Fatty acid desaturase regulation in adipose tissue by dietary composition is independent of weight loss and is correlated with the plasma triacylglycerol response[1–3]

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ABSTRACT

Background: Atherogenic dyslipidemia associated with elevated plasma triacylglycerol concentrations and reduced HDL is improved by both caloric restriction and reduced carbohydrate consumption.

Objective: We aimed to identify the molecular pathways responsive to both caloric restriction and dietary composition within adipose tissue by monitoring transcriptional expression.

Design: Subcutaneous adipose tissue biopsy specimens were obtained from 131 moderately overweight men [body mass index (in kg/m^2): 29.2 ± 2.0] after 1 wk of a basal diet [54%, 16%, and 30% carbohydrate, protein, and fat, respectively; 7% saturated fat), after 3 wk with 1 of 4 randomized diets [basal diet; moderate-carbohydrate (39%) diet; low-carbohydrate (26%) and low-saturated-fat (9%) diet; low-carbohydrate (26%) and high-saturated-fat (15%) diet], after 5 wk of acute weight loss with a randomized diet (−1103.0 ± 216.5 kcal/d, which resulted in a loss of 10.0 ± 3.3 lb, or 4.5 ± 1.5 kg), and after 4 wk of being stabilized at a reduced weight. Transcriptional response was identified by using expression array analysis and was confirmed by using real-time polymerase chain reaction analysis.

Results: Of the 1473 transcripts significantly decreased in expression in response to acute weight loss, 30 were responsive to isocaloric alterations in dietary composition, including stearoyl-coenzyme A desaturase (*SCD*), fatty acid desaturases 1 and 2 (*FADS1* and *FADS2*), and diacylglycerol transferase 2 (*DGAT2*). Response was confirmed by real-time polymerase chain reaction analysis for these genes ($P < 0.003$). *SCD* expression in response to isocaloric dietary change was most strongly correlated with carbohydrate intake ($P = 0.019$) and, with the low-carbohydrate diet, *SCD* expression was inversely correlated with saturated fat intake ($P = 0.05$). Triacylglycerol responses to changes in dietary composition were independently correlated with *SCD* ($P = 0.003$) and *DGAT2* ($P = 0.05$) responses.

Conclusions: *SCD* expression in adipose tissue is independently regulated by weight loss and by carbohydrate and saturated fat intakes. Moreover, *SCD* and *DGAT2* expression may be involved in dietary regulation of systemic triacylglycerol metabolism. *Am J Clin Nutr* 2007;86:759–67.

KEY WORDS Gene expression, gene expression microarray, gene transcription, adipose tissue, weight loss, dietary carbohydrate, dietary saturated fat, fatty acid desaturases, triacylglycerol

INTRODUCTION

Excess adiposity contributes to an elevated risk of many disorders, such as atherogenic dyslipidemia, which is a major risk factor for cardiovascular disease (1, 2). Atherogenic dyslipidemia comprises an interrelated cluster of metabolic alterations, most notably, an elevation in triacylglycerols, a decrease in HDL cholesterol, and an increase in small, dense LDL particles (3, 4).

Whereas the primary nutritional intervention for achieving weight reduction is a restriction in total energy intake, there has been recent interest in the effects of diets restricted in carbohydrate intake (5–7). Such diets are reported to have the dual benefit of promoting weight reduction, at least over the short term, and improving lipid profiles (8, 9). Whereas a high carbohydrate intake is known to increase plasma triacylglycerol concentrations, in most studies, the effects on dyslipidemia of carbohydrate restriction per se have not been separated from those of concomitant weight loss (10).

We recently showed that both isoenergetic carbohydrate restriction and diet-induced weight loss in the absence of carbohydrate restriction separately improved the markers of atherogenic dyslipidemia in moderately overweight but otherwise healthy men (11). The effects of these interventions were equivalent but nonadditive, which suggested that they acted through shared pathways. Adipose tissue is the major site of storage for excess systemic energy, in the form of triacylglycerols, and is the major source of plasma nonesterified fatty acids used for hepatic triacylglycerol synthesis and triacylglycerol-rich lipoprotein secretion during times of fasting (12). Dysregulation of the lipolytic and endocrine functions of adipose tissue is associated with abnormal plasma lipid and lipoprotein markers, and inhibition of lipolysis in adipose tissue by niacin is an established pharmaceutical approach for treating hyperlipidemia (13–15). The goal of the present study was to use genome-wide expression array

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TABLE 1
Plasma lipid and lipoprotein measurements at baseline and in response to dietary intervention in subjects[1]

			26% Carbohydrate	
	56% Carbohydrate	39% Carbohydrate	Low saturated fat	High saturated fat
Baseline (54% carbohydrate)				
n	17	23	27	15
BMI (kg/m^2)	28.5 ± 0.4[2]	29.4 ± 0.4	29.1 ± 0.4	29.5 ± 0.5
Triacylglycerol (mg/dL)	165.5 ± 21.1	184.9 ± 20.0	154.2 ± 14.0	174.5 ± 22.2
Total cholesterol (mg/dL)	210.3 ± 9.1	205.5 ± 4.9	211.9 ± 5.6	196.4 ± 8.1
LDL cholesterol (mg/dL)	134.9 ± 7.5	126.7 ± 5.1	138.6 ± 4.4	121.0 ± 9.1
HDL cholesterol (mg/dL)	41.9 ± 1.9	41.9 ± 1.9	42.5 ± 2.0	40.6 ± 2.8
ΔDiet, stable weight				
n	10	14	15	11
BMI (kg/m^2)	−0.2 ± 0.08	−0.3 ± 0.06	−0.4 ± 0.07	−0.4 ± 0.1
Triacylglycerol (mg/dL)	−34.1 ± 14.7	−17.4 ± 24.4	−52.5 ± 15.6	−61.4 ± 21.7
Total cholesterol (mg/dL)	−10.7 ± 6.2	−4.3 ± 6.0	−16.5 ± 7.1	1.4 ± 5.1
LDL cholesterol (mg/dL)	−2.4 ± 6.1	−1.6 ± 6.1	−6.9 ± 5.8	10.1 ± 6.2
HDL cholesterol (mg/dL)	−1.64 ± 2.2	1.6 ± 1.1	1.0 ± 1.3	3.5 ± 1.4
ΔWeight, acute weight loss				
n	10	13	19	15
BMI (kg/m^2)	−1.4 ± 0.1	−1.4 ± 0.1	−1.4 ± 0.1	−1.4 ± 0.1
Triacylglycerol (mg/dL)	−31.9 ± 13.2	−51.5 ± 20.9	−21.6 ± 8.1	−23.5 ± 6.5
Total cholesterol (mg/dL)	−9.6 ± 5.1	−10.3 ± 3.7	−10.4 ± 3.9	−3.3 ± 5.1
LDL cholesterol (mg/dL)	−4.9 ± 5.9	−3.1 ± 4.1	−2.5 ± 4.0	−8.2 ± 3.8
HDL cholesterol (mg/dL)	1.9 ± 1.1	2.3 ± 1.8	2.1 ± 1.2	4.0 ± 1.5
ΔWeight, reduced stable weight				
n	9	11	20	14
BMI (kg/m^2)	−1.8 ± 0.2	−1.6 ± 0.2	−1.7 ± 0.1	−1.5 ± 0.2
Triacylglycerol (mg/dL)	2.7 ± 14.8	−16.4 ± 16.1	−2.0 ± 9.2	−10.4 ± 5.9
Total cholesterol (mg/dL)	−13.6 ± 3.1	6.2 ± 7.0	−2.0 ± 4.9	1.1 ± 6.2
LDL cholesterol (mg/dL)	−13.3 ± 4.0	7.9 ± 5.8	−3.0 ± 4.3	0.4 ± 4.5
HDL cholesterol (mg/dL)	−0.6 ± 2.4	0.4 ± 1.0	2.8 ± 1.7	1.5 ± 1.4

[1] Δ, change. No statistical differences between diets were detected for any lipid or lipoprotein measurement or response within this subset of subjects. Differences were observed in the full cohort (178 subjects) under Δdiet, stable weight conditions (BMI, triacylglycerol, total cholesterol, and LDL cholesterol); under Δweight, acute weight loss (triacylglycerol and total cholesterol); and under Δweight, reduced stable weight (triacylglycerol, total cholesterol, and LDL cholesterol; 11).

[2] $\bar{x}$ ± SEM (all such values).

analysis in adipose tissue to identify shared mechanisms of adaptation to weight loss and to isocaloric changes in dietary composition, including reduced carbohydrate intake that may be involved in systemic metabolic response.

SUBJECTS AND METHODS

Study design and sample isolation

The study used a randomized, 4-arm design to examine the effect on lipid markers of dietary composition under isocaloric and hypocaloric conditions, as described previously (11). All subjects consumed a basal diet (54%, 16%, and 30% carbohydrate, protein, and fat, respectively; 7%, 13%, and 8% saturated, monounsaturated, and polyunsaturated fats, respectively) for 1 wk before being randomly assigned to 1 of 4 diets [basal diet; moderate-carbohydrate (39%) diet [29% protein and 31% fat (8% saturated fat and 13% monounsaturated fat)]; low-carbohydrate (26%) and low-saturated-fat (9%) diet [29% protein and 46% fat (27% monounsaturated fat)]; low-carbohydrate (26%) and high-saturated-fat (15%) diet [29% protein and 46% fat (20% monounsaturated fat)] for the remainder of the study. The diets were controlled for cholesterol (150 mg/1000 kcal), *trans* fatty acids (<2% total energy), fiber (25 g/2000 kcal plus 2.5 g/500 kcal above 2000), carbohydrate content (50% simple and 50% complex), protein content (50% animal and 50% vegetable), and dairy product intake (3 portions of milk, yogurt, or cheese per day). The diets were consumed under isocaloric, weight-stable conditions for 3 wk, followed by a 5-wk period of acute caloric deprivation (−1103.0 ± 216.5 kcal/d) that resulted in a cumulative weight loss of 4.5 ± 1.5 kg (−10.0 ± 3.3 lb), and a final 4-wk stabilization period at reduced weight. Baseline body mass index (BMI), triacylglycerols, and plasma cholesterol concentrations did not differ across diets (**Table 1**; 11). Significant differences in lipid and lipoprotein changes between diets in response to both isocaloric dietary composition and weight reduction were observed in the entire population (11), but these changes were not statistically detectable in the subset of the population for which adipose tissue was available (Table 1). Participants were free-living and received menus designed to provide nutrient intake over 6-d cycles. Nutrient calculations were performed by using THE FOOD PROCESSOR software (version 7.3; ESHA Research, Salem, OR). Frozen prepared entrées fortified with vitamins and minerals to meet the Recommended Dietary Allowances (LifeSpring Home Nutrition, Irvine, CA) were provided

for lunch and dinner, and participants prepared their own breakfasts and snacks according to menus. Weight was measured weekly by the staff, and the prescribed daily energy intake was adjusted as necessary to achieve weight goals. Compliance was assessed via analysis of a daily checklist of foods eaten, and no participant was excluded for noncompliance (deviation from daily diet >5% of total energy). Daily energy intake specific to carbohydrate, protein, or fat was calculated by using total energy intake and the percentage of the energy prescribed to be consumed for each macronutrient. Because the present study was performed in an outpatient setting, nutrient and energy intakes could not be fully controlled.

Fasting blood samples and adipose tissue biopsy samples were obtained at 4 time points: after the basal diet (week 1), during the randomized diet at stable weight (week 4), after acute weight loss (week 9), and after stabilization at a reduced weight (week 13). Adipose tissue was collected by needle biopsy from the subcutaneous flanking region after injection of 1% lidocaine hydrochloride with adrenaline and sodium bicarbonate. Biopsy samples were washed with phosphate-buffered saline, flash frozen in liquid nitrogen, and stored at −80 °C for future use.

Subject and sample selection

Subjects were selected for inclusion in the nutritional intervention trial as described previously (11). Briefly, subjects were recruited through mailed solicitations. Inclusion criteria included the absence of a history of cardiovascular disease, of other chronic disease, or of the use of lipid-altering medications; a BMI (in kg/m^2) range of 26–35; LDL-cholesterol concentrations below the 95th percentile for age and sex; triacylglycerol concentrations <500 mg/dL (5.65 mmol/L); fasting glucose concentrations <125 mg/dL (6.94 mmol/L); systolic blood pressure <150 mm Hg and diastolic blood pressure <90 mm Hg; nonsmoking status; and an agreement to consume no alcohol during the course of the study.The study was limited to male participants to satisfy statistical requirements for powering hypotheses related to lipid responses. All subjects gave informed consent under a protocol approved by the Institutional Review Boards of Children's Hospital Oakland and the University of California, Berkeley. Subjects were randomly assigned to dietary groups according to directions in sealed, sequentially numbered envelopes that were the result of random permutations of subjects into treatment conditions. Of 256 subjects found eligible after screening, 35 declined to enroll, and 43 discontinued after beginning the dietary protocol, typically because of difficulties meeting the time requirements of the study.

A total of 712 adipose samples from 178 subjects were collected and stored at −80 °C. Before RNA isolation, samples were stored for 24–48 h at −20 °C in RNALater ICE (Ambion, Austin, TX); 88 samples were excluded from RNA isolation because the amount of tissue was inadequate (<5 mg); 32 samples were excluded because of being overcontaminated with blood, as judged by sight; and 6 samples were excluded because of mislabeling. Total RNA was isolated from the remaining 586 samples by using Qiagen Lipid Tissue Mini Kit (Qiagen, Valencia, CA). Total RNA was quantified by spectroscopy by using an ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). An additional 133 samples were discarded for insufficient material (<200 ng total RNA), which resulted in viable RNA samples from 453 adipose tissue biopsies.

Laboratory measurements

Plasma triacylglycerol and total cholesterol concentrations were measured with enzymatic procedures on an Express 550 Plus analyzer (Ciba Corning, Oberlin, OH). These measurements were consistently monitored by the Centers for Disease Control and Prevention–National Heart, Lung, and Blood Institute standardization program. HDL cholesterol was measured after dextran sulfate precipitation, and LDL cholesterol was calculated with the formula of Friedewald et al (16).

Expression array analysis

Total RNA samples were selected for genome-wide expression array analysis on the basis of quantity and quality of RNA. The original experimental design called for analysis of RNA from 12 subjects (3 subjects per study arm) at each of the 4 time points, for a total of 48 arrays. For all subjects from which RNA was obtained at all 4 time points, RNA quality was assessed by using the RNA 6000 Pico Total RNA Kit on a 2100 Bioanalyzer (Agilent, Santa Clara, CA). Samples exceeding at least a 28S:18S ratio of 1.3 were obtained at all 4 time points in 10 subjects, and these 40 samples were selected for array analysis. To ensure that 2–3 subjects were available for comparison between each set of interventions for all 4 diets, an additional 7 samples from 3 subjects were selected for inclusion, which resulted in a set of 47 samples from 13 subjects.

Total RNA samples were first treated with DNase and then purified by silicon-based clean-up column. Purified total RNA (50 ng) was used for target preparation according to the 2-cycle target labeling method in the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA). Briefly, double-stranded complementary DNA (cDNA) was generated to undergo an initial in vitro transcription (IVT) with unlabeled ribonucleotides. This IVT product was then used as a template to synthesize double-stranded cDNA. This, in turn, was biotin labeled by IVT to produce labeled complementary RNA. After fragmentation of the complementary RNA, the samples were hybridized overnight onto Affymetrix Human Genome (HG) U133 Plus 2.0 arrays. The arrays were then washed and stained on a Fluidics Station 450 (Affymetrix) and were scanned on a GeneChip Scanner 3000 (Affymetrix). The HG-U133 Plus 2.0 array represents 52 563 probe sets mapped to 38 500 well-characterized genes.

Quality control of array data was determined by using MAS 5.0 software (Affymetrix). Forty-six of 47 arrays passed quality control and were used in subsequent analyses. Within each array, genes were called as missing or present by using a Wilcoxon's signed-rank–based gene expression presence or absence detection algorithm first implemented in MAS 5.0 software (17). Only probe sets called as present at $P < 0.05$ on 36 of 46 arrays (78.3% of samples) were considered to be expressed in adipose tissue. A total of 15 667 of 52 563 probe sets met these criteria and were included in further analyses.

Probe-level gene expression data were normalized by quantile normalization. Background correction and calculation of gene expression values were performed by using the robust multichip average algorithm implemented in the Bioconductor affy package (18–20). Transcriptional responses to acute weight loss, isocaloric dietary composition, and stable weight reduction were analyzed by the Significance Analysis of Microarrays (21) as

implemented in the Bioconductor samr package. The false discovery rate (FDR), which was calculated by using 500 random permutations of the data set reflecting nonspecific sample variance, was set at 0.03 for these analyses (22). Within the Significance Analysis of Microarrays, comparisons across interventions were analyzed by Student's paired *t* test by using log(2) expression data before and after intervention. Effects of acute weight loss were analyzed by using samples from 11 subjects (3 subjects, basal diet; 3 subjects, 39%-carbohydrate diet; 2 subjects, 26%-carbohydrate, low saturated fat diet; and 3 subjects, 26%-carbohydrate plus high saturated fat diet). Response to stable weight reduction was analyzed by using samples from 10 subjects (3 subjects, basal diet; 3 subjects, 39%-carbohydrate diet; 2 subjects, 26%-carbohydrate, low saturated fat diet; and 2 subjects, 26%-carbohydrate, high saturated fat diet). Responses to altered dietary composition were analyzed by using samples from 9 subjects (3 subjects, 39%-carbohydrate diet; 3 subjects, 26%-carbohydrate, low saturated fat diet; and 3 subjects, 26%-carbohydrate, high saturated fat diet). Categorical differences in response to intervention (weight loss, weight reduction, and altered dietary composition) between diet arms were examined by multiple class analysis by using the calculated difference in log(2) expression values between time points. To identify genes responsive to both weight loss and alterations in dietary composition, responses to variation in dietary composition were examined in the subset of transcripts that were significantly responsive to acute weight loss. This analysis used a less stringent FDR threshold (FDR < 0.05) to be more inclusive of genes responsive to both interventions. Expression values are reported as means ± SEMs of the ratios of final expression to initial expression (fold change).

Responses of biological pathways to interventions were assessed by using gene set enrichment analysis (GSEA) software (version 1.0; Broad Institute, Boston, MA; 23, 24). This program calculates the ranked change in response to biological stimuli for each gene within an expression data set by using the signal-to-noise difference metric and calculates enrichment near the extreme of this list of genes within each gene set by using the Kolmogorov-Smirnov statistic. FDR was calculated for each gene set by using 1000 random permutations of the data set. Gene set responses to interventions were analyzed in 15 336 transcripts across 292 gene sets, which contained 15–425 genes per set. The FDR threshold was set at 0.25, as recommended by the authors of the software.

RNA preparation for real-time polymerase chain reaction

cDNA was synthesized from 453 total RNA samples (200 ng per sample, selected as described above) by using the High Capacity cDNA Archive Kit (Applied Biosystems, Santa Clara, CA). Six putative normalization genes were identified as unresponsive to dietary composition, weight loss, or weight reduction by using expression array data: *RPS14*, *SNX11*, *PRK*, *FARSLB*, *VCAR3*, and *DNCL12*. SYBR Green-based gene expression assays (Applied Biosystems, Foster City, CA) were developed for each of these genes, and expression stability was assessed across 36 samples, which varied in diet and intervention, by using geNorm (25). Two genes, *SNX11* and *FARSLB*, were identified as sufficient for normalization within this study population. All subsequent real-time polymerase chain reaction (PCR) analyses were performed in triplicate by using TaqMan Assays on Demand (Applied Biosystems, Foster City, CA). Data for each gene were quantified by using a standard curve (for PCR primers used to construct standards and exact assays purchased, please contact authors) and were normalized to the geometric mean of *SNX11* and *FARSLB* expression. Stability and quality of cDNA from each of the 453 samples were assessed by comparison of expression of *SNX11* and *FARSLB*. Because expression of these 2 genes should be concordant across all samples, samples with data that deviated >1 SD from the mean ratio of expression (*FARSLB* to *SNX11*) were considered of poor quality and were subsequently removed from further analysis. A total of 338 samples remained for analysis.

Statistical analysis

All statistical procedures were performed by using JMP software (version 6.0.2; SAS Institute Inc, Cary, NC), and graphical representations were drawn by using PRISM software (version 4.0; GraphPad, San Diego, CA). Transcript concentrations, energy intakes, and BMIs were expressed as means ± SEMs, and transcriptional responses were expressed as the median and 95% median CI of the fold change in expression (ratio of preintervention to postintervention). Comparisons of transcriptional expression across interventions were analyzed by Student's paired *t* test. Samples from subjects who remained on the basal diet were excluded when responses to isocaloric alteration in dietary composition were assessed. Correlations between basal expression and response to acute weight loss were determined by using initial expression (week 1) and response (week 9 – week 4) data to avoid issues of regression to the mean. Cross-diet comparisons within each intervention and the correlations with lipid and lipoprotein responses were determined by analysis of variance (ANOVA) or covariance by using expression and concentration differences (posttreatment expression minus pretreatment expression). Log transformations of plasma triacylglycerol concentrations and all transcript quantities were used in the analyses to attain normal distributions and equal variance. Changes in BMI and caloric intake were used as covariates in all ANOVA analyses.

RESULTS

Expression array analysis

Comparison of transcriptional response with acute weight loss called 1473 (9.4%) of the analyzed gene probe sets as significantly changing (*see* Table 1 under "Supplemental data" in the online issue). Each of these transcripts showed decreased expression in response to acute weight loss. The greatest reduction was observed in the gene encoding stearoyl-coenzyme A desaturase (*SCD*; −3.65 ± 0.93 fold change). No differences in response were observed between diets (data not shown). Analysis of response to stable weight reduction identified only 2 significantly responsive transcripts (FDR < 0.03, Significance Analysis of Microarrays): ATP-binding cassette, subfamily G, member 1 (*ABCG1*, 2.4 ± 0.33) and malate dehydrogenase (*MDH1*, 1.4 ± 0.04). Neither of these transcripts was significantly altered by acute weight loss. Transcriptional response to isocaloric change in dietary composition identified 4 significantly altered gene transcripts: membrane metalloendopeptidase (*CD10*, −1.60 ± 0.15), adiponutrin (*ADPN*, −1.50 ± 0.15), WW domain containing E3 ubiquitin protein ligase 1 (*WWP1*, −1.17 ± 0.06), and cDNA clone IMAGE:5260162, a transcribed locus of unknown function (−1.29 ± 0.07).

To identify the transcripts responsive to both weight loss and dietary composition, the responses to isocaloric change in dietary

TABLE 2
Genes responsive to both acute weight loss and isocaloric alteration in dietary composition[1]

Gene symbol[1]	Gene name	Acute weight loss[2]	Altered dietary composition[2]	Affymetrix probe identifier[3]
Energy metabolism				
SCD	Stearoyl-coenzyme A desaturase	−3.65 ± 0.93	−1.53 ± 0.29	200832_s_at
ECHDC1	Enoyl-coenzyme A hydratase domain containing 1	−2.02 ± 0.35	−1.24 ± 0.11	233124_s_at
ECHDC1	Enoyl-coenzyme A hydratase domain containing 1	−1.96 ± 0.33	−1.26 ± 0.11	219974_x_at
FADS1	Fatty acid desaturase 1	−1.87 ± 0.33	−1.22 ± 0.14	208964_s_at
FADS1	Fatty acid desaturase 1	−1.86 ± 0.29	−1.44 ± 0.19	208962_s_at
FADS1	Fatty acid desaturase 1	−1.80 ± 0.33	−1.44 ± 0.17	208963_x_at
PLA2G4A	Phospholipase A2, group IVA	−1.81 ± 0.30	−1.20 ± 0.09	210145_at
DGAT2	Diacylglycerol *O*-acyltransferase 2	−1.81 ± 0.37	−1.30 ± 0.09	226064_s_at
LPGAT1	Lysophosphatidylglycerol acyltransferase 1	−1.63 ± 0.22	−1.19 ± 0.08	202651_at
ACBP	Acyl-coenzyme A binding protein	−1.54 ± 0.19	−1.18 ± 0.09	209389_x_at
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	−1.51 ± 0.18	−1.15 ± 0.04	225424_at
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	−1.45 ± 0.16	−1.17 ± 0.06	225420_at
SPOT14	Thyroid hormone responsive	−1.48 ± 0.14	−1.38 ± 0.07	1553583_a_at
PPP2R1B	Protein phosphatase 2, regulatory subunit A, beta	−1.42 ± 0.16	−1.32 ± 0.11	202884_s_at
ME1	Malic enzyme 1, $NADP^+$-dependent, cytosolic	−1.38 ± 0.12	−1.15 ± 0.05	204059_s_at
GLS	Glutaminase	−1.41 ± 0.15	−1.20 ± 0.09	221510_s_at
Apoptosis				
NME1	Nonmetastatic cells 1, protein (NM23A) expressed in	−1.46 ± 0.18	−1.33 ± 0.12	201577_at
CYCS	Cytochrome *c,* somatic	−1.40 ± 0.12	−1.16 ± 0.05	208905_at
Other				
BCAP29	B-cell receptor-associated protein 29	−1.43 ± 0.15	−1.27 ± 0.13	205084_at
SUB1	Activated RNA polymerase II transcription cofactor 4	−1.41 ± 0.16	−1.23 ± 0.08	214512_s_at
OSTM1	Osteopetrosis-associated transmembrane protein 1	−1.38 ± 0.13	−1.13 ± 0.06	218196_at
CD10	Membrane metalloendopeptidase	−1.35 ± 0.13	−1.28 ± 0.09	203435_s_at
MRPL13	Mitochondrial ribosomal protein L13	−1.58 ± 0.20	−1.24 ± 0.13	218049_s_at
PEX11A	Peroxisomal biogenesis factor 11A	−1.27 ± 0.09	−1.22 ± 0.10	205160_at
SLC25A16	Solute carrier family 25 (mitochondrial carrier)	−1.21 ± 0.07	−1.13 ± 0.06	209910_at
CNIH4	HSPC163 protein	−1.30 ± 0.11	−1.32 ± 0.14	218728_s_at
Unknown				
NA	Transcribed locus	−1.88 ± 0.22	−1.22 ± 0.16	241925_x_at
NA	Hypothetical protein FLJ22104	−1.60 ± 0.22	−1.24 ± 0.08	222209_s_at
NA	Transcribed locus	−1.47 ± 0.13	−1.15 ± 0.08	239398_at
NA	Transcribed locus	−1.43 ± 0.17	−1.13 ± 0.06	227278_at

[1] All values are $\bar{x}$ ± SEM. NA, not available. Genes are listed by magnitude of response within each ontologic category.
[2] Response values were identified by expression array and are expressed as a ratio of preintervention to postintervention transcript concentrations (fold change). Negative values indicate reduction in expression.
[3] Affymetrix (Santa Clara, CA).

composition were analyzed within the subset of gene probes responsive to acute weight loss. These analyses were performed by using data from all subjects who underwent isocaloric alteration of dietary composition ($n = 9$) and identified 30 gene probe sets that significantly decreased in expression (**Table 2**). Differences in response were not observed between diets by expression array analysis. These transcripts map to 26 separate genes, of which 22 have known function. Twelve of these 22 genes are involved in energy metabolism (54.5%), 2 are involved in apoptosis (9.1%), and 8 are involved in other functions (36.4%; Table 2). Of the genes related to energy metabolism, 5 were involved in lipogenesis [*SCD*; fatty acid desaturase 1 (*FADS1*); diacylglycerol transferase 2 (*DGAT2*); lysophosphatidylglycerol acyltransferase 1 (*LPGAT1*); and glycerol-3-phosphate acyltransferase, mitochondrial (*GPAM*)], and 4 were involved in lipid metabolism [enoyl-coenzyme A hydratase domain containing 1 (*ECHDC1*); phospholipase A2 group IVA (*PLA2G4A*); acyl-coenzyme A binding protein (*ACBP*/*DBI*); and thyroid hormone responsive protein (*SPOT14*/*THRSP*)].

Responsive biological pathways were identified by using GSEA to statistically rank responses across gene sets, defined by biological function, for all queried transcripts (23). Expressions of 100 gene sets were significantly attenuated in response to acute weight loss, and these sets mapped to 4 major functional categories: energy metabolism (40.6% of responsive gene sets), cell cycle and proliferation (22.8%), insulin signaling (9.9%), and immune function or inflammatory response (6.0%; *see* Table 2 under "Supplemental data" in the online issue). Of the 16 gene sets most significantly altered (FDR < 0.03), 13 were related to energy metabolism. No gene set was identified as significantly responsive following stabilization after weight reduction. GSEA identified only one gene set significantly altered by isocaloric dietary composition: fatty acid synthesis. This gene set was also decreased in response to acute weight loss.

Real-time PCR analysis

Response was confirmed by real-time PCR for the 5 most responsive genes (*SCD*, *ECHDC1*, *DGAT2*, *FADS1*, and *PLA2G4A*) and, as a negative control, for 1 gene that did not show transcriptional response to either intervention by expression array [adiponectin (*APM1*)]. Transcriptional responses were indistinguishable between expression array and real-time PCR analyses for these genes ($P = 0.57$; *see* Figure 1 under "Supplemental data" in the online issue).

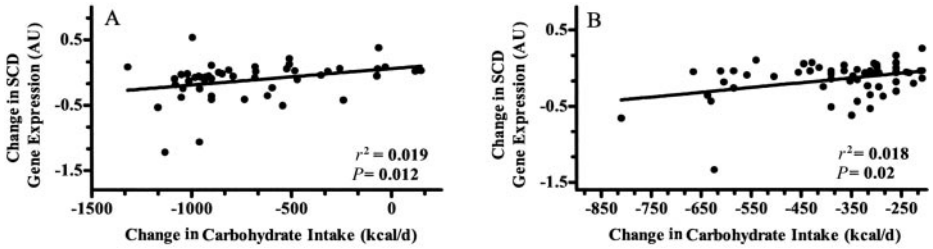

FIGURE 1. Transcriptional response of stearoyl-coenzyme A desaturase (*SCD*) in adipose tissue to altered dietary composition is correlated with change in carbohydrate consumption under (A) isocaloric and (B) hypocaloric conditions. AU, arbitrary units. Transcriptional response is represented as the difference (posttreatment expression minus pretreatment expression). For the isocaloric analysis, samples ($n = 50$) were isolated after 1 wk of the basal diet (pretreatment, 54%, 16%, and 30% carbohydrate, protein, and fat, respectively; 7% saturated fat) and after 4 subsequent weeks of the treatment diet [$n = 10$, basal diet; $n = 14$: 39%, 29%, and 31% carbohydrate, protein, and fat, respectively (8% saturated fat); $n = 15$: 26%, 29%, and 46% carbohydrate, protein, and fat, respectively (9% saturated fat); and $n = 11$, 26%, 29%, and 46% carbohydrate, protein, and fat, respectively (15% saturated fat)]. For the hypocaloric analyses, samples ($n = 57$) were isolated after 3 wk of the randomization diet and after 5 wk of acute weight loss with the randomized diet [$n = 10$, basal diet; $n = 13$, 39%, 29%, and 31% carbohydrate, protein, and fat, respectively (8% saturated fat); $n = 19$, 26%, 29%, and 46% carbohydrate, protein, and fat, respectively (9% saturated fat); and $n = 15$, 26%, 29%, and 46% carbohydrate, protein, and fat, respectively (15% saturated fat)]. Statistical analyses were performed by ANOVA with change in BMI and total caloric intake as covariates.

To further confirm response, real-time PCR analyses were expanded to a larger subset of the study population, which comprised 338 samples from 131 subjects selected as described in Subjects and Methods, allowing for comparison of response in 37–58 subjects per intervention. Repeated-measures ANOVA detected significant changes in transcriptional expression of *SCD*, *DGAT2*, *FADS1*, and *FADS2* over time ($P < 0.0001$). Response to acute weight loss was confirmed for all genes selected from expression array analysis except *APM1*, as expected (**Table 3**). In addition, expression of *FADS2* was significantly decreased by acute weight loss (Table 3). *FADS2* was excluded from expression array analysis owing to low transcript expression but was included in real-time PCR analysis because its expression was previously shown to be reduced in response to weight loss in human adipose tissue (26). Dietary composition during weight loss had no detectable influence on transcriptional response for any of the genes examined (data not shown). *SCD* response to acute weight loss was highly correlated with basal *SCD* expression ($r^2 = 0.38$, $P < 0.0001$). A similar but nonsignificant correlation was also observed for *FADS1* ($r^2 = 0.10$, $P = 0.07$). Transcriptional expression after reduced weight stabilization remained attenuated for *FADS1* and *FADS2* but was indistinguishable from preweight loss expression for *SCD*, *DGAT2*, *ECHDC1*, and *PLA2G4A* (Table 3). Comparison across all adipose samples indicated that expression of *FADS1* and *FADS2* was correlated with BMI ($r^2 = 0.06$, $P = 0.0006$ and $r^2 = 0.03$, $P = 0.008$, respectively), but no significant correlations with BMI were observed for the other genes analyzed.

Transcriptional responses to changes in isocaloric dietary composition were confirmed by real-time PCR for *SCD*, *DGAT2*, and *FADS1* but not for *ECHDC1* or *PLA2G4A* (Table 3). *FADS2* expression was also responsive to change in dietary composition (Table 3). The observed reduction in *SCD* expression was significantly different between the low saturated fat diets that differed in carbohydrate content [0.01 ± 0.09 arbitrary units (AU; 54% carbohydrate) compared with −0.06 ± 0.07 AU (39% carbohydrate) compared with −0.09 ± 0.04 AU (26% carbohydrate); $P = 0.04$, ANOVA]. *SCD* expression response correlated with changes in energy consumed as carbohydrate but not as protein or as total fat, under both isocaloric ($P = 0.012$; **Figure 1**A) and hypocaloric ($P = 0.02$; Figure 1B) conditions. In addition, *SCD* response to isocaloric diet change was significantly different between the two 26%- carbohydrate diets that differed in saturated fat content [**Figure 2**; −0.09 ± 0.04 AU compared with −0.33 ± 0.15 AU; 26%, 29%, and 46% (9%, 27%, and 5%) carbohydrate, protein, and fat (saturated, monounsaturated, and polyunsaturated fats), respectively) compared with 26%, 29%, and 46% (15%, 20%, and 6%) carbohydrate,

TABLE 3
Confirmation of transcriptional response to dietary composition, acute weight loss, and stable weight reduction by real-time polymerase chain reaction analysis for top changing genes[1]

Gene symbol	Altered dietary composition			Acute weight loss			Stable weight reduction		
	n	Response	P^2	*n*	Response	P^2	*n*	Response	P^2
SCD	39	−1.60 (−2.39, −1.40)[3]	0.0007	56	−2.38 (−3.16, −1.97)	< 0.0001	53	1.14 (−1.47, 1.24)	0.91
ECHDC1	38	1.03 (−1.33, 1.14)	0.53	53	−1.32 (−1.85, −1.23)	0.015	48	−1.08 (−1.13, 1.29)	0.92
FADS1	39	−1.44 (−2.07, −1.30)	0.003	55	−1.75 (−2.45, −1.58)	< 0.0001	53	−1.19 (−1.68, −1.12)	0.01
DGAT2	39	−1.51 (−1.82, −1.22)	0.0004	56	−1.25 (−1.95, −1.23)	0.0022	53	1.02 (−1.29, 1.17)	0.75
PLA2G4A	36	−1.10 (−1.31, −1.01)	0.11	50	−1.16 (−1.37, −1.11)	0.0016	46	−1.04 (−1.24, 1.06)	0.56
FADS2	39	−1.67 (−2.45, −1.37)	0.0030	54	−2.44 (−3.60, −2.15)	< 0.0001	51	−1.29 (−1.85, −1.09)	0.04
APM1	29	0.96 (−1.14, 1.20)	0.56	40	1.01 (−1.09, 1.20)	0.57	39	1.04 (−1.08, 1.19)	0.37

[1] *SCD,* stearoyl-coenzyme A desaturase; *ECHDC1,* enoyl-coenzyme A hydratase domain containing 1; *FADS1,* fatty acid desaturase 1; *DGAT2,* diacylglycerol transferase 2; *PLA2G4A,* phospholipase A2, group IVA; *FADS2,* fatty acid desaturase 2; and *APM1,* adiponectin.

[2] Paired *t* test on log-transformed expression data.

[3] Median fold changes; 95% median lower and upper CIs in parentheses (all such values).

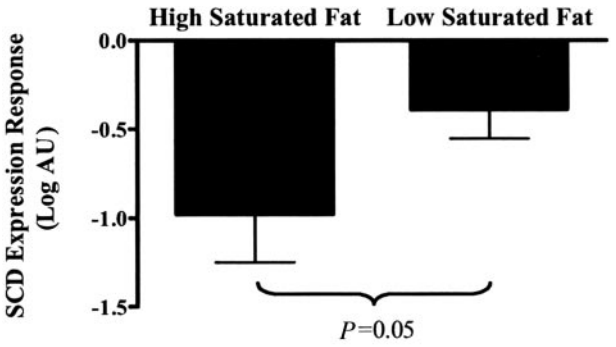

FIGURE 2. Mean (±SEM) stearoyl-coenzyme A desaturase (*SCD*) expression in response to carbohydrate restriction was enhanced by the high saturated fat content of the diet. AU, arbitrary units. Change in *SCD* expression, represented as log(2) difference (treatment expression minus basal expression) in response to isocaloric reduction in carbohydrate from the basal diet [pretreatment, 54% carbohydrate (7% saturated fat) to low-carbohydrate (26%) diets either high in saturated fat (15% saturated fat; $n = 11$) or low in saturated fat (9% saturated fat; $n = 15$). Statistical analyses were performed by ANOVA with change in BMI and total caloric intake as covariates. Both diets resulted in reduced *SCD* expression in comparison with the basal diet (low-carbohydrate and high saturated fat diet, $P = 0.005$; low-carbohydrate and low saturated fat diet, $P < 0.03$), with a significant difference in the response between the 2 diets ($P < 0.05$).

protein, and fat (saturated, monounsaturated, and polyunsaturated fats), respectively; $P = 0.05$]. This change was correlated with changes in energy consumed as saturated fat ($r^2 = 0.18$, $P = 0.002$) but not as monounsaturated fat ($P > 0.10$; data not shown). Transcriptional responses of *DGAT2*, *FADS1*, and *FADS2* could not be distinguished between diets. Expressions of *SCD*, *DGAT2*, *FADS1*, and *FADS2* were correlated with one another ($P < 0.0005$ for all pairwise comparisons) but were not significantly correlated with the expression of the other queried genes (*ECHDC1*, *PLA2G4A*, or *APM1*).

We next examined the correlations between gene expression and plasma triacylglycerol, total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations. For each gene, correlations were modeled by using all available expression data, which represented 338 samples from 131 subjects across dietary interventions. To correct for the potential confounding effects of repeated subject sampling and multiple dietary interventions, subject identifiers and intervention status (modeled by change in weight per week, BMI, and caloric intake) were included as covariates in the regression model. Triacylglycerol concentrations were correlated significantly with expression of *SCD*, *DGAT2*, *FADS1*, and *FADS2* (**Table 4**) but not with expression of the other queried genes (*ECHDC1*, *PLA2G4A*, or *APM1*). Because expression levels of these 4 genes were correlated and because *SCD* activity is known to correlate with plasma triacylglycerol concentrations (27), correlations of triacylglycerol with expression of *FADS1*, *FADS2*, and *DGAT2* were adjusted for *SCD* expression. Only *DGAT2* was correlated with triacylglycerol independently of *SCD* ($P = 0.04$), which suggests coordinated regulation of the desaturase genes and disparate regulation of *DGAT2*. Expression of neither *SCD* nor *DGAT2* was correlated with total cholesterol, LDL-cholesterol, or HDL-cholesterol concentrations. However, LDL-cholesterol concentrations were strongly correlated with *FADS1* expression (β-coefficient: -5.50 ± 1.61; $P < 0.0008$); this relation was independent of BMI, triacylglycerol concentrations, or *SCD* expression. *FADS2* expression was also correlated with LDL-cholesterol concentrations (β-coefficient: -2.60 ± 1.28; $P < 0.04$), but this relation was reduced to nonsignificance after adjustment for *FADS1* expression ($P = 0.20$).

Correlations were also performed to examine the relation between plasma lipid responses and gene expression response for each intervention. Triacylglycerol response to isocaloric change in dietary composition was correlated with *SCD* ($P = 0.0003$) and *DGAT2* ($P = 0.046$) response. This relation was not dependent on carbohydrate consumption (*SCD* × carbohydrate interaction, $P = 0.25$; *DGAT2* × carbohydrate interaction, $P = 0.40$). Triacylglycerol responses to acute weight loss were not correlated with gene expression responses, but triacylglycerol responses to stabilized weight reduction were correlated with both *SCD* ($P = 0.04$) and *FADS2* ($P = 0.03$) response (Table 4). In addition, *FADS1* response to altered dietary composition was correlated with changes in LDL cholesterol (β-coefficient: -9.3 ± 4.3; $P = 0.036$), total cholesterol (β-coefficient: -10.3 ± 4.5; $P = 0.026$), and HDL cholesterol (β-coefficient: -2.3 ± 1.0; $P = 0.026$).

DISCUSSION

Adipose tissue is a metabolically active tissue responsible for energy storage and is involved in the regulation of systemic lipid transport. Dietary interventions, which include weight loss and alterations in macronutrient composition, are indicated for the

TABLE 4
Plasma triacylglycerol concentrations correlate with adipose gene expression[1]

	Triacylglycerol											
	Across interventions[2]			Dietary response[3]			Acute weight loss[4]			Weight reduction[4]		
	n	β-coefficient	*P*	*n*	β-coefficient	*P*	*n*	β-coefficient	*P*	*n*	β-coefficient	*P*
SCD	338	0.10 ± 0.03[5]	0.0001	39	0.34 ± 0.08	0.0003	58	0.03 ± 0.04	0.53	55	0.09 ± 0.04	0.04
DGAT2	338	0.13 ± 0.04	0.001	39	0.22 ± 0.11	0.05	58	-0.014 ± 0.05	0.80	55	0.06 ± 0.06	0.37
FADS1	338	0.08 ± 0.04	0.034	38	0.11 ± 0.10	0.29	56	-0.05 ± 0.05	0.30	55	0.08 ± 0.07	0.28
FADS2	338	0.07 ± 0.03	0.012	38	0.12 ± 0.08	0.12	55	-0.05 ± 0.04	0.17	55	0.13 ± 0.06	0.03

[1] *SCD,* stearoyl-coenzyme A desaturase; *DGAT2,* diacylglycerol transferase 2; *FADS1,* fatty acid desaturase 1; and *FADS2,* fatty acid desaturase 2.
[2] Statistical analysis by ANCOVA included the following covariates: subject, BMI, total calories, change in weight (in pounds) per week.
[3] Statistical analysis by ANCOVA included the following covariates: change in BMI.
[4] Statistical analysis by ANCOVA included the following covariates: change in BMI and change in total calories.
[5] $\bar{x} \pm$ SEM (all such values).

reduction of excess adiposity and improvement in the associated markers of cardiovascular disease risk, which include plasma lipid and lipoprotein concentrations. In the present study, we used expression array analysis to assess the transcriptional responses of genes in human adipose tissue to these 2 interventions to delineate pathways that may mediate their metabolic effects.

Active weight loss resulted in significantly reduced expression of 1473 genes and was independent of dietary composition. Pathway analysis indicated that genes related to energy metabolism were the most responsive, including genes involved in oxidative phosphorylation, the electron transport chain, glycolysis, and gluconeogenesis. Reduction in expression of genes active in cell cycle and proliferation, insulin signaling, and immune or inflammatory response was also observed. These results are similar to previous reports (26, 28–31). In contrast with previous reports, these analyses did not detect any transcripts that significantly increased in expression in response to weight loss. This may reflect differences in subject characteristics because this is the first study to examine transcriptional response in nonobese subjects (75% of subjects had BMI $<$ 30.0 on enrollment). Obesity alters lipolytic and inflammatory regulation in adipose tissue, and response to weight loss in obese subjects reflects a normalization of these processes in addition to more generalized response to energy restriction (31). In contrast with weight loss, transcription after stabilization at reduced weight was characterized by alteration in transcription of only 2 genes (increased expression of *ABCG1* and *MDH1*), which indicates that changes observed after active weight loss were related to energy restriction rather than to weight reduction.

Transcriptional responses to altered macronutrient content were modest in comparison with responses to weight loss, in both the number of genes and the magnitude of response per gene. Twenty-six of the genes responsive to weight loss were also responsive to altered macronutrient composition in the absence of weight loss. Response was confirmed for 3 desaturases (*SCD*, *FADS1*, and *FADS2*) and for *DGAT2*. In adipose tissue, *SCD* converts saturated fatty acids, derived from de novo lipogenesis or plasma lipoproteins, into monounsaturated fatty acids. Studies in animal models showed that *SCD* is highly regulated by diet (32–34), but data in humans are limited (26, 27, 33). *SCD* products, predominantly oleate (18:1), are substrates for endogenous triacylglycerol synthesis, mediated by *DGAT2* (35). *FADS1* and *FADS2* catalyze endogenous synthesis of highly unsaturated fatty acids, which include arachidonic acid and eicosanoids (36, 37).

Because there were multiple differences in macronutrient composition in the 4 experimental diets within the present study, it is difficult to draw conclusions as to the specific nutritional determinants of the observed changes in adipose tissue gene expression. However, *SCD* expression was directly correlated with total carbohydrate consumption both with and without weight loss, consistent with evidence of an effect of dietary carbohydrate on lipogenesis and the critical role of *SCD* in this process (38, 39). It is likely that the effects of carbohydrate restriction also contributed to changes in *SCD* expression observed with total energy reduction. Surprisingly, *SCD* expression was inversely related to saturated fat intake with the low-carbohydrate diet. In mice, saturated fat induces hepatic *SCD* expression, which stimulates de novo lipogenesis (40). This observation may reflect tissue or species specificity of saturated fat–mediated regulation of *SCD* expression or, alternatively, may suggest a combined requirement of carbohydrate and saturated fat for induction of *SCD* expression, although there is as yet no experimental evidence to support this hypothesis.

Plasma triacylglycerol concentrations, which are independently reduced by weight loss and by carbohydrate restriction in the absence of weight loss (11), were independently correlated with *SCD* and *DGAT2* expression. In humans, systemic *SCD* activity, measured by a plasma desaturation index (18:0/18:1 fatty acid ratio) and presumably reflecting activity in both liver and adipose tissue, is higher with a high-carbohydrate, low-fat diet than with a low-carbohydrate, high-fat diet and is predictive of plasma triacylglycerol response (27). However, the observed correlation between diet-induced change in plasma triacylglycerol and adipose tissue *SCD* expression was independent of dietary carbohydrate. Moreover, in preliminary analyses, we confirmed that changes in systemic *SCD* activity as assessed by the desaturation index were correlated with changes in plasma triacylglycerol but that these were not correlated with changes in adipose *SCD* expression (L Mangravite and M Mytosaki, unpublished data, 2004). These findings suggest that the correlation of adipose tissue *SCD* expression with plasma triacylglycerol reflects the action of mechanisms not reflected in systemic *SCD* activity, which includes a substantial contribution from hepatic *SCD* (27). We also cannot preclude the contribution to systemic activity of *SCD* expressed in visceral adipose depots, which are thought to be more metabolically active than are subcutaneous depots (41). *DGAT2* plays a critical role in lipogenesis and is required in the liver for triacylglycerol secretion and in adipose tissue for lipid accumulation (42, 43).

In summary, we showed that transcriptional expression of lipogenic genes, particularly that of *SCD*, in subcutaneous adipose tissue is influenced by dietary macronutrient composition and by energy restriction and that the magnitude of *SCD* and *DGAT2* gene expression across diets is correlated with plasma triacylglycerol concentrations. Gene expression responses to changes in dietary composition were small in comparison with responses to energy restriction. These data suggest that weight reduction programs that involve restriction of specific macronutrients would not differentially affect transcriptional expression within adipose tissue provided that they achieved similar amounts of weight loss. However, restriction of specific macronutrients under weight-stable conditions can alter fatty acid processing within adipose tissue and may contribute to altered systemic regulation of the metabolism of lipids and lipoproteins, particularly triacylglycerol and triacylglycerol-rich lipoproteins. Determination of the mechanisms responsible for the diet-induced changes in adipose gene expression observed in the present study will be required to gain a more complete understanding of the consequences of these dietary effects for human lipid metabolism.

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The authors' responsibilities were as follows—LMM: was responsible for experimental procedures, data analysis, and manuscript preparation; KD: performed statistical analysis of expression arrays; RRD: prepared RNA for expression array analysis and performed array hybridizations; JPG: managed the expression array laboratory; RMK: was the principal investigator; and all authors: prepared the manuscript. None of the authors had any conflicts of interest.

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