Single nucleotide polymorphisms in ABCG5 and ABCG8 are associated with changes in cholesterol metabolism during weight loss[S]

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Abstract **The purpose of this study was to examine whether changes in cholesterol metabolism after weight loss were affected by single nucleotide polymorphisms (SNPs) in ABCG5 and ABCG8 genes. Thirty-five hypercholesterolemic women lost 11.7 ± 2.5 kg ($P < 0.001$). Cholesterol kinetics were assessed using stable isotope techniques. TaqMan PCR was used to detect SNPs in ABCG5/G8. Homozygous Q604E variants in ABCG5 had larger ($P < 0.05$) reductions in cholesterol absorption and greater increases ($P < 0.05$) in synthesis in contrast to heterozygous and homozygous wild-type carriers. Heterozygous C54Y carriers had smaller declines ($P = 0.047$) in synthesis compared with homozygous variant individuals. The presence of at least one Y54 variant was associated with higher ($P = 0.042$) post-weight-loss synthesis compared with carriers of the C54 genotype. The direction of the results is consistent with cross-sectional studies on the effects of Q604E and C54Y polymorphisms on plasma cholesterol. SNPs in ABCG5/G8 were found to be associated with the response of cholesterol metabolism to weight loss. The evidence for associations between SNPs in ABCG5/G8 and various parameters of cholesterol metabolism indicates the potential effectiveness of establishing genetic screening tools to determine optimal lipid-lowering treatment routes for individuals during weight reduction.**—Santosa, S., I. Demonty, A. H. Lichtenstein, J. M. Ordovas, and P. J. H. Jones. **Single nucleotide polymorphisms in ABCG5 and ABCG8 are associated with changes in cholesterol metabolism during weight loss.** ***J. Lipid Res.*** **2007.** 48: **2607–2613.**

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A common comorbidity of obesity is cardiovascular disease, often resulting from chronic underlying dyslipidemia. Obesity-associated dyslipidemia is likely caused by perturbed cholesterol metabolism, which is commonly seen in these individuals (1–3). It has been well established that weight loss ameliorates dyslipidemia in obese individuals (4). Weight loss has been associated with changes in cholesterol metabolism, including decreases in cholesterol synthesis that are not compensated for by corresponding increases in cholesterol absorption (1, 2, 5, 6). On the other hand, the extent to which individuals are able to decrease cholesterol levels through weight loss may be genetically predetermined (7).

Cholesterol homeostasis is maintained through the balance of cholesterol absorption, biosynthesis, and turnover. An outstanding characteristic of cholesterol absorption is its large range among individuals (8–10). If human variability is as large as it appears to be, a great potential exists for other exogenous and endogenous factors such as genetics to affect cholesterol absorption. ABCG5 (MIM 605459) and ABCG8 (MIM 605460) are cholesterol half-transporters that have been identified to form functional heterodimers (11) in apical membranes of hepatocytes and enterocytes in the proximal small intestine. ABCG5 and ABCG8 function together to regulate cholesterol kinetics in humans (12). Indeed, expression of these transporters from 13 exon genes serves to reduce net cholesterol absorption by promoting the efflux of cholesterol from the enterocyte into the intestinal lumen. This, in turn, results in increased hepatic cholesterol synthesis (12). Genetic mutations in DNA that encodes these proteins may affect the efficacy with which ABCG5 and ABCG8 function, as mutations in these transporters have been associated with sitosterolemia and hypercholesterolemia (11, 13–15). Thus,

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genetic mutations in ABCG5 and ABCG8 may affect the response to cholesterol-lowering therapies (14, 15).

Recently, several single nucleotide polymorphisms (SNPs) were identified in introns and exons of ABCG5 and ABCG8 (11, 13, 16, 17). More specifically, these SNPs include Q604E (RS6720173), I18427 (RS4148189), I7892 (RS4131229), and M216 (RS3806471) in ABCG5 and C54Y (RS4148211), D19H (RS11887534), T400K (RS4148217), and I14222 (RS6709904) in ABCG8 (11, 13, 16, 17). Data are now available on the potential relationship between these SNPs and dyslipidemia. Observational data suggest that total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) concentrations are higher in individuals with the D19H wild type compared with individuals with at least one mutant variant (18). Again, focusing on the D19H SNP treatment with 10 mg of atorvastatin showed that individuals who had at least one variant allele had lower pretreatment TC concentration and lower posttreatment TC and LDL-C concentrations (15). In a cross-sectional study, Weggemans et al. (19) reported that individuals homozygous for the wild type of the Q604E SNP of ABCG5 had TC concentrations that were lower than those of carriers of at least one mutant allele. Another trial that examined the effects of decreased animal fat intake over an 8 year period found that the C54Y variant in ABCG8 was associated with greater decreases in TC and LDL-C concentrations in female carriers compared with the wild type (13). Using the same data set, Hubacek et al. (13) reported that male participants homozygous for the threonine allele had a greater decrease in TC and LDL-C concentrations over 8 years compared with individuals who had at least one K400 mutation.

Although dyslipidemia and perturbed cholesterol metabolism are common in overweight individuals, no study has examined the role of SNPs in ABCG5 and ABCG8 on cholesterol metabolism across weight loss. Understanding the role that these SNPs may play in affecting the responsiveness of cholesterol kinetics during weight loss will help to establish a model of cholesterol metabolism as well as genetic screening tools to determine optimal treatment routes for these patients. Therefore, the objective of this study was to examine whether changes in metabolism across weight loss were affected by common SNPs in ABCG5 and ABCG8 genes.

METHODS

Subjects

Women from the Montreal area were recruited to participate in a 24 week weight loss study. To be selected for the study, women were required to be 35–60 years of age with body mass indices of 28–39 kg/m^2. Subjects who used oral antihypertensive agents, thyroid hormones, and hormone replacement therapy were also included, regardless of menopausal status, provided that they were stable and continued on the same dose throughout the duration of the study. Potential subjects were screened for fasting plasma LDL-C concentrations of 3.4–6.7 mmol/l and triacylglycerol concentrations of >1.5 mmol/l. Exclusion criteria included treatment with oral hyperlipidemic therapy <6 months before the start of the study, any history of chronic illness, and previous history of eating disorders. All selected subjects were given an outline of the study protocol and were required to sign a consent form before starting the study. The Faculty of Medicine Ethics Review Board at McGill University and the Human Investigation Review Committee of Tufts University approved the experimental protocol for ethics.

Experimental design

Subjects served as their own controls in a 24 week longitudinal study. During the 24 weeks, participants were required to undergo three consecutive dietary periods: a 2 week preloss stabilization period, a 20 week weight loss period, and a 2 week postloss stabilization period.

During the 2 week stabilization periods, subjects were required to maintain stable weight as well as their usual food and exercise habits. To ensure that subjects were maintaining their usual food and exercise habits, the subjects were counseled and closely monitored by the dietician/nutritionist. Subject compliance during the postloss stabilization phase was measured by whether any significant changes in weight occurred as well as by stabilization in lipid levels. Blood was drawn on days 1, 8, and 15 for lipid analysis during the stabilization periods to assess stability. Analysis of cholesterol kinetics was measured using stable isotopes between days 11 and 15 in each phase. To measure cholesterol absorption and turnover, baseline fasting blood samples were collected on day 11 of each stabilization phase before subjects received an intravenous injection of 15 mg of [25,26,26,26,27,27,27-D_7]cholesterol and a 75 mg oral dose of [3,4-^{13}C] cholesterol (>99 atom percent excess for both; CDN Isotopes, Montreal, Canada). Cholesterol fractional absorption was then determined by comparing the ratio of ingested [^{13}C]cholesterol and intravenous [D_7]cholesterol enrichment in red blood cells on days 13 and 14. Cholesterol turnover was calculated as the rate of decay in the enrichment of D_7 in red blood cell cholesterol between day 11, hour 12 and day 14. Cholesterol biosynthesis was measured on days 14 and 15 using the deuterium incorporation method, which involved ingestion of 0.7 g of D_2O/kg estimated body water (99.8 atom percent excess; CDN Isotopes). Body water was estimated at 60% of total body weight.

Weight loss protocol

Weight loss was accomplished in a free-living environment by decreasing dietary energy intake by 20% and increasing energy expenditure by 10% through physical activity. The diet was taught via an exchange system and consisted of 50–60% of energy from carbohydrates, 20% of energy from protein, and <30% of energy from fat. To help participants achieve a caloric deficit of 10% by energy expenditure, semiprivate group sessions with a personal trainer were held at the beginning of and midway through the weight loss period. Throughout the weight loss period, compliance was determined at weekly weigh-ins, to which subjects wore the same clothes. Participant motivation was encouraged using an award point system and visual graphs by which they were able to monitor weekly weight loss.

End point analyses

After phlebotomy, blood samples were promptly centrifuged at 1,500 rpm for 15 min to separate red blood cells and plasma and immediately stored at −20°C until analysis.

Cholesterol absorption

A description of cholesterol absorption determination by the dual isotope ratio methodology may be found elsewhere (20). Briefly, lipids from red blood cells were extracted using a modified method of Folch, Lees, and Sloane Stanley (21), isolated,

and combusted to produce CO_2 enriched with ^{13}C and water enriched with D_7. The CO_2 and water generated by combustion were separated using vacuum distillation. D_7-enriched water was further reduced with zinc reagent (Biogeochemical Laboratories, Indiana University, Bloomington, IN) to produce D_7-labeled hydrogen gas. Enrichment of free cholesterol with D_7 and ^{13}C was measured by differential isotope ratio mass spectrometry (VG Isomass 903D and SIRA 12, respectively; Isomass, Cheshire, UK). The cholesterol absorption coefficient was calculated for days 13 and 14 with the following equation (22):

$$\text{cholesterol absorption (\%)} = \frac{\Delta^{13}C \times 7/46 \times [D_7]\text{cholesterol iv dose (mg)} \times 0.0112}{\Delta D_7 \times 2/27 \times [^{13}C]\text{cholesterol oral dose (mg)} \times 0.000155} \times 100$$

where Δ for ^{13}C and D_7 is the difference between samples at 48 and 72 h and the baseline abundance (time zero) in parts per thousand relative to PDB and SMOW standards, respectively. The factors 7/46 and 2/27 reflect the ratio of labeled atoms/mg dose, and the constants 0.0112 and 0.000155 are conversion factors of the parts per thousand units into atom percent excess for the PDB and SMOW scales, respectively.

Calculated absorption of cholesterol at days 13 and 14 were then averaged to determine overall cholesterol absorption.

Cholesterol biosynthesis

The deuterium incorporation method was used to assess cholesterol biosynthesis, as described previously (20), as this method has been shown to accurately measure in vivo cholesterol synthesis (23–25). Briefly, erythrocyte cholesterol was extracted and water from the extracted cholesterol was isolated, at which point enrichment was determined using differential isotope ratio mass spectrometry (VG Micromass 903D) for days 14 and 15 of each stabilization period, as described above (21, 26). In addition, deuterium enrichment of plasma water was measured.

The following equation, which corrects for cholesterol deuterium-protium ratio, was used to determine the fractional synthesis rate (FSR) over 24 h (26):

$$\text{FSR (\%/day)} = (\Delta_{\text{cholesterol}}/\Delta_{\text{plasma}}) \times 0.478 \times 100$$

where Δ refers to deuterium enrichment above baseline level over 24 h in parts per thousand relative to a SMOW standard. The factor 0.478 reflects to ratio of labeled H atoms replaced by deuterium during in vivo biosynthesis (26).

Cholesterol turnover

Turnover of plasma free unesterified cholesterol is defined as the difference between the rate of influx from synthesis and dietary absorption and the rate of efflux from esterification, excretion, or transfer from other body pools (27). Thus, the rate of erythrocyte free cholesterol turnover was assessed as the rate of decay of injected $[D_7]$cholesterol over 12 to 72 h.

Assessment of ABCG5 and ABCG8 SNPs

Leukocyte DNA was extracted from 5 to 10 ml of whole blood as described by Miller, Dykes, and Polesky (28). SNPs Q604E, I18429, I7892, and M216 in ABCG5 and C54Y, D19H, I14222, and T400K in ABCG8 were determined using PCR-based TaqMan allele discrimination assays (Applied Biosystems, Foster City, CA) (29). The primers used for PCR have been described previously (13, 15). A GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) was used for PCR analysis. A 7900HT sequence detection system (Applied Biosystems) was used to carry out PCR analysis. Reactions were subjected to 50°C for 2 min, 95°C for 10 min, and 40 cycles each of 95°C for 15 s and 60°C for 1 min.

Statistics

All data are expressed as means ± SD. Paired Student's *t*-tests were used to determine statistically significant changes in cholesterol metabolism. Repeated-measures ANOVA was used to examine whether cholesterol levels were stable during the stabilization periods. The Wilcoxon signed rank test was applied where appropriate. To test whether there was a difference in cholesterol kinetic changes within an SNP, one-way ANOVA with post hoc Scheffe multiple comparisons was applied. The Kruskal-Wallis test was used where appropriate. Data from homozygous variant carriers and heterozygous individuals were then combined and tested for associations. Homozygous variant groups with less than three individuals were automatically collapsed into the heterozygous group. A two-sample Student's *t*-test or a Mann-Whitney U test was then applied to the regrouped data, depending on whether a normal distribution condition was met. The Kruskal-Wallis test was used to determine statistical significance in the I7892 to examine change in synthesis and initial turnover and in the C54Y to test for change in absorption, synthesis, and turnover. A Mann-Whitney U test was used to determine whether differences existed among the I18429 genotypes in final synthesis, initial turnover, and change in turnover, among the I1422 genotypes in final synthesis, final turnover, and change in turnover, and among the T400K genotypes in final synthesis. Significance level was set at $\alpha = 0.05$. Data were analyzed using SPSS for Windows (version 12.0.0; SPSS, Inc., Chicago, IL).

RESULTS

Forty-two subjects were accepted into the protocol, with 35 completing the study. Participants withdrew because of inability to fulfill study requirements (n = 2) and difficulty adhering to the weight loss protocol and scheduled study visits (n = 5). The 35 participants who completed the study had an average age of 49.4 ± 6.7 years, body mass index of 31.4 ± 2.8 kg/m^2, and body weight of 81.4 ± 9.5 kg. **Figure 1** shows individual weights of participants at the beginning and end of the study period. Overall, participants lost an average of 11.7 ± 2.5 kg (14.5 ± 3.1% of original body weight) ($P < 0.001$).

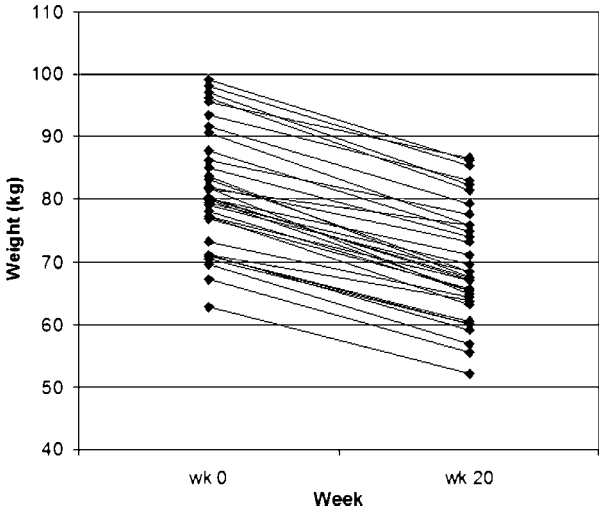

Fig. 1. Individual weights of participants before and after the weight loss period.

Changes in cholesterol metabolism as a result of weight loss

As reported previously (30), no significant differences were observed between cholesterol levels on days 1, 8, and 15 of the preloss and postloss stabilization periods. In response to weight loss, FSR decreased ($P = 0.003$) by 3.86 ± 9.33 %/day. No differences were seen in the shifts in cholesterol absorption (3.31 ± 19.4%; $P = 0.32$) or the rates of cholesterol turnover (−0.17 ± 9.56%; $P = 0.92$).

SNP distribution and frequency

Individual genotypes for each subject are shown in **Table 1**. Genotype distribution and frequency of SNPs in ABCG5 and ABCG8 are shown in **Table 2**. All genotypes were distributed according to the Hardy-Weinberg equilibrium.

Effects of genotype on cholesterol metabolism

Changes in cholesterol absorption were related to the Q604E SNP in ABCG5 (**Table 3**). More specifically, heterozygous (7.33 ± 15.6%) and homozygous wild-type (5.94 ± 18.3%) carriers had larger increases ($P = 0.017$ and $P = 0.010$) in absorption than those who were homozygous for the variant (−30.8 ± 1.90%). Individuals who were homozygous for the Q604E SNP also had higher initial ($P = 0.026$ and $P = 0.039$) cholesterol absorption (86.5 ± 13.3%) compared with heterozygous (57.2 ± 11.8%) and homozygous wild-type (59.7 ± 18.5%) subjects. Although the standard deviation of cholesterol absorption in the E604 SNP implies that values were >100%, all subjects had absorption values of <100%, the lowest and highest being 73.1% and 99.7%, respectively. **Table 4** provides individual data for these volunteers. Changes in cholesterol FSR were also seen, where those who were homozygous for the E604 genotype had increases (1.69 ± 10.0%/day) as a result of weight loss and subjects who were heterozygous decreased in FSR across weight loss (−7.39 ± 9.36%/day) ($P = 0.041$). When individuals who were heterozygous were grouped with individuals who were homozygous for the Q604E SNP variant, no significant differences were identified in indicators of cholesterol metabolism.

A trend existed in the I7892 SNP in ABCG5, where initial FSR values tended to be higher ($P = 0.058$) in participants who had at least one mutation (12.4 ± 9.03%/day) than in those who were homozygous for the wild-type SNP (7.75 ± 5.08%/day).

The C54Y SNP in ABCG8 was also associated with changes in FSR. More specifically, heterozygous carriers had smaller reductions ($P = 0.047$) in FSR (−0.17 ± 7.60%/day) compared with participants who were homozygous for the Y54 genotype (−8.09 ± 10.3%/day) (Table 3). Lower post-weight-loss FSR was found in homozygous wild-type subjects (5.18 ± 4.88%/day) in relation to subjects who carried at least one variant (7.76 ± 4.90%/day) ($P = 0.042$). No further differences in cholesterol metabolism were found to be associated with SNPs in ABCG5 or ABCG8.

TABLE 1. Individual subject genotypes for each SNP in ABCG5 and ABCG8

ABCG5				ABCG8			
Q604E	I18429	I7892	M216	C54Y	D19H	I14222	T400K
11	11	12	12	12	11	11	11
11	11	11	11	11	11	11	12
12	12	11	11	11	12	12	12
11	11	12	12	12	11	11	12
11	11	22	22	22	11	11	11
11	11	22	12	12	11	12	11
12	12	11	11	11	11	11	12
11	11	11	11	11	11	11	22
22	12	11	11	11	12	12	11
11	11	12	12	12	12	12	12
11	11	22	22	22	11	11	11
12	12	12	12	22	11	11	11
12	12	11	11	11	12	12	12
11	11	11	11	12	11	11	11
12	12	11	11	11	12	12	11
22	12	11	11	11	12	12	11
11	11	12	12	12	11	11	11
11	11	12	11	11	12	12	11
11	11	12	12	22	11	11	11
12	12	11	11	11	11	11	12
12	11	11	11	11	11	11	12
12	12	22	22	12	11	11	12
11	12	11	11	11	11	11	22
11	11	22	22	22	11	11	11
11	11	12	12	12	11	11	11
12	12	12	11	11	12	12	11
22	12	12	11	11	11	12	11
12	11	12	12	12	11	11	12
11	11	22	22	22	11	11	11
12	11	22	22	22	11	11	11
11	11	11	11	12	11	11	11
11	11	12	22	12	11	11	11
12	11	11	11	11	12	11	11
11	11	11	11	11	11	11	11
12	12	12	12	12	11	11	12

SNP, single nucleotide polymorphism. 1 represents the wild type and 2 represents a mutation. Each row represents one subject.

DISCUSSION

This study is the first to provide evidence that weight-related changes in cholesterol metabolism are associated with SNPs in ABCG5 and ABCG8. The results indicate that homozygous variant carriers of the Q604E SNP in ABCG5 experienced larger decreases in cholesterol absorption and increased FSR after weight loss. Individuals homozygous for the C54Y SNP in ABCG8 exhibited lower post-weight-loss FSR than those who carried at least one Y54 genotype.

A unique aspect of this study is that the stabilization periods incorporated into the study design help to minimize variation and to isolate the effects of weight loss. The existing literature and the outcome of the repeated-measures ANOVA on cholesterol concentrations during the stabilization periods support the notion that the subjects were in a steady state. Cholesterol levels during this period are provided in supplementary Table I. Other studies have indicated 2 weeks as a sufficient period to produce changes in circulatory cholesterol levels by means of dietary changes (31, 32).

As part of this study, SNPs in introns were examined to determine whether they might be associated with func-

TABLE 2. Genotype distribution and frequency of SNPs in introns and exons of ABCG5 and ABCG8 in the studied population

	Homozygous Wild Type			Heterozygous			Homozygous Variant		
SNP	n	Age	BMI	n	Age	BMI	n	Age	BMI
	%	*years*	kg/m^2	%	*years*	kg/m^2	%	*years*	kg/m^2
ABCG5									
Q604E	19 (54.3)	48.6 ± 6.56	31.0 ± 2.92	13 (37.1)	50.2 ± 7.24	32.0 ± 2.79	3 (8.6)	51.0 ± 6.56	30.6 ± 2.45
I18429	22 (62.9)	49.1 ± 6.91	31.2 ± 2.81	13 (37.1)	49.9 ± 6.51	31.7 ± 2.91	0 (0)		
I7892	15 (42.9)	50.1 ± 7.09	30.6 ± 2.55	13 (37.1)	46.9 ± 6.59	32.1 ± 3.02	7 (20.0)	52.3 ± 4.96	31.5 ± 2.98
M216	18 (51.4)	50.3 ± 6.89	30.6 ± 2.33[a]	10 (28.6)	45.0 ± 5.19[b]	33.2 ± 2.99[a]	7 (20.0)	53.1 ± 5.21[b]	30.6 ± 2.85
ABCG8									
C54Y	16 (45.7)	50.4 ± 7.10	30.8 ± 2.42	12 (34.3)	46.9 ± 6.33	31.5 ± 2.28	7 (20.0)	51.3 ± 5.88	32.5 ± 4.26
D19H	26 (74.3)	48.9 ± 7.06	31.7 ± 3.00	9 (25.7)	50.8 ± 5.89	30.3 ± 2.01	0 (0)		
I14222	25 (71.4)	48.6 ± 7.16	31.5 ± 3.06	10 (28.6)	51.2 ± 5.18	31.0 ± 2.17	0 (0)		
T400K	22 (62.9)	50.6 ± 5.67	31.2 ± 3.03	11 (31.4)	46.6 ± 8.43	31.9 ± 2.40	2 (5.7)	50.5 ± 3.54	31.0 ± 3.82

BMI, body mass index.
[a,b] Significant difference between groups ($P < 0.05$).

tional aspects of the ABCG5 and ABCG8 proteins. Although no strong effects of these intron SNPs were found, there were significant associations between selected exon SNPs and cholesterol metabolism. The functional assessment of amino acid substitutions that result from the examined exon SNPs in ABCG5 and ABCG8 is still unclear. The Q604E SNP is located on exon 13 of the ABCG5 gene and is encoded on a loop that faces the luminal or cell surface (16). Nonsynonymous SNPs on ABCG8, specifically, D19H, C54Y, and T400K, are located on exons 1, 2, and 8, respectively (33).

Changes in cholesterol metabolism have never been examined in the context of ABCG5 and ABCG8 SNPs. Only studies that investigated the effect of SNPs in ABCG5 and ABCG8 on baseline cholesterol absorption exist. In contrast with our results showing no relation between initial cholesterol kinetic levels and D19H, one study ascertained a connection with the D19H SNP, where the H19 allele was associated with higher cholesterol absorption (18). Other research has not detected any differences between cholesterol absorption and the SNPs in ABCG5 and ABCG8 that were examined in the present trial (34). One explanation for the disparities in these findings was that the subject population varied between studies. Both studies included men and women (30, 34), with one including only Japanese participants (34). A trial by Chan et al. (35) that included 47 overweight men, however, resulted in observations consistent with those of the present investigation, in that they found no differences in sterol indicators of cholesterol absorption with respect to the D19H SNP.

A novel finding of this trial is that changes in cholesterol absorption and synthesis after weight loss, measured by stable isotopes, were affected by the Q604E and C54Y SNPs. The changes in cholesterol kinetics moved in a direction appropriate to reconcile the observed shifts in lipid levels (see supplementary Table II). There were only three subjects who were homozygous carriers for the E604 allele, be-

TABLE 3. Cholesterol metabolism and change according to exon SNPs in ABCG5 and ABCG8

	Cholesterol Biosynthesis			Cholesterol Absorption			Cholesterol Turnover		
SNP	Initial	Final	Change	Initial	Final	Change	Initial	Final	Change
	%/*day*	%/*day*	%/*day*	%	%	%	%	%	%
Q604E									
QQ	8.48 ± 6.76	6.16 ± 5.66	−2.32 ± 8.86	59.7 ± 18.5[a]	65.7 ± 13.7	5.94 ± 18.3[c]	39.1 ± 5.99	36.5 ± 8.14	−2.45 ± 9.76
QE	13.9 ± 9.05	6.48 ± 3.95	−7.39 ± 9.36[a]	57.2 ± 11.8[b]	64.5 ± 18.0	7.33 ± 15.6[d]	37.5 ± 9.27	39.5 ± 6.31	1.93 ± 8.59
EE	7.91 ± 4.91	9.60 ± 5.09	1.69 ± 10.0[a]	86.5 ± 13.3[a,b]	55.7 ± 13.5	−30.8 ± 1.90[c,d]	40.8 ± 3.72	45.3 ± 8.63	4.47 ± 12.1
QE + EE	12.8 ± 8.63	7.07 ± 4.18	−5.69 ± 9.84	62.6 ± 16.6	62.8 ± 17.2	0.18 ± 20.7	38.2 ± 8.44	40.6 ± 6.86	2.43 ± 8.94
C54Y									
CC	9.96 ± 8.83	5.18 ± 4.88[a]	−4.78 ± 9.62	64.9 ± 15.4	67.5 ± 16.4	2.67 ± 22.0	37.0 ± 8.43	38.1 ± 7.68	1.13 ± 10.6
CY	8.95 ± 5.34	8.79 ± 5.35	−0.17 ± 7.60[a]	54.2 ± 13.5	65.4 ± 12.2	11.2 ± 12.9	38.3 ± 5.00	40.1 ± 8.42	1.77 ± 8.67
YY	14.1 ± 9.07	5.99 ± 3.74	−8.09 ± 10.3[a]	64.1 ± 25.6	55.3 ± 15.6	−8.80 ± 17.9	43.3 ± 6.57	36.2 ± 7.00	−7.03 ± 6.42
CY + YY	10.8 ± 7.16	7.76 ± 4.90[a]	−3.08 ± 9.28	57.9 ± 18.8	61.7 ± 14.0	3.85 ± 17.5	40.0 ± 5.87	38.8 ± 8.00	−1.17 ± 8.89
D19H									
DD	10.1 ± 6.68	6.54 ± 5.28	−3.55 ± 8.28	58.8 ± 15.9	61.8 ± 13.3	3.00 ± 17.2	39.1 ± 6.00	38.2 ± 7.59	−0.70 ± 8.41
DH	11.4 ± 11.0	6.68 ± 4.35	−4.76 ± 12.4	67.5 ± 21.2	71.7 ± 18.8	4.19 ± 25.9	37.5 ± 10.2	39.1 ± 8.57	1.45 ± 12.3
T400K									
TT	10.4 ± 8.91	6.91 ± 5.51	−3.53 ± 10.9	64.4 ± 19.4	63.6 ± 17.2	−0.76 ± 20.2	40.4 ± 5.15	38.9 ± 7.39	−1.61 ± 9.46
TK + KK	10.4 ± 5.99	6.01 ± 4.12	−4.42 ± 6.29	55.4 ± 12.2	65.6 ± 11.6	10.2 ± 16.5	35.8 ± 9.10	37.7 ± 8.52	2.24 ± 9.64

[a,b] Significant difference between groups ($P < 0.05$).
[c,d] Significant difference between groups ($P < 0.01$).

TABLE 4. Individual data for homozygous carriers of the E604 genotype

Characteristic	Participant 1	Participant 2	Participant 3
Age (years)	57	44	52
BMI (kg/m^2)	28.0	32.9	30.7
Initial weight (kg)	70.9	82.1	70.8
Weight loss (kg)	10.5	8.9	10.6
Initial total cholesterol (mmol/l)	5.34	4.74	5.26
Initial LDL cholesterol (mmol/l)	2.85	3.16	3.03
Initial HDL cholesterol (mmol/l)	1.09	0.80	1.11
Initial absorption (%)	99.7	86.6	73.1
Final absorption (%)	70.1	53.6	43.3
Initial fractional synthesis rate (%/day)	6.76	3.68	13.3
Final fractional synthesis rate (%/day)	10.5	14.2	4.13
Initial turnover (%)	43.7	36.6	42.2
Final turnover (%)	42.1	55.0	38.7

cause the estimated population frequency of the allele is only 3.5%. It would be difficult to obtain a large number of homozygous individuals, and despite their small number, those who were homozygous for the variant were examined as a group in the present study to determine whether there were any differences in cholesterol metabolism among these individuals. Participants who were homozygous for the glutamic acid allele had decreases in cholesterol absorption and increases in cholesterol synthesis, whereas carriers of at least one wild-type allele had increases in cholesterol absorption and decreases in cholesterol synthesis.

The individuals who were homozygous for the E604 allele had unusually high cholesterol absorption values, according to historical data (8–10). In the calculation of cholesterol absorption, the isotopic enrichment of deuterium and C13 was determined in duplicate at each of the two time points of days 13 and 14. The data from the two days were then averaged to come to the absorption values obtained. Therefore, the absorption values were calculated using four individual enrichment values. The data for the subjects for whom cholesterol absorption was very high were reexamined, and no reason was found to exclude these individuals. There is always the possibility that a systemic error might have occurred; however, any such error would have occurred across all time points for all samples, as the before and after time series were analyzed simultaneously. The majority of the rest of the values obtained for cholesterol absorption fall into predicted ranges, between 40%/day and 80%/day.

Homozygous carriers of the variation that results in the translation of tyrosine in the C54Y SNP were observed to have larger reductions in FSR in response to weight loss than those who were heterozygous for the mutation. Additionally, participants with at least one variant had smaller post-weight-loss FSR than homozygous wild-type subjects. Because individuals who had at least one variant also had lower post-weight-loss LDL-C concentrations than homozygous wild-type participants (see supplementary Table II), it appears that lower cholesterol FSR in the carriers of the C54Y mutation was responsible for the lower post-weight-loss circulating concentrations of LDL-C.

The use of stable isotopes for the determination of cholesterol kinetics in this study is unique and allows for the direct quantification of associated parameters. However, the time and cost related to using stable isotopes do not allow this technique to be applied to large sample sizes. Thus, further studies that examine cholesterol metabolism are required to confirm these conclusions in larger groups of individuals. One way this could be done is to use plant sterols and cholesterol precursors as indicators of cholesterol metabolism (36–39). Although these measurements are limited in that they do not provide direct quantification of cholesterol absorption and synthesis, they are less invasive and expensive. Therefore, the precursor method can be feasibly applied to larger populations to indicate how SNPs might affect the efficacy of interventions targeted toward changing cholesterol metabolism (6).

One of the limitations of the present study was that the smaller sample size used did not allow us to conduct haplotype analyses. The sample size, however, was sufficient to detect significant differences among various SNPs. The effect shown by the data is consistent enough such that even with a small sample size, the directional change follows the expected change. Thus, the directional change of the data is concordant with what the literature suggests. The present trial is the only existing study that demonstrates the potential effect of SNPs in ABCG5 and ABCG8 as a regulator of cholesterol metabolism during weight loss. The statistically significant results lend testimony to the notion that the genetic influences on the kinetics may be larger than the same genetic effect on the cholesterol biomarker that is modified by these kinetics.

Recently, Niemann-Pick C1 Like-1 (NPC1L1) was identified as a transporter that regulates intestinal cholesterol absorption (40). Since we conducted the present study, several new SNPs in NPC1L1 have been identified. Variant alleles were associated with a 10% lower LDL cholesterol in a study by Cohen et al. (41), whereas another trial found improved cholesterol-lowering response with ezetimibe, a cholesterol absorption inhibitor that targets NPC1L1 (42). Although decreases in body weight affect cholesterol synthesis, future studies might be considered to elucidate the functional effects of SNPs in NPC1L1 on cholesterol metabolism.

In conclusion, SNPs in ABCG5 and ABCG8 were found to be associated with the response of cholesterol metabolism to weight loss. Understanding the effects of these SNPs on cholesterol risk factors will help establish genetic screening tools to determine optimal lipid-lowering treatment routes for individuals during weight reduction.

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REFERENCES

1. Di Buono, M., J. S. Hannah, L. I. Katzel, and P. J. Jones. 1999. Weight loss due to energy restriction suppresses cholesterol biosynthesis in overweight, mildly hypercholesterolemic men. *J. Nutr.* **129:** 1545–1548.
2. Simonen, P., H. Gylling, A. N. Howard, and T. A. Miettinen. 2000. Introducing a new component of the metabolic syndrome: low cholesterol absorption. *Am. J. Clin. Nutr.* **72:** 82–88.
3. Stahlberg, D., M. Rudling, B. Angelin, I. Bjorkhem, P. Forsell, K. Nilsell, and K. Einarsson. 1997. Hepatic cholesterol metabolism in human obesity. *Hepatology.* **25:** 1447–1450.
4. Dattilo, A. M., and P. M. Kris-Etherton. 1992. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *Am. J. Clin. Nutr.* **56:** 320–328.
5. Simonen, P., H. Gylling, and T. A. Miettinen. 2002. Acute effects of weight reduction on cholesterol metabolism in obese type 2 diabetes. *Clin. Chim. Acta.* **316:** 55–61.
6. Santosa, S., K. A. Varady, S. Abumweis, and P. J. Jones. 2007. Physiological and therapeutic factors affecting cholesterol metabolism: does a reciprocal relationship between cholesterol absorption and synthesis really exist? *Life Sci.* **80:** 505–514.
7. Aberle, J., D. Evans, F. U. Beil, and U. Seedorf. 2005. A polymorphism in the apolipoprotein A5 gene is associated with weight loss after short-term diet. *Clin. Genet.* **68:** 152–154.
8. Borgstrom, B. 1969. Quantification of cholesterol absorption in man by fecal analysis after the feeding of a single isotope-labeled meal. *J. Lipid Res.* **10:** 331–337.
9. Grundy, S. M., and E. H. Ahrens, Jr. 1969. Measurements of cholesterol turnover, synthesis, and absorption in man, carried out by isotope kinetic and sterol balance methods. *J. Lipid Res.* **10:** 91–107.
10. Quintao, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. An evaluation of four methods for measuring cholesterol absorption by the intestine in man. *J. Lipid Res.* **12:** 221–232.
11. Berge, K. E., H. Tian, G. A. Graf, L. Yu, N. V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science.* **290:** 1771–1775.
12. Klett, E. L., M. H. Lee, D. B. Adams, K. D. Chavin, and S. B. Patel. 2004. Localization of ABCG5 and ABCG8 proteins in human liver, gall bladder and intestine. *BMC Gastroenterol.* **4:** 21.
13. Hubacek, J. A., K. E. Berge, J. C. Cohen, and H. H. Hobbs. 2001. Mutations in ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. *Hum. Mutat.* **18:** 359–360.
14. Kajinami, K., M. E. Brousseau, J. M. Ordovas, and E. J. Schaefer. 2004. Interactions between common genetic polymorphisms in ABCG5/G8 and CYP7A1 on LDL cholesterol-lowering response to atorvastatin. *Atherosclerosis.* **175:** 287–293.
15. Kajinami, K., M. E. Brousseau, C. Nartsupha, J. M. Ordovas, and E. J. Schaefer. 2004. ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin. *J. Lipid Res.* **45:** 653–656.
16. Lee, M. H., K. Lu, S. Hazard, H. Yu, S. Shulein, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuma, R. Pegoraro, et al. 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat. Genet.* **27:** 79–83.
17. Lee, M. H., K. Lu, and S. B. Patel. 2001. Genetic basis of sitosterolemia. *Curr. Opin. Lipidol.* **12:** 141–149.
18. Gylling, H., M. Hallikainen, J. Pihlajamaki, J. Agren, M. Laakso, R. A. Rajaratnam, R. Rauramaa, and T. A. Miettinen. 2004. Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. *J. Lipid Res.* **45:** 1660–1665.
19. Weggemans, R. M., P. L. Zock, E. S. Tai, J. M. Ordovas, H. O. Molhuizen, and M. B. Katan. 2002. ATP binding cassette G5 C1950G polymorphism may affect blood cholesterol concentrations in humans. *Clin. Genet.* **62:** 226–229.
20. Vanstone, C. A., M. Raeini-Sarjaz, W. E. Parsons, and P. J. Jones. 2002. Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. *Am. J. Clin. Nutr.* **76:** 1272–1278.
21. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
22. Bosner, M. S., R. E. Ostlund, Jr., O. Osofisan, J. Grosklos, C. Fritschle, and L. G. Lange. 1993. Assessment of percent cholesterol absorption in humans with stable isotopes. *J. Lipid Res.* **34:** 1047–1053.
23. Jones, P. J., L. M. Ausman, D. H. Croll, J. Y. Feng, E. A. Schaefer, and A. H. Lichtenstein. 1998. Validation of deuterium incorporation against sterol balance for measurement of human cholesterol biosynthesis. *J. Lipid Res.* **39:** 1111–1117.
24. Jones, P. J., A. S. Pappu, D. R. Illingworth, and C. A. Leitch. 1992. Correspondence between plasma mevalonic acid levels and deuterium uptake in measuring human cholesterol synthesis. *Eur. J. Clin. Invest.* **22:** 609–613.
25. Matthan, N. R., M. Raeini-Sarjaz, A. H. Lichtenstein, L. M. Ausman, and P. J. Jones. 2000. Deuterium uptake and plasma cholesterol precursor levels correspond as methods for measurement of endogenous cholesterol synthesis in hypercholesterolemic women. *Lipids.* **35:** 1037–1044.
26. Jones, P. J., C. A. Leitch, Z. C. Li, and W. E. Connor. 1993. Human cholesterol synthesis measurement using deuterated water. Theoretical and procedural considerations. *Arterioscler. Thromb.* **13:** 247–253.
27. Goodman, D. S., R. P. Noble, and R. B. Dell. 1973. Three-pool model of the long-term turnover of plasma cholesterol in man. *J. Lipid Res.* **14:** 178–188.
28. Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16:** 1215.
29. McGuigan, F. E., and S. H. Ralston. 2002. Single nucleotide polymorphism detection: allelic discrimination using TaqMan. *Psychiatr. Genet.* **12:** 133–136.
30. Santosa, S., I. Demonty, K. Cianflone, A. H. Lichtenstein, and P. J. H. Jones. 2007. An investigation of hormone and lipid interactions after weight loss in women. *J. Am. Coll. Nutr.* **26:** 250–258.
31. Jenkins, D. J., C. W. Kendall, A. Marchie, D. A. Faulkner, J. M. Wong, R. de Souza, A. Emam, T. L. Parker, E. Vidgen, K. G. Lapsley, et al. 2003. Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. *J. Am. Med. Assoc.* **290:** 502–510.
32. Hodson, L., C. M. Skeaff, and J. E. McKenzie. 2002. Maximal response to a plasma cholesterol-lowering diet is achieved within two weeks. *Nutr. Metab. Cardiovasc. Dis.* **12:** 291–295.
33. Hazard, S. E., and S. B. Patel. 2007. Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. *Pflugers Arch.* **453:** 745–752.
34. Miwa, K., A. Inazu, J. Kobayashi, T. Higashikata, A. Nohara, M. Kawashiri, S. Katsuda, M. Takata, J. Koizumi, and H. Mabuchi. 2005. ATP-binding cassette transporter G8 M429V polymorphism as a novel genetic marker of higher cholesterol absorption in hypercholesterolaemic Japanese subjects. *Clin. Sci. (Lond.).* **109:** 183–188.
35. Chan, D. C., G. F. Watts, P. H. Barrett, A. J. Whitfield, and F. M. van Bockxmeer. 2004. ATP-binding cassette transporter G8 gene as a determinant of apolipoprotein B-100 kinetics in overweight men. *Arterioscler. Thromb. Vasc. Biol.* **24:** 2188–2191.
36. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesaniemi. 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am. J. Epidemiol.* **131:** 20–31.
37. Tilvis, R. S., and T. A. Miettinen. 1986. Serum plant sterols and their relation to cholesterol absorption. *Am. J. Clin. Nutr.* **43:** 92–97.
38. Kempen, H. J., J. F. Glatz, J. A. Gevers Leuven, H. A. van der Voort, and M. B. Katan. 1988. Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J. Lipid Res.* **29:** 1149–1155.
39. Pfohl, M., I. Schreiber, H. M. Liebich, H. U. Haring, and H. M. Hoffmeister. 1999. Upregulation of cholesterol synthesis after acute myocardial infarction—is cholesterol a positive acute phase reactant? *Atherosclerosis.* **142:** 389–393.
40. Altmann, S. W., H. R. Davis, Jr., X. Yao, M. Laverty, D. S. Compton, L. J. Zhu, J. H. Crona, M. A. Caplen, L. M. Hoos, G. Tetzloff, et al. 2002. The identification of intestinal scavenger receptor class B, type I (SR-BI) by expression cloning and its role in cholesterol absorption. *Biochim. Biophys. Acta.* **1580:** 77–93.
41. Cohen, J. C., A. Pertsemlidis, S. Fahmi, S. Esmail, G. L. Vega, S. M. Grundy, and H. H. Hobbs. 2006. Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. *Proc. Natl. Acad. Sci. USA.* **103:** 1810–1815.
42. Simon, J. S., M. C. Karnoub, D. J. Devlin, M. G. Arreaza, P. Qiu, S. A. Monks, M. E. Severino, P. Deutsch, J. Palmisano, A. B. Sachs, et al. 2005. Sequence variation in NPC1L1 and association with improved LDL-cholesterol lowering in response to ezetimibe treatment. *Genomics.* **86:** 648–656.