Original Article
EPIDEMIOLOGY/GENETICS

Obesity

Genetic Predisposition to an Adverse Lipid Profile Limits the Improvement in Total Cholesterol in Response to Weight Loss

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Objective: Overweight and obesity are associated with a dyslipidaemia which can be improved by weight loss. Whether genetic predisposition to an adverse lipid profile modifies such beneficial effects of weight loss on lipid levels in overweight and obese individuals was examined.

Design and methods: White European participants ($n = 374$) who completed a 12-month weight loss trial were genotyped for 36 lipid-associated single nucleotide polymorphisms (SNPs), previously identified in genome-wide association studies (GWAS). Genetic predisposition scores (GPSs) were calculated for four lipid traits by summing the number of risk alleles (RA) for each participant. The associations of each GPS with four lipid traits were assessed at baseline, and with lipid changes in response to weight change after 12 months.

Results: At baseline, the trait-specific GPSs were associated with 0.11 ± 0.04 mM higher total cholesterol/RA ($P = 0.004$), 0.05 ± 0.02 mM higher low density lipoprotein cholesterol/RA ($P = 0.005$), 0.03 ± 0.007 mM lower high density lipoprotein cholesterol/RA ($P = 0.00002$) and 0.04 ± 0.01 mM higher triglyceride/RA ($P = 0.00002$). After the intervention, weight loss was associated with improvements in all lipids ($P < 0.01$). GPS attenuated the weight loss-associated reduction in TC so those with a higher GPS had less improvement (interaction = 0.01 ± 0.005 mM/GPS/kg weight loss, $P = 0.003$). A similar pattern was observed for LDLC (interaction = 0.004 ± 0.002 mM/GPS/kg weight loss, $P = 0.07$). There was no evidence of a GPS-modifying effect for change in HDLC or TG.

Conclusion: Genetic predisposition is an important determinant of lipid levels and appears to limit the improvement in TC and to some extent LDLC levels, but not in other plasma lipids, in response to weight loss. © 2013 American Institute of Chemical Engineers AIChE J, 2013

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Introduction

Overweight and obesity are associated with an adverse lipid profile characterized by high levels of low density lipoprotein cholesterol (LDLC), triglycerides (TG), and low levels of high density lipoprotein cholesterol (HDLC), which contributes to the increased risk of cardiovascular disease (1). However, weight loss can result in an improvement in lipid profile (2,3).

Besides lifestyle factors, genetic factors also exert a strong influence on the inter-individual variation in plasma lipids, with heritability estimates ranging from 35 to 60% (4,5). Genome-wide association studies (GWAS) have identified a number of single nucleotide polymorphisms (SNPs) robustly associated with lipid traits in cross-sectional studies (6–10). However it is not clear whether these common lipid-associated SNPs also alter the responses to interventions designed to improve lipids. Using a composite genetic predisposition

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Additional Supporting Information may be found in the online version of this article.

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score (GPS) from these SNPs, we have shown previously that genetic predisposition to an adverse lipid profile does not modify the improvements in lipid levels from an isoenergetic reduction in dietary saturated fat, indicating that participants are able to improve their lipid profile by the same magnitude regardless of their genetic predisposition (11). Here we examine whether genetic predisposition to an adverse lipid profile modifies the beneficial effects of weight reduction on lipids in overweight and obese participants in a 12-month community weight loss intervention trial.

Methods

Original trial study design

Full details of the original intervention trial (ISRCTN: 85485463) have been published previously (12). Briefly, participants ($n = 772$) aged 18 years or older and who had a body mass index (BMI) of 27-35 kg m^{-2} and at least one risk factor for obesity-related disorders were randomized to receive 12 months of free access to a commercial weight loss program or to standard weight loss treatment in primary care defined by national treatment guidelines. This trial was conducted in three countries: Australia, Germany, and the UK. Ethical approval for the study was granted from the Ethics Review Committee of the Sydney South West Area Health Service, Australia, from the Ethical Committee of the Faculty of Medicine of the Techniche Universität München, Germany and from the Nottingham Research Ethics Service, UK and written informed consent from participants was obtained including for subsequent genetic analyses.

Participant body weight, waist circumference and fat mass were assessed according to standardized methods at 0, 2, 4, 6, 9, and 12 months of the study, with fasting blood taken at 0, 2, 6, and 12 months (12). In Australia, analyses were conducted at Laverty Pathology (North Ryde, New South Wales, Australia). Total cholesterol (TC) was determined by CHOD-PAP method, and HDLC and TG were each measured by enzymatic methods (Siemens Advia Centaur, Siemens Advia 2400, Siemens Australia, Bayswater, Australia). LDLC was calculated using the Friedewald equation. In Germany, all analyses were carried out at Labor München Zentrum (Medizinisches Versorgungszentrum, Munich, Germany). TC was determined by CHOD-PAP method and LDLC, HDLC and TG with an enzymatic colorimetric assay (Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany). In the UK, lipid analyses were conducted at Northampton General Hospital. TC, HDLC and TG were analyzed using enzymatic colorimetric assays using the Vitros 5.1FS platform (Ortho Clinical Diagnostics). LDLC was calculated using the Friedewald equation.

Study cohort

Of the 772 participants who enrolled in the study, 444 (58%) completed the 12-month assessment of the study. The current analysis was limited to those participants who did complete this final assessment. To reduce heterogeneity in genetic background individuals of white European ancestry only (based on self-reported ethnicity, $n =$ 401 completers) were included in the analysis. All other ethnic subgroups were excluded from these analyses due to their limited size. Of these completers, genotyping for 374 participants met the quality control criteria (see below) and were included in the analyses. Participant characteristics are shown in Supporting Information Table 1.

SNP selection and genotyping

All participants were genotyped using a panel of 41 SNPs within 31 loci. The genotyped SNPs were shown in previous GWAS to be associated with an adverse lipid profile or were proxies for the lead SNPs (linkage disequilibrium (LD) $r^2 > 0.8$) (6–10). Samples were genotyped with the Mass ARRAY system using the iPLEX Gold Chemistry (Sequenom, San Diego, CA). The samples were analyzed in a matrix-assisted laser desorption ionization time of flight mass spectrometer (MALDI TOF MS, Bruker Daltonik, Leipzig, Germany). The minor allele frequency in our sample was consistent with previous studies (6–10).

SNPs with a call rate of <95% were excluded from analyses (5 SNPs: rs2304130-NCAN; rs28927680-APO (A1,A4,A5,C3); rs4420638-APO (E,C1,C4,C2); rs6857-TOMM40-APOE; rs6987702-TRIB1). Individuals were excluded if genotyping was unsuccessful in >10% of SNPs (27 participants). Genotype distributions of all SNPs were tested for deviation from the Hardy–Weinberg Equilibrium (HWE) using the log likelihood ratio chi-square test (1 df) for association. No SNPs were excluded for deviation using a cut-off of $P < 0.001$, based on a Bonferroni correction for 41 tests. SNPs within the same locus were only included in this analysis if they were in low LD ($r^2 < 0.3$). As such 36 SNPs were included in the analyses: 7 SNPs that had been established for association with TC; 16 SNPs for LDLC; 17 SNPs for HDLC, and 12 SNPs for TG. Some SNPs were associated with more than one trait (Supporting Information Table 2).

Genetic predisposition score

We defined the risk-allele of a SNP as the allele associated with higher TC, LDLC, TG or lower HDLC levels in previous GWAS (6–10). An individual's genotype was coded as 0, 1, or 2 depending on the number of the risk alleles an individual carried for that particular SNP. For each individual, a GPS was calculated separately for each lipid trait by adding the number of risk alleles (Supporting Information Table 2). As there is currently no evidence for interaction between SNPs, a simple addition of the associated risk alleles for each trait has been commonly adopted (13) and used in this study. For participants missing individual genotyping data, the average count of risk alleles for the respective SNP was substituted for the missing genotype for the purposes of calculating the GPS. No participants had missing data for more than four genotypes (10%). The distribution of each GPS for the cohort and the number of participants with each GPS is shown in Figure 1.

Statistical analysis

Distributions of traits were tested for normality and because of right-skewness, the TG data were log(n)-transformed for analyses, while geometric mean and 95% confidence intervals were presented in the figures. For interpretation of the effect of GPS on TG, the exponential of the co-efficient of association from the linear regression analysis was used, where indicated.

Because of limited power to examine single-SNP associations, we focused our study on the GPS which provides more power. Linear regression analysis was used to test for associations between each GPS (a continuous variable according to the number of risk alleles) and lipid traits at baseline, assuming an additive effect of each

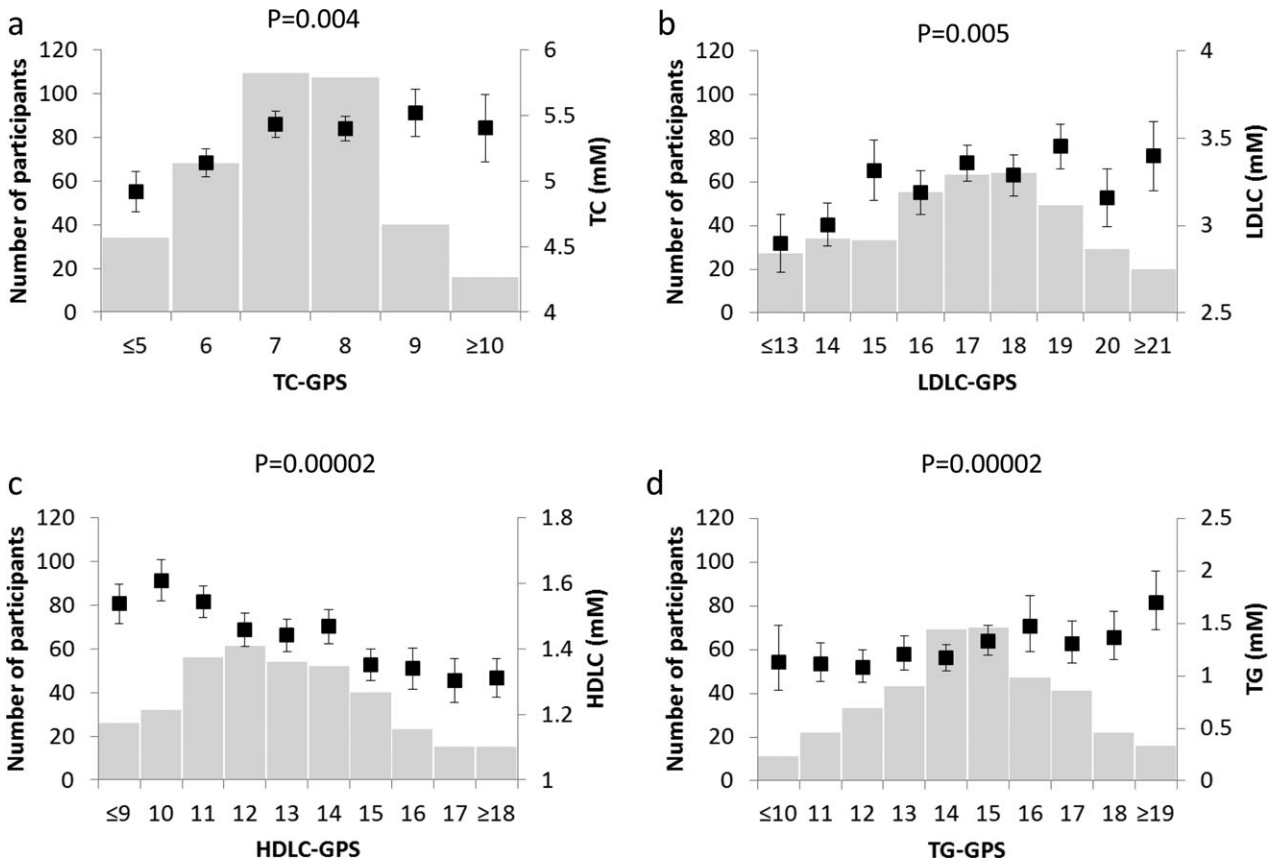

FIGURE 1 Variation of (**a**) total cholesterol (TC), (**b**) LDL cholesterol (LDLC), (**c**) HDL cholesterol (HDLC) and (**d**) triglycerides (TG) at baseline by trait-specific genetic predisposition score (GPS). The number of participants for each GPS is indicated by the grey bars corresponding to the left-hand Y axis. Data points are the mean and standard error (SE) values of (**a**) HDLC and (**b**) LDLC (**c**) TC and the geometric mean and 95% confidence intervals (CI) of (**d**) TG for each GPS score category defined by the number of risk alleles per individual, with units indicated on the right-hand Y-axis. For depiction in this figure, GPS at the lower and upper ends for each trait were grouped due to small n. The analysis was performed by linear regression of trait at baseline using the ungrouped GPS and adjusted for age, gender, baseline weight, country and lipid medication, and the P value for the association is shown.

additional risk allele, while adjusting for age, gender, country, weight at baseline, and lipid medication.

We tested for association between the GPS for each trait and weight loss to examine whether each GPS was related to weight loss ($P > 0.3$ for each trait). Next, we tested for whether there was an association between each GPS and the change in each lipid trait from baseline to 12 months. We then tested whether each GPS modified the change in its respective lipid trait in response to weight loss following the 12 month intervention by an interaction term of each GPS and change in weight in the regression model. This was used in a linear regression model of the association between the change in TC, LDLC, HDLC and TG at 12 months with weight loss and trait-specific GPS, adjusted for baseline weight, age, gender, country, and lipid medication. For the purposes of this analysis we concentrated on the weight loss (kg) regardless of the intervention group. Whilst weight loss was different between groups, there was no evidence of a modifying effect of treatment group on the weight loss-associated change in any lipid variables ($P > 0.3$ for treatment x weight loss interactions). To illustrate the effects of GPS to moderate weight loss-associated changes in lipids, values were estimated from the regression equations using fixed values of 5 kg for weight loss and a low and high GPS value, and averaging or otherwise integrating over the remaining covariates. For illustrative purposes the effect of weight loss on change in lipids was calculated by linear regression analysis (using the covariates listed above) for participants in each GPS stratum.

The proportion of variance in the change in lipids from baseline to 12 months explained by each GPS and by each individual SNP was estimated from the r^2 value of each regression model. This was compared to the r^2 values of the regression models for the effect of weight loss on change in lipids to determine whether genetic predisposition explains some of the variance in lipid responses to weight loss.

Statistical analysis was conducted using Stata 11 (StataCorp, TX). The data are presented as the coefficient ± standard error (SE) of the regression equations, unless indicated otherwise. We performed eight main tests but elected to report the findings with no correction for multiple testing as this was an exploratory study of the effects of GPS on lipids from a weight loss intervention trial. We decided to only report the summary statistics for associations of individual SNPs for future research rather than to interpret them on their own

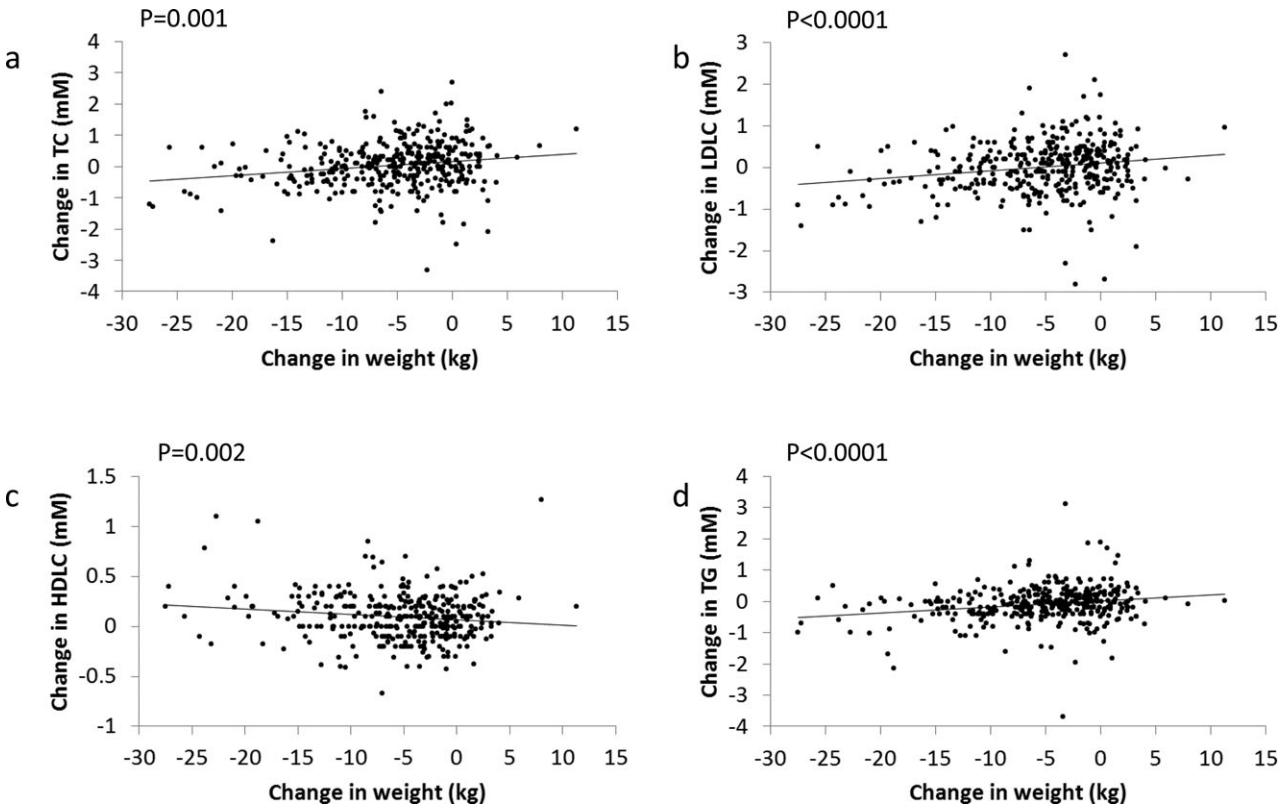

FIGURE 2 The association of weight loss with change in (**a**) total cholesterol (TC), (**b**) LDL cholesterol (LDLC), (**c**) HDL cholesterol (HDLC), and (**d**) triglycerides (TG) at 12 months. Data points are change in weight compared with change in lipid trait for each individual. The P value for the association assessed by linear regression analysis adjusted for age, gender, country, and lipid medication is shown.

due to the limited statistical power to detect small individual effects when corrected for false positive chance.

Results

Effect of genotype on lipids at baseline

The trait-specific GPSs were all associated with the respective traits, i.e. the higher the score the less favorable the lipid profile (Figure 1). TC was 0.11 ± 0.04 mM higher per TC risk allele ($P = 0.004$); LDLC was 0.05 ± 0.02 mM higher per LDLC risk allele ($P = 0.005$); HDLC was 0.03 ± 0.007 mM lower per HDLC risk allele ($P = 0.00002$); and TG was 0.04 ± 0.01 mM higher per TG risk allele ($P = 0.00002$).

We observed association coefficients for the individual SNPs that were directionally consistent with previous GWAS for 6 out of the 7 TC-SNPs; 14 out of the 16 LDLC-SNPs; 14 out of the 17 HDLC-SNPs and 10 out of the 12 TG-SNPs (Supporting Information Table 2). The effect size of each SNP was small with a cumulative effect toward each GPS.

Lipid responses to weight loss

The mean lipid values in the participants remaining in the trial are shown for each time point in Supporting Information Figure 1. An initial reduction in TC, HDLC and LDLC at 2 months was followed by a rebound in values by 6 months.

At 12 months, the mean weight loss in participants was 5.2 ± 0.3 kg. Weight loss was associated with improvements in all lipid traits (Figure 2) with a reduction in TC (0.02 ± 0.006 mM kg^{-1} weight lost, $P = 0.001$) and LDLC (0.02 ± 0.006 mM kg^{-1} weight lost, $P < 0.0001$), an increase in HDLC (0.006 ± 0.002 mM per kg of weight lost, $P = 0.002$) and a decrease in TG (0.02 ± 0.004 mM kg^{-1} weight lost, $P < 0.0001$).

Effect of GPS on lipid responses to weight loss

There was no association ($P > 0.1$) between each GPS alone and the change in each lipid trait after 12 months. In response to the 12 month intervention, TC-GPS attenuated the change in TC associated with weight loss, such that weight loss was associated with a greater reduction in TC the lower the GPS (Figure 3a). More specifically in individuals with a low GPS (TC-GPS of 5), a 5 kg weight-loss resulted in a reduction in TC of 0.60 ± 0.15 mM whereas with a higher TC-GPS of 10 the same weight loss was estimated to result in an increase in TC of 0.12 ± 0.2 mM (interaction = 0.01 ± 0.005 mM per GPS per kg weight loss, $P = 0.003$). A similar but non-significant pattern was seen for LDLC-GPS (interaction = 0.004 ± 0.002 mM per GPS per kg weight loss, $P = 0.07$, depicted in Figure 3b). There was no evidence for an effect of HDLC-GPS to modify

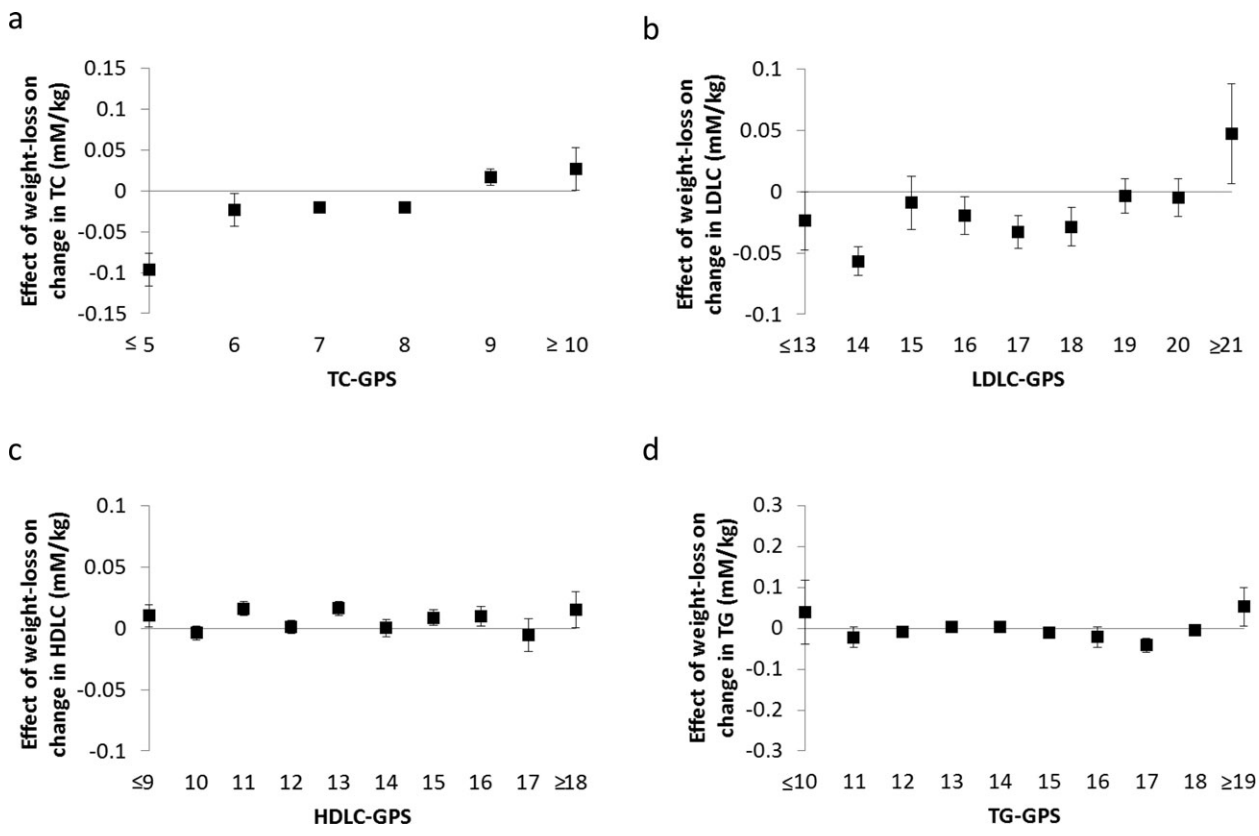

FIGURE 3 The association of weight loss with change in (**a**) total cholesterol (TC), (**b**) LDL cholesterol (LDLC), (**c**) HDL cholesterol (HDLC), and (**d**) triglycerides (TG) at baseline by trait-specific genetic predisposition score (GPS). Data are the coefficient and standard error (SE) derived from linear-regression models of change in trait with change in weight adjusted for age, gender, country, and lipid medication calculated for participants in each GPS stratum. For depiction in this figure GPS at the lower and upper ends for each trait were grouped due to small n.

the change in HDLC (P for weight-loss × HDLC-GPS interaction = 0.6) or TG-GPS to modify the change in TG (P for weight-loss × TG-GPS interaction = 0.3) associated with weight loss (depicted in Figure 3c and d).

The proportion of variance in the change in lipids after 12 months intervention explained by weight loss was small: 4% for TC and LDLC, 5% for HDLC and 7.5% for TG. The inclusion of GPS in each case increased the proportion of variance explained to 6.5% for TC, 6% for LDLC, 5.5% for HDLC and 8.5% for TG. TC-GPS, LDLC-GPS and TG-GPS explained more of the variance in change in lipids than the individual SNPs associated with each trait; however some individual HDLC-SNPs helped explain more of the variance of change in HDLC than using the HDLC-GPS (Supporting Information Table 3).

Because of the potentially complex effects of lipid medication and weight loss on the change in lipids, we repeated the analysis excluding participants on lipid medication (n = 52). These findings remained consistent for TC: interaction = 0.01 ± 0.004 mM per GPS per kg weight loss, P = 0.002), whereas the pattern seen for LDLC became significant: 0.006 ± 0.002 mM per GPS per kg weight loss, P = 0.008), There remained no evidence of a modifying effect on the change in TG or HDLC.

Although not significant, we observed directionally consistent effects of individual TC-SNPs to those of the total TC-GPS to modify weight loss associated changes in TC and (Supporting Information Table 3). There was no consistent directional effect modification of individual LDLC-SNPs, HDLC-SNPs and TG-SNPs on LDLC, HDLC or TG changes (Supporting Information Table 3).

Discussion

We confirmed the genetic predisposition (defined by the GPS) to high TC and LDLC, TG and low HDLC in this cohort of overweight and obese participants in a weight loss intervention trial. Furthermore genetic predisposition to high TC impaired the weight loss-associated improvement in TC after 12 months of the intervention. A genetic predisposition to high LDLC impaired the weight-loss associated improvement in LDLC in participants not on lipid-lowering medication. However, we observed no effect modification on the improvements in HDLC and TG.

A moderate reduction in body weight is sufficient to improve lipids (3,14), and this was shown in our weight loss trial (12). A systematic review on the effectiveness of weight loss on long-term (>2 years) lipid outcomes demonstrated a consistent, sustained reduction in TC, LDLC and TG following weight loss, but no clear effect on HDLC (15). However, in a meta-analysis of subjects at a stable

weight following weight loss, HDLC was increased (3). Changes in lipids vary with the duration of intervention and the trajectory of weight loss. A study on the pattern of cholesterol metabolism in response to weight loss diets showed a down-regulation of cholesterol synthesis in the first 6 months of active weight loss (16). This may be a direct consequence of energy reduction which has been shown to reduce cholesterol synthesis acutely even in the absence of weight loss (17,18). This was followed by a rebound during a subsequent weight maintenance/weight regain period (16). A similar pattern of cholesterol levels and change was seen in the current study over the course of the intervention. We sought to examine the modifying effect of genetic predisposition on the sustained effects of weight reduction rather than the effects of energy restriction on change in lipids. Therefore we chose to investigate this following 12 months of intervention, when the weight loss had largely stabilized (12). Whilst it was recognized that participants would have been on a variety of weight change trajectories at the 12-month time point, we elected to use the change from baseline to 12 months to allow for interpretation of the results. Genetic predisposition to TC (assessed by TC-GPS) attenuated the weight loss-associated reduction in TC, such that those with a greater genetic predisposition to high TC had less improvement in TC. A similar pattern was seen for LDLC, but was less apparent when complicated by lipid lowering medication. It is of note that a similar pattern of GPS to modify weight loss-associated change in lipids was seen at the other two time points where lipids were measured: at 2 months (TC interaction: 0.02 $\pm$ 0.008 mM per GPS per kg weight loss, $P = 0.005$; LDLC interaction: 0.01 $\pm$ 0.004 mM per GPS per kg weight loss, $P = 0.02$) and 6 months (TC interaction: 0.01 $\pm$ 0.005 mM per GPS per kg weight loss, $P = 0.01$; LDLC interaction: 0.003 $\pm$ 0.002 mM per GPS per kg weight loss, $P = 0.1$). There was no evidence of a modifying effect of GPS on TG or HDLC at any time point.

We focused on the cumulative genetic predisposition using the GPS, as the opportunities to examine single SNPs were limited due to small sample size and the expected small effect size of the SNPs. We were therefore unable to study the biological role of specific genetic loci to modify lipid responses to weight loss. However, this preliminary analysis indicated that the effect of the individual SNPs was cumulative rather than any particular locus driving the effect. The selection of SNPs for this study was conducted prior to the meta-analysis by Teslovich et al. which described 95 loci associated with lipid traits (19). Whilst the SNPs included in our study do not cover the full range of lipid-related SNPs which are now known, 17 out of the top 20 loci identified in the meta-analysis by Teslovich et al. (20) are included in our study. The included loci are involved in a number of different metabolic pathways (including cholesterol efflux, cholesterol synthesis, lipoprotein docking and triglyceride hydrolysis). That these loci appear to be acting cumulatively to modify a change in TC and possibly LDLC warrants further exploration. These findings helped to explain a small amount of the variance in lipid responses to weight loss. However, as the improvements in HDLC and TG were unimpeded by the GPS profile it should be stressed that there was an overall improvement in the lipid profile associated with weight loss, regardless of genetic predisposition.

Very few studies have investigated whether the lipid-associated SNPs identified in GWAS are also important modulators of lipid responses to interventions designed to lower cardiovascular disease risk. Using a similar approach, we demonstrated that genetic predisposition (defined by GPS) does not modify the improvement in lipids in response to an isoenergetic reduction in dietary saturated fat (11). In a related approach, a composite score of 9 LDLC- and HDLC-associated SNPs identified in GWAS was tested for associations with the magnitude of LDLC and HDLC response to fluvastatin therapy in men and women. In this instance women with higher genetic susceptibility had a more pronounced improvement in lipids (21). In a study that investigated the effect of 60 HDL-associated SNPs (identified in GWAS) on the variation in HDLC reduction following weight loss from bariatric surgery, none of these SNPs were significantly associated with the change in HDLC (a similar finding to that of our study), however no other lipid measures were investigated (22). Thus, the susceptibility of lipid response to modification by these genetic factors may depend on the lipids studied, and the mechanism by which the lipids are altered.

While most GWAS have been performed in cross-sectional data, some GWAS have recently been conducted to identify SNPs most strongly associated with lipid changes in intervention studies. In a combined analysis of three GWAS performed on the change in lipid response to statin therapy there was no overlap between SNPs which were associated with the statin-induced change in lipids and those associated with lipid levels from cross-sectional analyses (23). It might be that SNPs which account for the most variation within a population (cross-sectionally) might not be the most important moderators of change in lipids. Previous candidate gene studies have shown that variation in some genes can moderate the impact of weight-loss associated change in lipids. Carriers of the $\varepsilon 2$ genotype of the Apolipoprotein E (APOE) polymorphism were found to have a greater reduction in TC and LDLC in response to 12 weeks on an energy restricted diet than participants with $\varepsilon 3/\varepsilon 3$ genotype and $\varepsilon 4$ carriers (24,25). A study which investigated SNPs in the cholesterol transporters ABCG5 and ABCG8 found that these SNPs were associated with a modification of biosynthesis and absorption of cholesterol following weight loss (26). The expansion of GWAS to intervention studies will identify more comprehensively the SNPs important for change in lipids in addition to those previously studied through a candidate gene approach (20).

Because of the nature of controlled trials, we were limited to a small sample size for investigating gene $\times$ weight loss interactions. However, this controlled trial provided the opportunity to undertake a preliminary investigation into whether SNPs associated robustly with an adverse lipid profile modified responses of these lipids to changes in weight. Future opportunities may arise to combine the findings of this study with other weight loss studies, allowing these findings to be explored further. A limitation of our study was that the lipids were analyzed in the separate study centers, using different analysis methods. Whilst there was variability in the lipid values for each center, the magnitude and direction of effect of GPS in modifying the weight-loss associated change in lipids was consistent when analyzed by center (data not shown). A further limitation of our study was the use of lipid-lowering medication, which was taken by 11% of the participants. However, the sensitivity analysis which excluded these participants confirmed the moderating effect of GPS on weight loss for TC and LDLC, and also confirmed no association with TG and HDLC. Furthermore, there are other factors such as exercise frequency and/or intensity, alcohol intake, and dietary composition which may change during the course of a weight loss intervention, and which may also contribute to changes in lipid levels.

This study shows that genetic predisposition to an adverse lipid profile is an important determinant of lipid traits in overweight and

obese individuals. Whilst weight loss was associated with improvements in all lipid traits, genetic predisposition to high TC and LDLC appears to impair the weight loss-associated reduction in TC and in LDLC for participants not on lipid-lowering medication, but did not modify the improvements in HDLC or TG. However, further research into the underlying mechanisms is required. O

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