9:84–8.
3. **Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A.** Desaturation and elongation of fatty acids and insulin action. *Ann NY Acad Sci.* 2002;967:183–95.
4. **Warensjö E, Riserus U, Vessby B.** Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia.* 2005;48:1999–2005.
5. **Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH.** Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr.* 2003;78:91–8.
6. **Ntambi JM, Miyazaki M.** Regulation of stearoyl-CoA desaturases and role in metabolism. *Prog Lipid Res.* 2004; 43:91–104.
7. **Vessby B, Uusitupa M, Hermansen K, et al.** Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU Study. *Diabetologia.* 2001;44:312–9.
8. **Ntambi JM, Miyazaki M, Stoehr JP, et al.** Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci USA.* 2002;99:11482–6.
9. **Cohen P, Miyazaki M, Soccia ND, et al.** Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science.* 2002;297:240–3.
10. **Voss MD, Beha A, Tennagels N, et al.** Gene expression profiling in skeletal muscle of Zucker diabetic fatty rats: implications for a role of stearoyl-CoA desaturase 1 in insulin resistance. *Diabetologia.* 2005;48:2622–30.
11. **Wang J, Yu L, Schmidt RE, et al.** Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. *Biochem Biophys Res Commun.* 2005;332:735–42.
12. **Hulver MW, Berggren JR, Carper MJ, et al.** Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. *Cell Metab.* 2005;2:251–61.
13. **Dobrzyn A, Ntambi JM.** Stearoyl-CoA desaturase as a new drug target for obesity treatment. *Obes Rev.* 2005;6: 169–74.
14. **Vessby B, Tengblad S, Lithell H.** Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. *Diabetologia.* 1994;37:1044–50.

15. **Byberg L, Siegbahn A, Berglund L, et al.** Plasminogen activator inhibitor-1 activity is independently related to both insulin sensitivity and serum triglycerides in 70-year-old men. *Arterioscler Thromb Vasc Biol.* 1998;18:258–64.
16. **Öhrvall M, Tengblad S, Gref CG, et al.** Serum alpha tocopherol concentrations and cholesterol ester fatty acid composition in 70-year-old men reflect those 20 years earlier. *Eur J Clin Nutr.* 1996;50:381–5.
17. **DeFronzo RA, Tobin JD, Andres R.** Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237:E214–23.
18. **Pollare T, Vessby B, Lithell H.** Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. *Arterioscler Thromb.* 1991;11:1192–203.
19. **Bell PA, Chaturvedi S, Gelfand CA, et al.** SNPstream UHT: ultra-high throughput SNP genotyping for pharmacogenomics and drug discovery. *Biotechniques.* 2002;(suppl):70–7.
20. **Stephens M, Smith NJ, Donnelly P.** A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet.* 2001;68:978–89.
21. **Barrett JC, Fry B, Maller J, Daly MJ.** Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21:263–5.
22. **Nyholt DR.** A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet.* 2004;74:765–9.
23. **Palmer LJ, Cardon LR.** Shaking the tree: mapping complex disease genes with linkage disequilibrium. *Lancet.* 2005;366:1223–34.
24. **Bouchard C, Tremblay A, Despres JP, et al.** The response to long-term overfeeding in identical twins. *N Engl J Med.* 1990;322:1477–82.
25. **Liew CF, Groves CJ, Wiltshire S, et al.** Analysis of the contribution to type 2 diabetes susceptibility of sequence variation in the gene encoding stearoyl-CoA desaturase, a key regulator of lipid and carbohydrate metabolism. *Diabetologia.* 2004;47:2168–75.
26. **Hattersley AT, McCarthy MI.** What makes a good genetic association study? *Lancet.* 2005;366:1315–23.