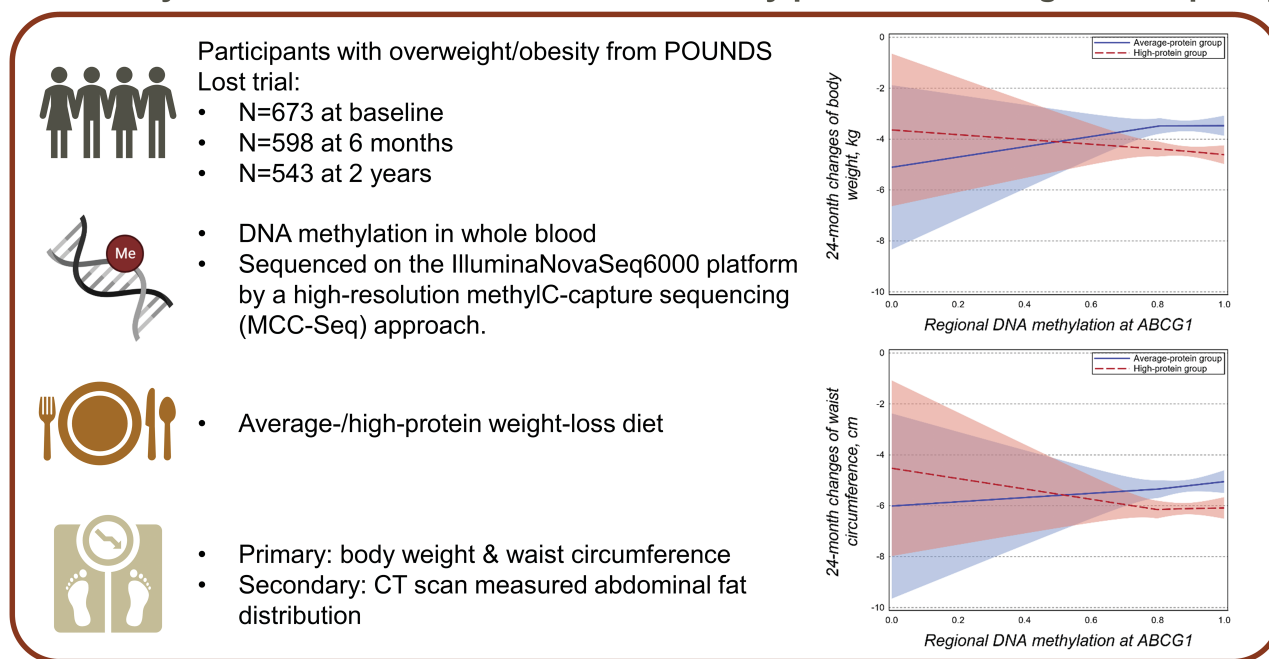# DNA Methylation at *ABCG1* and Long-term Changes in Adiposity and Fat Distribution in Response to Dietary Interventions: The POUNDS Lost Trial

Xiang Li, Xiaojian Shao, Minghao Kou, Xuan Wang, Hao Ma, Elin Grundberg, Lydia A. Bazzano, Steven R. Smith, George A. Bray, Frank M. Sacks, and Lu Qi

DNA methylation at *ABCG1* interacted with dietary protein on changes in adiposity

Participants with overweight/obesity from POUNDS Lost trial:
- N=673 at baseline
- N=598 at 6 months
- N=543 at 2 years

- DNA methylation in whole blood
- Sequenced on the IlluminaNovaSeq6000 platform by a high-resolution methylC-capture sequencing (MCC-Seq) approach.

- Average-/high-protein weight-loss diet

- Primary: body weight & waist circumference
- Secondary: CT scan measured abdominal fat distribution

ARTICLE HIGHLIGHTS

- Dietary weight-loss interventions are effective in reducing adiposity; however, large interindividual variability exists. Evidence has suggested that DNA methylation may at least partly explain this.
- We sought to investigate the association of diabetes-related DNA methylation at *ABCG1* with long-term changes in adiposity and how dietary protein may interact with DNA methylation.
- Participants with lower methylation at *ABCG1* benefitted more in long-term reductions in body weight, waist circumference, and body fat distribution when consuming an average-protein diet.
- Our data indicate that baseline DNA methylation at *ABCG1* interacted with dietary protein intake to enhance long-term decreases in body weight, adiposity, and body fat distribution.

DNA Methylation at *ABCG1* and Long-term Changes in Adiposity and Fat Distribution in Response to Dietary Interventions: The POUNDS Lost Trial

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OBJECTIVE

To examine whether participants with different levels of diabetes-related DNA methylation at *ABCG1* might respond differently to dietary weight loss interventions with long-term changes in adiposity and body fat distribution.

RESEARCH DESIGN AND METHODS

The current study included overweight/obese participants from the POUNDS Lost trial. Blood levels of regional DNA methylation at *ABCG1* were profiled by high-resolution methylC-capture sequencing at baseline among 673 participants, of whom 598 were followed up at 6 months and 543 at 2 years. Two-year changes in adiposity and computed tomography–measured body fat distribution were calculated.

RESULTS

Regional DNA methylation at *ABCG1* showed significantly different associations with long-term changes in body weight and waist circumference at 6 months and 2 years in dietary interventions varying in protein intake (interaction *P* < 0.05 for all). Among participants assigned to an average-protein (15%) diet, lower baseline regional DNA methylation at *ABCG1* was associated with greater reductions in body weight and waist circumference at 6 months and 2 years, whereas opposite associations were found among those assigned to a high-protein (25%) diet. Similar interaction patterns were also observed for body fat distribution, including visceral adipose tissue, subcutaneous adipose tissue, deep subcutaneous adipose tissue, and total adipose tissue at 6 months and 2 years (interaction *P* < 0.05 for all).

CONCLUSIONS

Baseline DNA methylation at *ABCG1* interacted with dietary protein intake on long-term decreases in adiposity and body fat distribution. Participants with lower methylation at *ABCG1* benefitted more in long-term reductions in body weight, waist circumference, and body fat distribution when consuming an average-protein diet.

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Various weight loss diets are effective in reducing adiposity; however, considerable interindividual variability has been noted (1,2). Identifying the factors that predict individual variability in response to interventions could enhance efficacy while

ORIGINAL ARTICLE

reducing the burden on the health care system by treating only those who have known responsive phenotypes to an intervention. Emerging evidence has suggested that DNA methylation may at least partly explain such interindividual variability (3–5). DNA methylation is an epigenetic modification that can influence gene expression without altering the underlying genetic sequence. Previous studies with small sample sizes have reported several differentially methylated regions that distinguish responders from nonresponders to dietary interventions (4,5).

ATP binding cassette subfamily G member 1 (*ABCG1*), a protein-coding gene, has an established role in the reverse cholesterol transport from cells to high-density lipoprotein (6,7). Several recent epigenome-wide association studies have linked DNA methylation at CpG site cg06500161 with diabetes (8), BMI (9–12), waist circumference (10,13), and quantitative measures of body fat distribution (14). Mendelson et al. (11) conducted a comprehensive analysis, wherein they identified 83 CpG sites associated with BMI, with cg06500161 at *ABCG1* being the strongest in the replication cohorts. Three-way association analysis confirmed the association of DNA methylation at *ABCG1* with gene expression in whole blood (mRNA level) and gene expression with BMI (11). In another longitudinal study, Sun et al. (15) also reported that DNA methylation at *ABCG1* was the consequence of obesity. Geurts et al. (16) showed that both baseline DNA methylation and changes in methylation at *ABCG1* were associated with obesity in a longitudinal setting. The previous investigations were conducted among the general population. However, among overweight or obese participants, whose DNA methylation may be altered, whether baseline DNA methylation at *ABCG1* is associated with longitudinal changes in adiposity in response to dietary weight loss interventions is not clear.

DNA methylation acts in the interface of gene and environmental stressors, such as diet, and subsequently influences phenotypic changes. Various studies have shown that dietary factors, such as macronutrient components, could change DNA methylation, which then influences gene expression (17–19). However, to date, no study has assessed whether DNA methylation at *ABCG1* is associated with changes in adiposity in response to dietary interventions.

In the current study, we investigated the relationship between baseline DNA methylation at *ABCG1* with long-term changes in adiposity (i.e., body weight, waist circumference, and abdominal fat distribution). We were particularly interested in the possible interaction between DNA methylation and dietary protein and fat on long-term changes in the above outcomes.

RESEARCH DESIGN AND METHODS

Study Population

The study was conducted among participants from the POUNDS Lost trial (Clinical trial reg. no. NCT00072995, clinicaltrials.gov), a 2-year randomized trial that compared the weight loss effects of four energy-reduced diets with varying macronutrient compositions (carbohydrate, fat, and protein) (20). The POUNDS Lost trial was conducted at two clinical sites, the Harvard T.H. Chan School of Public Health and Brigham and Women's Hospital (Boston, MA) and the Pennington Biomedical Research Center of the Louisiana State University System (Baton Rouge, LA), from October 2004 to December 2007. Major exclusion criteria were the presence of diabetes, unstable cardiovascular disease, the use of medications that affect body weight, or less motivation for the intervention. In brief, a total of 811 participants with overweight or obesity were randomly assigned to one of four weight loss diets. The targeted macronutrient compositions were *1)* 20% fat, 15% protein, and 65% carbohydrates; *2)* 20% fat, 25% protein, and 55% carbohydrates; *3)* 40% fat, 15% protein, and 45% carbohydrates; and *4)* 40% fat, 25% protein, and 35% carbohydrates. Two diets were low fat (20%) and two were high fat (40%), and two diets were average protein (15%) and two were high protein (25%), constituting a 2 × 2 factorial design. The four diets consisted of similar foods, in line with cardiovascular health guidelines, including ≤8% saturated fat, dietary fiber ≥20 g/day, and ≤150 mg cholesterol ≤150 mg/1,000 kcal (20). To evaluate adherence to the assigned diets, dietary intake was assessed in a random sample of 50% of the total participants by a 5-day diet record at baseline and by 24-h diet recall on 3 nonconsecutive days at 6 months and 2 years. Biomarkers of nutrient intake were used to validate the self-reported adherence to macronutrient targets as follows: urinary nitrogen excretion for protein

(21) and respiratory quotient for fat (22). Detailed information on the study design and methods has been published elsewhere (20). The study was approved by the human subjects committees at the Harvard T.H. Chan School of Public Health and Brigham and Women's Hospital and the Pennington Biomedical Research Center of the Louisiana State University System and by a data and safety monitoring board appointed by the National Heart, Lung, and Blood Institute. All participants provided written informed consent.

In the current analysis, we included 673 participants at baseline, based on the availability of DNA methylation at *ABCG1*. Of the included participants, 598 had complete data on DNA methylation and main outcome variables at 6 months, and 543 had these data at 2 years. The follow-up rate was similar to that in the entire POUNDS Lost trial (i.e., 80%).

DNA Methylation

DNA samples were extracted from the buffy coat fraction of centrifuged peripheral blood samples using the QIA Blood Kit (Qiagen, Chatsworth, CA). DNA methylation in the peripheral blood samples was sequenced on the Illumina NovaSeq 6000 platform using a high-resolution methylC-capture (MCC-seq) approach at Children's Mercy Research Institute (23). Detailed methods for processing sequencing reads have been described previously (23–25). Cell-type composition (B cell, T cell, megakaryocyte/erythroblast, monocyte/macrophage, and neutrophil) was computed according to the Houseman approach using a custom panel of 30,455 cell type–specific hyper-/hypomethylated CpGs (26). Because DNA methylation is correlated with neighboring CpGs, a regional approach is more biologically meaningful. Previous evidence has also shown that regional DNA methylation is more strongly associated with phenotypic traits than individual CpG sites (14). Moreover, a regional method is preferred when methylation levels are measured using the MCC-seq method, which consists of shotgun sequencing targeted on random staggered positions in the genome (27). Therefore, we fetched CpG sites within the ±250-bp window of the probe CpG cg06500161 at *ABCG1* (chr21 43,656,337–43,656,837; seven CpGs in the tested window). Regional DNA methylation at *ABCG1* was calculated as the percentage of the total number of pooled methylation

reads of the total number of pooled sequencing reads covering CpGs over the region.

Measurement of Adiposity Traits

Body weight was measured by calibrated hospital scales in the morning before breakfast on 2 nonconsecutive days at baseline and every 6 months during the intervention, and averaged values were used. Height was measured at baseline. BMI was calculated as weight divided by the square of height (kg/m^2). Waist circumference was measured on the same days as body weight using a nonstretchable tape at 4 cm above the iliac crest. A random sample of 25% of the total participants was selected to undergo computed tomography for the abdominal fat distribution measurement at baseline, 6 months, and 2 years. A series of eight single-slice images was obtained every 10 cm from 2 cm below and 5 cm above the fourth and fifth lumbar vertebrate interspaces (28). Visceral adipose tissue (VAT) mass, subcutaneous adipose tissue (SAT) mass, and deep subcutaneous adipose tissue (dSAT) mass were derived from these eight scans, and total adipose tissue (TAT) mass was calculated as the sum of the individual slices (29).

Statistical Analysis

The primary outcomes were changes in body weight and waist circumference. Secondary outcomes included changes in abdominal fat distribution: VAT, SAT, dSAT, and TAT. Baseline characteristics of the study population across the tertile categories of regional DNA methylation at *ABCG1* are presented. Generalized linear regression models (PROC GLM) for continuous variables and χ^2 tests (PROC FREQ) for categorical variables were performed for comparison of the characteristics between average- and high-protein groups, as well as across the regional DNA methylation at *ABCG1* tertiles. Dietary intake and biomarkers of adherence were also compared across the tertiles of regional DNA methylation at *ABCG1* at 6 months and 2 years. Regional DNA methylation at *ABCG1* was analyzed as a continuous variable. Generalized linear regression models were fitted to examine the association of regional DNA methylation at *ABCG1* and the changes in outcome variables over 2 years. The interaction between DNA methylation and diet was tested based on continuous DNA

methylation by introducing the product term DNAm × diet in the above model. For the main outcomes (body weight and waist circumference), we adjusted for age, race, sex, use of lipid-lowering medications, baseline values of respective outcomes, and blood cell composition in the generalized linear regression model. For the secondary outcomes (abdominal fat distribution), we further adjusted baseline BMI on the above model. Linear mixed models (PROC MIXED) with a compound symmetry covariance structure were performed to test the effect of pretreatment DNA methylation at *ABCG1* on the trajectory of changes in outcomes according to diet by including DNA methylation–time interactions. In line with previous studies (24,25), we used tertiles of regional DNA methylation at *ABCG1* for better visualization of the differences between DNA methylation levels. The interaction patterns did not depend on the choice of tertile or other category. All statistical analyses were performed using SAS software (version 9.4; SAS Institute). All *P* values are two sided, and a *P* value <0.05 was considered statistically significant. We also provide *P* values adjusted for multiple testing by controlling for the false discovery rate (30).

RESULTS

Study participants' mean age was 51 ± 9 years, mean BMI was 32.7 ± 3.9 kg/m^2. Among participants, 61% were female, and 80% were White. Table 1 lists the baseline characteristics of the study population according to average- and high-protein groups. There were no differences in age, race, sex, body weight, waist circumference, or abdominal fat distributions between the groups. Characteristics according to tertile category of regional DNA methylation at *ABCG1* are listed in Supplementary Table 1. Dietary intake and biomarkers of adherence confirmed that the participants modified their macronutrient intake in the direction of the intervention (Table 2). Nutrient intake and biomarkers of adherence did not differ across tertiles of DNA methylation. There were no differences between the average-protein and high-protein groups in changes in adiposity or abdominal fat distribution at 6 months or 2 years (Supplementary Table 2). We did not find significant associations between baseline regional DNA methylation at *ABCG1* and changes in adiposity

or abdominal fat distribution, except for 2-year changes in dSAT (Supplementary Table 3).

Interestingly, we found significant interactions between baseline regional DNA methylation at *ABCG1* and dietary protein intake on changes in body weight (interaction *P* = 0.0072 at 6 months and 0.030 at 2 years) and waist circumference (interaction *P* = 0.007 at 6 months and 0.034 at 2 years) (Table 3 and Fig. 1). At 6 months, in the average-protein diet group, a lower level of regional DNA methylation at *ABCG1* was associated with greater reductions in body weight (*P* = 0.011) and waist circumference (*P* = 0.010), after adjustment for multiple variables, whereas an opposite association was found in the high-protein diet group. The interaction pattern persisted at 2 years (Table 3 and Fig. 1). No significant interactions were found between baseline regional DNA methylation at *ABCG1* and dietary fat or carbohydrates.

We then examined the relationship between regional DNA methylation at *ABCG1*, dietary protein intake, and changes in abdominal fat distribution (Table 3). We found that regional DNA methylation at *ABCG1* significantly modified the association between weight loss diets varying in protein composition and changes in abdominal fat distribution at 6 months and 2 years (interaction *P* < 0.05 for all). At 6 months, among participants consuming an average-protein diet, significant positive associations were found between regional DNA methylation at *ABCG1* and changes in dSAT (*P* = 0.007), SAT (*P* = 0.013), and TAT (*P* = 0.043). In other words, lower baseline DNA methylation at *ABCG1* was associated with greater reductions in abdominal fat distribution, including dSAT, SAT, and TAT. However, nonsignificant opposite associations were observed between DNA methylation and abdominal fat distribution among those consuming a high-protein diet. Similar interaction patterns were consistently found at 2 years.

We also used linear mixed models to test whether the association between pretreatment DNA methylation at *ABCG1* and adiposity traits differed by time during the 2-year intervention in average- and high-protein diet groups (Supplementary Figs. 1 and 2). We found significant interactions between regional DNA methylation at *ABCG1* and intervention time for trajectories of body weight and waist circumference in the average-protein group (Supplementary Fig. 1). In the average-protein group,

A 6 months

B 2 years

C

D
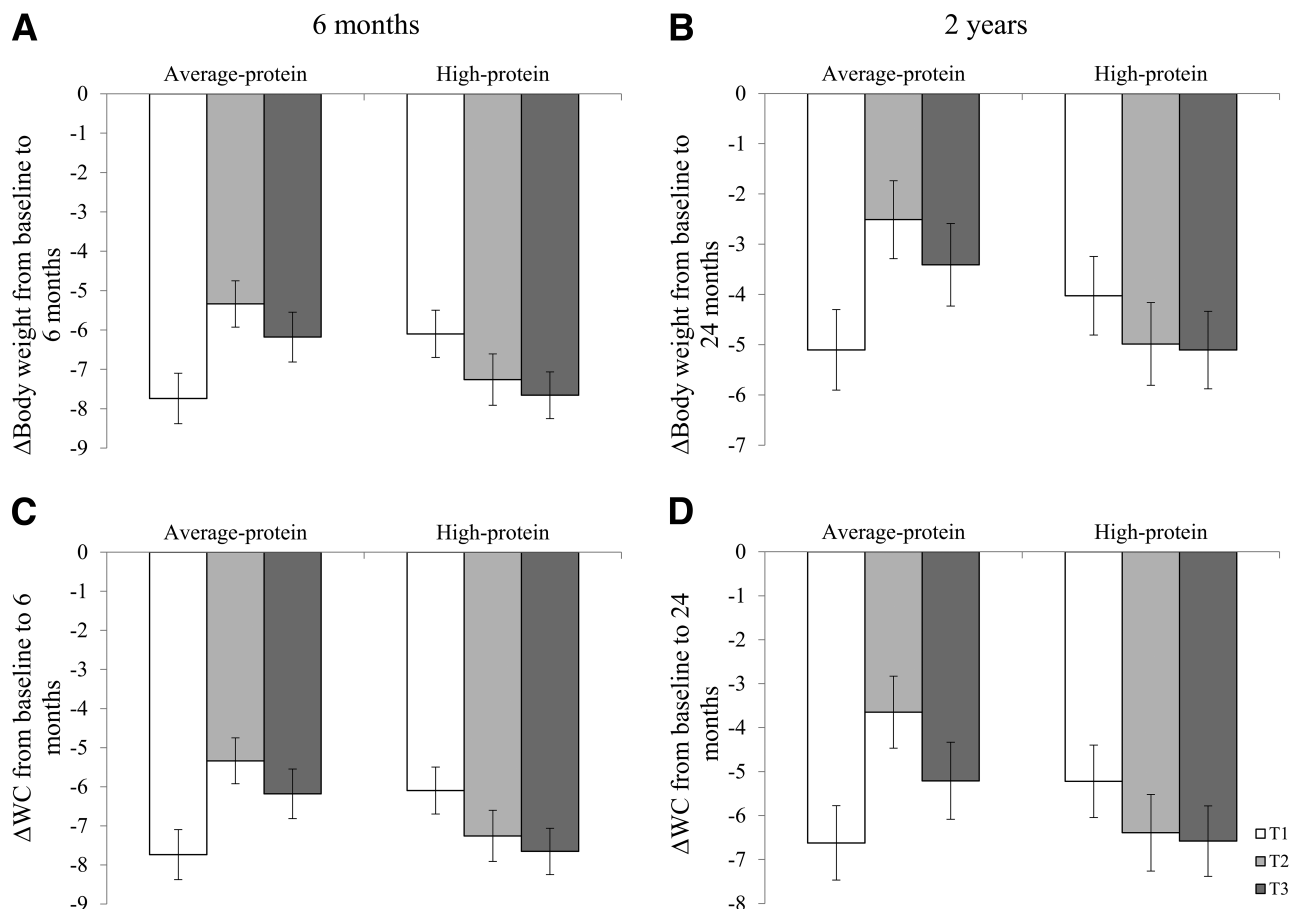
Figure 1—Association of regional DNA methylation at *ABCG1* and changes in weight (*A* and *B*) and waist circumference (WC) (*C* and *D*) in response to average- or high-protein diet at 6 months (*A* and *C*) and 2 years (*B* and *D*) of dietary intervention. Data are mean ± SE after adjustment for age, race, sex, use of lipid-lowering medications, baseline values of respective outcomes, and blood cell composition. T1 is the lowest tertile; T3 is the highest tertile. Sample size at 6 months: average-protein group T1 = 93, T2 = 109, and T3 = 94; high-protein group T1 = 104, T2 = 91, and T3 = 107. Sample size at 2 years: average-protein group T1 = 88, T2 = 97, and T3 = 85; high-protein group T1 = 91, T2 = 86, and T3 = 96.

compared with participants with higher levels (tertile 2 [T2] and T3) of DNA methylation at *ABCG1*, those in the lowest tertile (T1) had greater reductions in body weight and waist circumference from baseline to 12 months, but body weight and waist circumference then increased substantially thereafter to 2 years (Supplementary Fig. 1*A* and *C*). In the high-protein diet group, there was no difference across tertile categories of DNA methylation. Trajectories of changes in abdominal fat distribution are shown in Supplementary Fig. 2. In the average-protein group, significant DNA methylation–time interactions were observed on changes in VAT (interaction $P < 0.001$), SAT (interaction $P < 0.001$), dSAT (interaction $P < 0.001$), and TAT (interaction $P < 0.001$). Participants with lower baseline DNA methylation at *ABCG1* had greater reductions in VAT, SAT, dSAT, and TAT consistently from baseline to 2 years, whereas those with higher DNA methylation at *ABCG1* showed slight

reductions in abdominal fat distribution at 6 months, which they then regained remarkably from 6 months to 2 years.

CONCLUSIONS

Among the study participants from a 2-year randomized dietary weight loss intervention trial, we found that regional DNA methylation at *ABCG1* showed significantly different associations with long-term changes in body weight, waist circumference, and abdominal fat distribution among participants assigned to an average- or high-protein diet. In response to an average-protein diet, lower baseline regional DNA methylation at *ABCG1* was associated with greater reductions in body weight, waist circumference, and abdominal fat distribution across the 2 years, whereas no association was found in the high-protein group.

Several epigenome-wide association studies have consistently highlighted

associations between DNA methylation at *ABCG1* with BMI (9–12), waist circumference (10,13), and other adiposity tissues (14), wherein lower methylation at *ABCG1* (cg06500161) was correlated with favorable adiposity traits. Of note, DNA methylation at *ABCG1* (cg06500161) showed directional consistency for associations with BMI in both blood samples and adipose tissue (10,12). In addition, DNA methylation at *ABCG1* was linked to lipid metabolism and insulin resistance, which are common risk factors for obesity (31,32). However, whether DNA methylation at *ABCG1* is associated with changes in adiposity over time in response to dietary weight loss interventions among individuals with overweight/obesity has been less investigated. For the first time, we observed that regional DNA methylation at *ABCG1* significantly interacted with dietary protein intake on changes in adiposity in the 2-year dietary weight loss interventions.

Table 1—Baseline characteristics of study participants according to average- or high-protein group

	Average protein (n = 341)	High protein (n = 332)	P*
Regional DNA methylation at ABCG1	0.9 (0.1)	0.9 (0.1)	0.97
Age, years	51.4 (9.2)	50.4 (9.4)	0.14
White race, n (%)	270 (79.2)	268 (80.7)	0.62
Female sex, n (%)	204 (59.8)	208 (62.7)	0.45
BMI, kg/m²	32.6 (3.8)	32.9 (3.9)	0.26
Body weight, kg	93.5 (16.4)	93.5 (14.6)	0.98
Waist circumference, cm	103.9 (13.7)	103.6 (12.4)	0.80
SBP, mmHg	119.0 (13.1)	120.3 (13.9)	0.21
DBP, mmHg	75.3 (9.1)	75.8 (9.8)	0.51
LDL, mg/dL	126.7 (33.2)	125.6 (31.9)	0.65
HDL, mg/dL	48.1 (13.6)	49.6 (14.6)	0.17
Cholesterol, mg/dL	202.4 (38.3)	203.5 (36.3)	0.69
Triglycerides, mg/dL	121.0 [88, 175]	123.0 [81.5, 185.5]	0.97
Glucose, mg/dL	92.1 (11.9)	91.9 (11.8)	0.82
HbA$_{1c}$, %	5.4 (0.4)	5.4 (0.4)	0.78
Insulin, μU/mL	10.7 [7.1, 15.4]	10.3 [6.8, 14.9]	0.53
HOMA-IR	2.4 [1.5, 3.7]	2.3 [1.5, 3.4]	0.53
Dietary intake per day			
Energy, kcal	2,001.9 (556.2)	1,913.5 (545.4)	0.14
Carbohydrates, %	44.8 (7.6)	44.7 (7.6)	0.92
Fat, %	36.7 (6.0)	37.0 (6.0)	0.71
Protein, %	18.1 (3.2)	18.2 (3.5)	0.90
Biomarkers of adherence			
Respiratory quotient	0.8 (0.05)	0.8 (0.04)	0.54
Urinary nitrogen	12.4 (4.5)	12.1 (4.4)	0.45
Abdominal fat distribution, kg			
VAT	5.3 (2.5)	5.6 (2.6)	0.53
SAT	11.3 (2.5)	10.9 (2.7)	0.37
dSAT	5.8 (1.7)	5.6 (1.6)	0.50
TAT	16.6 (3.7)	16.6 (4.2)	0.93
Lipid-lowering medications	63 (18.5)	59 (17.8)	0.81

Data are given as mean (SD) or median [IQR] unless otherwise noted. DBP, diastolic blood pressure; HOMA-IR, HOMA of insulin resistance; SBP, systolic blood pressure. *P values were calculated by χ^2 test for categorical variables and F test for continuous variables.

We observed a significant association between lower baseline DNA methylation at ABCG1 and greater reduction in adiposity among participants assigned to an average-protein diet, but not among those assigned to a high-protein diet. Our findings add to the published literature and provide novel evidence that DNA methylation at ABCG1 interacts with dietary protein in relation to temporal changes in adiposity in response to dietary weight-loss interventions.

Although the underlying mechanisms for the interaction between DNA methylation and dietary protein are not well studied, we assume that the amino acid methionine, which is rich in dietary protein, may account for this observation (33,34). Firstly, previous studies have shown that DNA methylation at ABCG1 is associated with a decrease in gene expression of ABCG1 mRNA levels in whole blood and multiple tissues (11,35). This decrease in gene expression was subsequently linked to an increase in BMI (11). Indeed, growing evidence from animal/experimental studies has shown the functional role of ABCG1 in regulating adiposity (36,37). abcg1-knockout animal models exhibited reduced body weight and adipose tissue mass (36). In addition, functional single nucleotide polymorphisms at ABCG1 were also associated with BMI and fat mass index in an obese population (37). Next, methionine is an essential amino acid that is abundant in dietary protein sources. Methionine is a precursor for S-adenosylmethionine, which is the primary methyl donor to DNA methylation (38). High levels of methionine in the diet have been shown to increase S-adenosylmethionine levels and subsequently increase DNA methylation (33,39). Although we did not measure changes in DNA methylation during dietary interventions, we hypothesize DNA methylation levels may change in response to the interventions, differently in the average- versus high-protein group. Taken together, we speculate a high-protein diet may facilitate DNA methylation, which may mask the beneficial association of a lower methylation level at ABCG1 with adiposity and abdominal fat distribution. However, without DNA methylation levels after the interventions, such speculation should be interpreted with caution. We acknowledge the complexity of the relationship/interplay between DNA methylation, diet, and adiposity. Additional investigations are warranted to explore the underlying mechanisms.

Our study has several strengths. First, this is the first study to examine the interaction between regional DNA methylation at ABCG1 and macronutrient composition on long-term changes in adiposity in dietary weight loss interventions. The POUNDS Lost trial is among the largest and longest dietary weight loss intervention studies published. Second, we used a high-resolution MCC-seq approach to measure regional DNA methylation at ABCG1. Because DNA methylation is correlated in a region, our regional assessment is more biologically reasonable. Previous studies have also shown that regional DNA methylation is stronger than that at individual CpG sites (14). Third, computed tomography–measured abdominal fat distribution was assessed at multiple time points. Nonetheless, several limitations of the study should be noted. First, DNA methylation was measured only at baseline. Therefore, we were unable to investigate the changes in DNA methylation in response to dietary weight loss interventions or subsequently how the change in DNA methylation is associated with changes in adiposity traits. Second, DNA methylation was measured in peripheral blood samples rather than in adipose

Table 2—Nutrient intake and biomarkers of adherence according to regional DNA methylation at *ABCG1* and average- or high-protein diet group

	6 months				24 months			
	T1	T2	T3	*P*	T1	T2	T3	*P*
Average-protein group								
Dietary intake per day*								
Energy, kcal	1,627.8 (532.0)	1,663.8 (482.7)	1,580.2 (467.9)	0.90	1,653.8 (516.7)	1,489.8 (403.2)	1,501.6 (591.3)	0.56
Fat, %	30.8 (9.0)	29.1 (7.4)	31.1 (8.7)	0.38	31.7 (8.7)	31.0 (8.8)	29.3 (7.8)	0.52
Protein, %	16.9 (2.7)	18.4 (4.0)	18.6 (4.3)	0.06	18.7 (3.3)	20.9 (4.7)	19.9 (4.8)	0.31
Carbohydrates, %	54.4 (10.2)	53.0 (10.4)	51.1 (10.7)	0.39	51.1 (11.2)	48.2 (9.0)	49.8 (11.0)	0.59
Biomarkers of adherence								
Urinary nitrogen†	10.6 (4.4)	11.2 (4.8)	10.6 (4.2)	0.69	11.3 (3.7)	11.7 (5.1)	12.3 (4.1)	0.40
Respiratory quotient‡	0.8 (0.05)	0.8 (0.04)	0.8 (0.04)	0.18	0.8 (0.04)	0.8 (0.04)	0.8 (0.04)	0.51
High-protein group								
Dietary intake per day*								
Energy, kcal	1,613.1 (454.1)	1,603.8 (721.7)	1,609.4 (445.9)	0.88	1,487.3 (426.4)	1,478.9 (432.6)	1,523.0 (434.1)	0.78
Fat, %	29.8 (7.6)	29.8 (8.4)	32.3 (8.0)	0.35	32.3 (9.4)	30.3 (8.3)	31.9 (7.3)	0.68
Protein, %	21.6 (4.2)	22.2 (3.5)	22.5 (4.2)	0.54	20.5 (5.8)	21.2 (4.3)	21.6 (3.8)	0.91
Carbohydrate, %	49.8 (9.6)	48.3 (8.8)	44.7 (8.2)	0.05	47.9 (10.0)	47.9 (9.7)	45.8 (9.4)	0.92
Biomarkers of adherence								
Urinary nitrogen†	12.4 (4.9)	12.1 (4.6)	12.2 (4.6)	0.69	12.0 (5.1)	12.1 (4.6)	12.6 (4.8)	0.92
Respiratory quotient‡	0.8 (0.04)	0.8 (0.04)	0.8 (0.04)	0.49	0.8 (0.04)	0.8 (0.05)	0.8 (0.04)	0.51

Data are given as mean ± SD. *Sample size in average-protein group at 6 months: T1 = 42, T2 = 57, and T3 = 46; high-protein group at 6 months: T1 = 48, T2 = 43, and T3 = 53. Sample size in average-protein group at 2 years: T1 = 26, T2 = 23, and T3 = 28; high-protein group at 2 years: T1 = 25, T2 = 22, and T3 = 25. †Sample size in average-protein group at 6 months: T1 = 69, T2 = 83, and T3 = 79; high-protein group at 6 months: T1 = 88, T2 = 78, and T3 = 89. Sample size in average-protein group at 2 years: T1 = 47, T2 = 57, and T3 = 48; high-protein group at 2 years: T1 = 59, T2 = 57, and T3 = 64. ‡Sample size in average-protein group at 6 months: T1 = 80, T2 = 95, and T3 = 87; high-protein group at 6 months: T1 = 94, T2 = 84, and T3 = 99. Sample size in average-protein group at 2 years: T1 = 64, T2 = 73, and T3 = 62; high-protein group at 2 years: T1 = 73, T2 = 71, and T3 = 74.

tissue. However, DNA methylation at *ABCG1* in blood samples has been reported to be consistent with that in adipose tissues (10,12), indicating that blood-based DNA methylation could be a proxy for DNA methylation in adipose tissue. Third, the POUNDS Lost population is predominantly White middle-aged participants with overweight or obesity. Whether the results could be applied to the general population requires further investigation. Fourth, we acknowledge that power may be limited due to the interaction tests and sample sizes. Finally, we were unable to find another similar data set with both dietary intervention and DNA methylation information to replicate our findings.

Table 3—Association of regional DNA methylation at *ABCG1* and changes in body fat distribution in response to average- or high-protein diet at 6 months and 2 years of dietary intervention

	Average-protein group*		High-protein group*		*P*	
	β (SE)	*P*	β (SE)	*P*	Interaction	FDR
6 Months†						
Body weight	12.5 (4.9)	0.011	−9.4 (4.2)	0.027	0.0072	0.014
Waist circumference	13.3 (5.1)	0.010	−9.1 (4.6)	0.05	0.007	0.014
dSAT	5.4 (1.9)	0.007	−2.5 (1.6)	0.12	<0.001	0.011
SAT	8.8 (3.4)	0.013	−3.9 (2.6)	0.15	0.004	0.015
VAT	4.7 (2.7)	0.096	−3.7 (1.8)	0.04	0.006	0.015
TAT	11.1 (5.3)	0.043	−8.3 (4.1)	0.05	0.003	0.015
2 Years†						
Body weight	15.1 (6.5)	0.022	−8.4 (5.7)	0.14	0.03	0.034
Waist circumference	17.6 (7.0)	0.013	−7.8 (6.0)	0.20	0.034	0.034
dSAT	6.8 (2.9)	0.025	1.1 (2.3)	0.63	0.009	0.017
SAT	16.3 (5.9)	0.013	−3.6 (3.9)	0.37	0.025	0.031
VAT	7.5 (3.5)	0.044	−1.8 (2.7)	0.50	0.031	0.031
TAT	23.3 (9)	0.019	−4.2 (6.3)	0.51	0.021	0.031

FDR, false discovery rate. *Models were adjusted for age, race, sex, baseline BMI, use of lipid-lowering medications, baseline values for respective outcomes, and blood cell composition. †Sample size for 6-month changes: average-protein group *n* = 55; high-protein group *n* = 59. Sample size for 2-year changes: average-protein group *n* = 39; high-protein group *n* = 46.

In conclusion, our results indicate that lower pretreatment regional DNA methylation at *ABCG1* was associated with greater long-term reductions in body weight, waist circumference, and body fat distribution in response to an average-protein weight-loss diet. Our findings highlight the potential importance of epigenetic modifications in the relationship between dietary interventions and adiposity changes.

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