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Heavier smoking leads to relative increase in waist circumference: evidence for causal relationship from Mendelian Randomisation meta-analysis. The CARTA consortium

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Abstract

Objectives: To investigate, using a Mendelian Randomisation approach, whether heavier smoking is associated with a range of regional adiposity phenotypes, in particular those related to abdominal adiposity.

Design: Mendelian Randomisation meta-analyses using a genetic variant (rs16969968/rs1051730 in the *CHRNA5-CHRNA3-CHRNB4* gene region) as a proxy for smoking heaviness, of the associations of smoking heaviness with a range of adiposity phenotypes.

Participants: 148,731 current, former and never smokers of European ancestry aged ≥16 years from 29 studies in the consortium for Causal Analysis Research in Tobacco and Alcohol (CARTA).

Primary outcome measures: Waist and hip circumference, and waist-hip ratio.

Results: The data included up to 66,809 never smokers, 43,009 former smokers and 38,913 current daily cigarette smokers. Among current smokers, for each extra minor allele, the geometric mean was lower for waist circumference by -0.40% (95% confidence interval -0.57,-0.22), with effects on hip circumference, waist-hip ratio and body mass index (BMI) being -0.31% (95%CI -0.42,-0.19), -0.08% (-0.19,0.03) and -0.74% (-0.96,-0.51) respectively. By contrast, among never smokers, these effects were higher by 0.23% (0.09, 0.36), 0.17% (0.08, 0.26), 0.07% (-0.01, 0.15) and 0.35% (0.18, 0.52) respectively. When adjusting the three central adiposity measures for BMI, the effects among current smokers changed direction and were higher by 0.14% (0.05,0.22) for waist circumference, 0.02% (-0.05,0.08) for hip circumference and 0.10% (0.02,0.19) for waist-hip ratio, for each extra minor allele.

Conclusions: For a given BMI, a gene variant associated with increased cigarette consumption was associated with increased waist circumference. Smoking in an effort to control weight may lead to accumulation of central adiposity.

Strengths and limitations of this study

- This is a very large Mendelian randomisation study of the relationship between smoking and several anthropometric phenotypes relating to regional adiposity.
- Data included never, former and current smokers from a very wide spectrum of ages among 29 studies.
- By using a genetic variant associated with smoking heaviness as a proxy for smoking heaviness, bias from confounding is minimised and findings not affected by reverse causality.
- Data for direct measures of fat such as fat mass, and the biomarker leptin, were available for only about one fifth of the participants on whom weight, height, waist and hip were measured
- Participants were exclusively of self-reported European ancestry, and were mostly recruited in European countries.

Introduction

Tobacco is the single most important cause of preventable death globally: one in two young people taking up lifelong cigarette smoking will die of causes related to it [1]. Enormous efforts have gone into developing interventions for smoking cessation. Spontaneous cessation rates are low due to the high proportion of smokers that are dependent on nicotine, and effective treatments are still not widely available. One barrier to smoking cessation is the fear of weight gain. In a study of almost 2000 smokers in the USA, recruited into a trial of bupropion and/or nicotine inhalers to promote cessation, 50% of female and 26% of male smokers reported that gaining weight discouraged them from trying to quit [2], while among adults in Finland, daily smokers were found to report more weight concerns than former smokers or occasional smokers [3].

A genetic variant in the chromosome 15 *CHRNA5-CHRNA3-CHRNB4* gene region (rs16969968) codes for a functional amino acid change D398N in the nicotinic receptor alpha 5 subunit. This SNP and rs1051730, which is in perfect LD with rs16969968 in European populations, is associated with smoking quantity in smokers [4]. The minor allele of this variant is associated with an average increase in smoking amount of one cigarette per day in smokers and increases in cotinine (a metabolite of nicotine) levels [5 6]. It has also been found that the variant was associated with lower mean body mass index (BMI) [7-9], thus adding evidence that heavier smoking quantity leads to lower BMI. The latter study also noted lower waist and hip circumference among smokers with the variant [8]. However, prior observational evidence suggests that waist circumference and waist-hip ratio may be higher in smokers than in non-smokers after adjusting for BMI [10]. It has also been observed that smoking in adolescence predicts abdominal obesity in adulthood [11]. Moreover, heavy smokers exhibit greater central adiposity than light smokers, based on an analysis of middle aged smokers of European ancestry [12]. These studies suggest that smoking leads to a central fat accumulation at the expense of peripheral fat loss, particularly in women [13]. In addition, there are also suggestions that smoking may lead to loss of muscle mass as indicated by lower hip circumferences in smokers. This is of high public health relevance in view of the reported greater impact of increased central adiposity both on mortality [14 15], and on the development of diabetes especially among women [16 17]. and that smoking is associated with an increased risk of type 2 diabetes[18].

We previously used Mendelian Randomisation methods to investigate the effect of smoking quantity on BMI [7 9]. This method exploits Mendel's laws concerning the random assortment of alleles at the time of gamete formation so that individuals are allocated at random to having 0, 1 or 2 alleles in the rs1051730/rs16969968 genotype. The effect of this genotype on smoking quantity among smokers has been demonstrated [6], and thus the inverse relationship between allele count and BMI is not subject to effects of confounding and reverse causality. Using a substantial pool of studies in the consortium for Causal Analysis Research in Tobacco and Alcohol (CARTA), we have extended our use of Mendelian Randomisation methods to examine the effect of smoking quantity on a range of adiposity phenotypes. We test the hypotheses that (i) phenotypes representing central adiposity are affected by smoking quantity differentially from other phenotypes, and (ii) these effects are more marked among women than among men.

Methods

Study Populations

We used data on individuals (≥16 years) of self-reported European ancestry from 29 studies from the CARTA consortium (http://www.bris.ac.uk/expsych/research/brain/targ/research/collaborations/carta/): the 1958 Birth Cohort (1958BC) , the Avon Longitudinal Study of Parents and Children (ALSPAC, including both mothers and children), the British Regional Heart Study (BRHS), the British Women's Heart and Health Study (BWHHS), the Caerphilly Prospective Study (CaPS), the Christchurch Health and Development Study (CHDS), CoLaus, the Danish Monica study (Dan-MONICA), the Exeter Family Study of Child Health (EFSOCH), the English Longitudinal Study of Ageing (ELSA), the National FINRISK studies, GEMINAKAR, GS:SFHS (Generation Scotland: Scottish Family Health Study), the Genomics of Overweight Young Adults (GOYA) females, GOYA males, the Helsinki Birth Cohort Study (HBCS), Health2006, Health2008, the Nord-Trøndelag Health Study (HUNT), Inter99, MIDSPAN, the Northern Finland Birth Cohorts (NFBC 1966 and NFBC 1986), the National Health and Nutrition Examination Survey (NHANES), the MRC National Survey of Health & Development (NSHD), the Netherlands Twin Register (NTR), the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) and Whitehall II. All studies received ethics approval from local research ethics committees. Further details of these studies are provided in supplementary material.

Genotype

Within each study, individuals were genotyped for one of two single nucleotide polymorphisms (SNPs) in the *CHRNA5-A3-B4* nicotinic receptor subunit gene cluster, either rs16969968 or rs1051730. These single nucleotide polymorphisms are in perfect linkage disequilibrium with each other in Europeans (R^2 = 1.00 in HapMap 3, http://hapmap.ncbi.nlm.nih.gov/) and therefore represent the same genetic signal. Where studies had data available for both SNPs, we used the SNP that was genotyped in the largest number of individuals. Details of genotyping methods within each study are provided in supplementary material.

Adiposity measures

Direct physical measurements included weight, height, waist and hip circumference, arm circumference, triceps skinfold and subscapular skinfold thickness. Fat mass and fat free mass were available from bioimpedance measures, while leptin and adiponectin were the two biochemical markers related to fat mass.

Body mass index (weight/height^2) and waist-hip ratio (waist/hip) were calculated.

Waist circumference and waist-hip ratio were taken as key measures of central adiposity, while body mass index (BMI) acted as a non-specific measure of adiposity for purposes of adjustment in regression analysis.

Smoking status

Smoking status was self-reported (either by questionnaire or interview) at the same time as regional adiposity measures for all studies, with the exception of 1958 BC (see supplementary material). Individuals were classified as current, former, ever (i.e., current and former combined) or never cigarette smokers. Where information on pipe and cigar smoking was available, individuals reporting being current or former smokers of pipes or cigars but not cigarettes were excluded from all analyses.

For studies with adolescent populations (ALSPAC children and NFBC 1986), analyses were restricted to current daily smokers who reported smoking at least one cigarette per day (current smokers) and individuals who had never tried smoking (never smokers).

Statistical analysis

Analyses were conducted within each contributing study using Stata (Stata Corp, College Station, TX, USA) and R (R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org) software, following the same analysis plan. Analyses were restricted to individuals with full data on smoking status and rs1051730/rs16969968 genotype, and having data on at least one of the regional adiposity phenotypes.

Within each study, genotype frequencies were tested for deviation from Hardy Weinberg Equilibrium (HWE) using a chi-squared test. Mendelian randomisation analyses of the association between rs1051730/rs16969968 and each regional adiposity phenotype were performed using linear regression, stratified by smoking status (never, former and current) and sex, and adjusted for age. Apart from height, natural logarithmic transforms were taken of every anthropometric phenotype. An additive genetic model was assumed on log values, so that each effect size could be exponentiated to represent the percentage increase per minor (risk) allele. These analyses were presented separately for each smoking status category. All phenotypic measures were further adjusted for log (BMI) (apart from weight, height and BMI itself), thus assessing the effect of the particular adiposity measure after adjusting for this global weight measure. Log (weight) was adjusted for height instead of log (BMI). Since adjustment for ratio variables in anthropometric studies has been criticised [19], we further adjusted waist circumference for log(weight) and height. Finally we repeated analysis of waist circumference adjusted for BMI restricted to participants with BMI under 30 kg/m^2. 95% confidence intervals have been quoted for all effect sizes.

Meta analysis was also carried out of the relationship between reported daily cigarette consumption and rs1051730/rs16969968 genotype, among current smokers.

Although analyses were carried out for males and females separately, the estimates were combined where no evidence for separate sex effects was seen. For NHANES, which has a survey design, Taylor series linearization was implemented to estimate variances. For studies including related family members appropriate methods were used to adjust standard errors: in GEMINAKAR, twin pair identity was included as a cluster variable in the model, in MIDSPAN linear mixed effects regression models fitted using restricted maximum likelihood were used to account for related individuals, while in NTR, only unrelated individuals were included. ALSPAC mothers and children were analysed

as separate samples; as there are related individuals across these samples, sensitivity analyses were performed excluding each of these studies in turn.

Results from individual studies were meta-analysed in Stata (version 13) using the "metan" command from Stata. Where there was evidence of heterogeneity between studies ($I^2 > 50\%$), it was planned that both fixed and random effects analyses would be performed: however as this never occurred, results for fixed effects analysis only are shown. Meta-regression analysis, using the "metareg" command from Stata, was used to examine whether SNP effects varied by smoking status or by sex, or by a smoking by sex combination.

Results

Descriptive statistics

The maximum sample size available, with genotype recorded, was 148,731 for weight, height and BMI, over 29 studies. The data on individuals with weight, height, smoking status and genotype recorded, included 66,809 never smokers, 43,009 former smokers and 38,912 current smokers. Waist circumference was available in 28 studies (n=142,381), hip circumference and waist-hip ratio in 25 studies (n=139,667). Measures of fat mass and fat free mass were provided by 10 studies (n=28,231), arm circumference by nine studies (n=72,536), and the skinfolds by five studies (n=7,758). Finally leptin and adiponectin were measured in nine studies (n=23,630 and 19,191 respectively). Overall, 47% of the combined study population was male. The median age within the contributing studies ranged from 16-74 years. Descriptive statistics for each of the study populations are found in the supplementary material (Table S1).

Minor allele frequency for rs1051730/rs16969968 ranged between 0.31 and 0.36. There was no strong evidence for deviation from the Hardy-Weinberg Equilibrium in any of the studies (p-values all ≥ 0.09, Table S2).

Mendelian Randomisation analysis

Table 1 shows the per-allele increases in each phenotype, within each smoking status category. As previously shown [9], increase in BMI was positive in never smokers: +0.35% (95%CI 0.18, 0.52; $p=6.38*10^{-5}$), non-significant in former smokers: -0.14% (95%CI -0.34, +0.07; p=0.19) and significantly inverse in current smokers: -0.74% (95%CI -0.96, -0.51; $p=2*10^{-10}$). Full results for each contributing study are shown in Figure S1.

Waist circumference was higher per minor allele in never smokers: +0.23% (95%CI 0.09, 0.36; p=0.0012), non-significantly related in former smokers -0.07% (95%CI -0.24, 0.09; p=0.37), and lower in current smokers -0.37% (95%CI -0.55, -0.19; $p=1.69*10^{-5}$): differences among smoking groups were highly significant ($p=3.85*10^{-7}$), see Figure S2. The per-allele effect on waist circumference in current smokers was about half the magnitude of that seen for BMI. After adjustment for log(BMI), the minor allele of rs1051730-rs16969968 was not associated with waist circumference in either never smokers: +0.01% (95%CI -0.06, 0.08; p=0.72) or former smokers +0.06% (95%CI -0.02, 0.15; p=0.15). However in current smokers, the minor allele was associated with a 0.14% (95%CI 0.05, 0.22; p=0.003) higher waist circumference after adjustment for log(BMI). Very similar results were seen in all three smoking status categories after waist was adjusted for log(weight) and height instead of log(BMI). Effects of genotype on waist circumference were shown to differ between smoking status categories before adjustment ($p=3.85*10^{-7}$) but only weakly after adjustment for log(BMI) (p=0.102), and after adjustment for log(weight) and height (p=0.018). Little heterogeneity of study results was evident (I^2<=25% within all smoking groups). After restricting analysis to participants with BMI under 30 kg/m^2, we found that the percentage increases in waist circumference (after adjustment for log(BMI)) were 0.04% (95%CI -0.03, 0.12) for never smokers, 0.03% (95%CI -0.06, 0.13) for ex-smokers and 0.12% (95%CI 0.02, 0.21) for current smokers: however the test for difference in effects gave p=0.41.

Unadjusted results for hip circumference were very similar to that seen for waist, both in direction and magnitude, in all smoking status groups (Figure S3). However after adjustment for log(BMI), effects were not apparent in any of the three groups, and nor was the interaction of gene and smoking status.

Results for waist-hip ratio were similar to BMI, waist and hip circumference in direction but were smaller in magnitude: +0.07%, 0.00% and -0.08% increases in never-smokers, former smokers and current smokers respectively, (p=0.083 for differences between smoking categories), see Figure S4. After adjustment for log(BMI), increases remained non-significant for never smokers and former smokers (-0.01% and 0.04%) but increased significantly among current smokers (0.10%) (p=0.13 for differences among smoking groups).

For several other phenotypes, per-allele decreases were observed in current smokers that exceeded those seen either in former or never smokers (Table S4). However there was only statistical evidence for decreases among current smokers for arm circumference ($p=8.4*10^{-5}$) and leptin (p=0.025), while the difference between smoking groups was only significant for arm circumference ($p=3.29*10^{-4}$). Both effects became non-significant after adjustment for log(BMI). Fat mass and fat free mass, after adjustment by height, showed differences in effects by smoking group. These effects were more due to per-allele increases seen among never smokers than decreases among current smokers.

Meta-regression analyses showed no clear evidence for associations between genotype and each adiposity phenotype being modified by sex: p-values exceeded 0.1 for all phenotypes, adjusted or unadjusted, apart from hip circumference. The per-allele decreases in hip circumference among current smokers appeared more marked among women (p=0.067), but this effect was no longer apparent after adjusting for BMI (p=0.51).

The mean difference in daily cigarette consumption was 0.77 among current smokers (95%CI 0.67 to 0.88, I^2=17%).

Discussion

This meta-analysis of 29 studies comprising almost 150,000 participants with key adiposity phenotypes, has demonstrated firstly, that a variant associated with increased cigarette consumption was associated not only with lower BMI among current smokers, consistent with earlier findings [7 8], but also with lower waist and hip circumference. Secondly, the inverse association of the variant with lower waist circumference among current smokers changed direction after adjusting for BMI. The variant was positively associated with waist circumference but associated neither with hip circumference after BMI adjustment, nor waist-hip ratio. Our results suggest that for every copy of the minor allele associated with cigarette consumption (i.e. increasing cigarette per day consumption by approximately one cigarette), waist circumference will be increased by 0.14% if BMI were to remain constant. This suggests a preferential re-distribution towards central adiposity associated with higher cigarette consumption: this important finding is in keeping with our hypothesis and extends current observational data.

We also observed that none of the effects were modified by sex, contrary to our second hypothesis. Finally we have already noted among never-smokers an unexpected positive association of the gene variant with BMI [9] : the current analysis demonstrates this same association with waist and hip circumference. This occurred in the opposite direction to the inverse association of various adiposity measures with the gene variant seen in current smokers (before adjustment for BMI).

The analysis consisted of never, former and current smokers from a very wide spectrum of ages among the 29 studies. The sample size was very large for the primary phenotypes considered here. Participants were exclusively of self-reported European ancestry, and were mostly recruited in European countries. The data available for direct measures of fat such as fat mass, and the biomarker leptin, were available for only about one fifth of the participants on whom weight, height, waist and hip were measured. Effects according to genotype for these phenotypes showed broadly similar results for the three smoking categories to those seen for BMI.

Mendelian randomisation has proved a powerful tool for eliciting causal associations between phenotypic measures[20]. In the present analysis, Mendel's laws concerning random assignment of genotype should produce an unconfounded comparison between the genotype influencing smoking consumption and the outcomes of interest, namely anthropometric phenotypes. Furthermore, because this random assignment occurs at the very outset of life, the associations between genotype and anthropometric measures cannot be due to reverse causality. If the genotype only influences smoking consumption, and not the initiation of smoking, then the relationship between genotype and anthropometric outcomes would only be expected among smokers.

The reversal of the association between waist circumference and allele count from negative to positive among current smokers after adjustment for BMI may be consistent with alternative explanations. Firstly, heavy smokers may have less muscle mass; however no association between allele count and fat free mass could be detected in our analysis among smokers. Secondly, the test for interaction for smoking status and allele count on waist circumference after adjustment was of weak statistical significance. Thirdly, the adjustment of one measure of adiposity with another with which it is highly correlated may have caused a spurious association. We repeated our analysis for participants with BMI under 30 only, where the correlation was more modest, and obtained similar results albeit with reduced evidence for an effect.

Stratification of our analyses by smoking status, could in theory introduce bias by conditioning on a collider (rs1051730/rs16969968) [21]. This variant does not show strong evidence for association with smoking initiation (ever vs never smoking), but does show some evidence for association with smoking cessation (current vs former smoking) [22]. Whilst this is a possibility, no effect modifications of this variant with potential confounders by smoking status, were demonstrated among 56,625 participants in the HUNT study [8].

Cross sectional observational data from Switzerland has demonstrated that waist and hip circumference were more strongly related to number of cigarettes smoked per day than was BMI[13], while in Scotland being a smoker was associated with greater central adiposity among women[12] . In a Finnish longitudinal twin cohort study, smoking in adolescence predicted abdominal obesity in adulthood [11]. Observational data are however prone to confounding and reverse causality, and the present study adds some evidence that the associations reported are likely to be causal.

Some observational studies have noted that low fat free mass [23] and bone mineral density [24], were more common among smokers. The present analysis has not substantiated the association with fat free mass although our sample size was much more limited for this phenotype.

Our findings resonate with observational studies which have shown associations between smoking and risk of diabetes [17 18], especially as analysis of the British Women's Heart and Health Study showed that abdominal adiposity was a stronger predictor of diabetes than was BMI [16]. Waist circumference, and waist-to-hip ratio were strongly associated, independently of BMI, with the risk of death among 359,387 participants from nine countries in the European Prospective Investigation into Cancer and Nutrition [15]. Therefore the health hazards of smoking could well be enhanced, or partly mediated through increasing abdominal adiposity. In addition, the desire of many smokers to use smoking as a means of weight control [2] might be counterproductive, if a loss of weight is accompanied by a relative increase in waist circumference: this possibility could be used in counselling people seeking to quit smoking.

People who quit smoking appear to be at increased risk of acquiring diabetes in the short term and this was not explained by weight gain in a Japanese population [25]. The present study took place almost exclusively of white European participants, and replication of the findings among other ethnic populations would be of great value. This is especially urgent on a global scale since smoking levels are increasing among several non-white ethnic groups, and this is seen to be partly responsible for increases in coronary heart disease mortality in Beijing, China [26], in Syria [27], and in Tunisia among women [28]. In addition, increases in average waist circumferences have been observed even when average BMI levels have remained constant [29], and metabolic disorders especially diabetes have increased in prevalence[30]. It is thus possible increased CHD mortality will be partly fuelled by increasing smoking levels.

Mendelian Randomisation studies have more potential than traditional observational epidemiological studies to establish causality for specific exposures [20], and they should now be used to investigate other impacts of smoking, in particular on pathways leading to Type 2 diabetes, as well as on Type 2 diabetes itself. The findings of the current study could now be further tested by assembling data from randomised trials of smoking cessation, where post intervention data on measures of central adiposity are available. If confirmed, a tendency for smokers to acquire an "apple shape" due to increasing central adiposity might provide a novel health promotion message

to encourage smoking cessation, and appropriate new interventions should then be designed and evaluated as part of overall tobacco control policies in society.

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Author Contributions

RWM, AET, TIAK, MRM and NS conceived the study and contributed to the writing of the manuscript. RWM conducted the final analyses. All other authors conducted individual study analyses and contributed to the writing of the manuscript.

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Table 1. Per allele percentage increases in measures of regional adiposity (BMI, weigh, waist circumference, hip circumference, waist-hip ratio) among never, ex and current smokers, before and after adjustment for body mass index

		ADJUSTED FOR AGE				ADJUSTED FOR AGE AND BMI			
		Never smokers	Former smokers	Current smokers	*p for interaction**	Never smokers	Former smokers	Current smokers	*p for interaction**
BMI (kg/m^2)	*% increase*	0.35	-0.14	-0.74		-			
	95%CI	(0.18,0.52)	(-0.34,0.07)	(-0.96,-0.51)					
	p	6.38×10^{-5}	0.19	2.00×10^{-10}	4.95×10^{-13}				
	N	66,809	43,009	38,912					
	I^2	14%	0%	0%					
Waist circumference (cm)	*% increase*	0.23	-0.07	-0.40		0.01	0.06	0.14	
	95%CI	(0.09,0.36)	(-0.24,0.09)	(-0.57,-0.22)		(-0.06,0.08)	(-0.02,0.15)	(0.05,0.22)	
	p	0.0012	0.37	1.69×10^{-5}	3.85×10^{-7}	0.72	0.15	0.003	0.087
	N	64,265	40,756	37,360					
	I^2	14%	0%	10%		0%	0%	13%	
Hip circumference (cm)	*% increase*	0.17	-0.07	-0.31		0.02	0.02	0.02	
	95%CI	(0.08,0.26)	(-0.17,0.04)	(-0.42,-0.19)		(-0.03,0.07)	(-0.04,0.08)	(-0.05,0.08)	
	p	2.95×10^{-4}	0.23	2.55×10^{-7}	1.79×10^{-9}	0.38	0.54	0.59	0.99
	N	62,323	40,512	36,833					
	I^2	7%	0%	0%		16%	0%	0%	

Waist-hip ratio	*% increase*	0.07	0	-0.08		-0.01	0.04	0.1	
	95%CI	(-0.01,0.15)	(-0.10,0.10)	(-0.19,0.03)		(-0.08,0.06)	(-0.04,0.13)	(0.02,0.19)	
	p	0.087	0.97	0.14	0.083	0.78	0.30	0.02	0.13
	N	62,322	40,512	36,833					
	I^2	21%	9%	15%		0%	0%	13%	

*Interaction assessed by assessing heterogeneity between effect estimates according to smoking status, with fixed effects model

Table S1. Distribution of smoking status, sex and age, and availability of regional adiposity phenotypes, in each of the 29 studies

Study	Total N	Never smokers	Ex smokers	Current smokers	% male	Median age	Weight	Height	Waist	Hip	Arm	TSF	SSF	Fat mass	Fat free mass	Leptin	Adiponectin
1958 BC	5,022	2,353	1,449	1,220	50	42	*	*	*	*	*						
ALSPAC children	1,664	1,336	0	328	48	17.8	*	*						*	*		
ALSPAC mothers	1,530	1,004	395	131	0	48	*	*	*	*	*						
BRHS	3,576	1,040	2,072	464	100	68	*	*	*	*	*	*	*	*	*	*	*
BWHHS	3,615	2,043	1,180	392	0	68	*	*	*	*	*					*	*
Caerphilly	1,155	226	592	337	100	62	*	*	*	*							
CHDS	614	313	136	165	50	30	*	*	*	*							
CoLaus	4,305	1,817	1,393	1,095	43	53	*	*	*	*				*	*	*	*
Dan-MONICA	2,245	642	575	1,028	51	54	*	*	*	*	*			*	*		
EFSOCH	1,214	749	228	238	44	32	*	*	*	*	*	*	*				
ELSA	4,978	1,726	2,551	701	46	65	*	*	*	*							
Finrisk	20,368	9,755	5,493	5,120	46	51	*	*	*	*							

GEMINAKAR	1,120	556	185	379	48	38	*	*	*	*		*	*			*	*
Generation Scotland	7,294	3,893	2,421	980	41	57	*	*	*	*		*	*				
GOYA males	765	759	108	148	0	45	*	*	*	*				*	*	*	*
GOYA females	1,015	172	213	380	100	38	*	*	*								
HBCS	1,626	703	551	372	43	61	*	*	*	*				*	*	*	*
Health 2006	3,211	1,382	1,078	751	44	50	*	*	*	*				*	*		
Health 2008	624	280	221	123	44	47	*	*	*	*				*	*		
HUNT	55,476	24,302	14,144	17,030	48	47	*	*	*	*	*						
Inter 99	5,399	1,986	1,427	1,986	49	45	*	*	*	*						*	*
Midspan	2,099	994	574	531	45	45	*	*	*	*							
NFBC 1966	3,729	1,763	577	1,389	50	31	*	*	*	*							
NFBC 1986	1,171	752	0	419	48	16	*	*	*	*							
NHANES	2,045	987	616	442	38[1]	43[1]	*	*	*	*	*	*	*	*			
NSHD	1,751	776	639	336	48	53	*	*	*	*	*					*	*

NTR	3,718	1,822	1,081	815	36	39	*	*	*	*						
PROSPER	5,145	1,761	2,022	1,362	48	74	*	*							*	*
Whitehall II (phase 3)	2,836	1,383	1,088	365	75	48	*	*	*	*						
Whitehall II (phase 7)	2,921	1,426	1,278	217	77	59							*	*		

1. Weighted values from survey data

Table S2. Distribution of genotypes for SNP rs1051730/rs16969968, minor allele frequency and p-value from test for Hardy-Weinberg equilibrium (HWE)

Study	Major homozygotes	Heterozygotes	Minor homozygotes	MAF	HWE p-value
1958 BC	2,178	2,230	614	0.34	0.4
ALSPAC children	740	752	172	0.33	0.37
ALSPAC mothers	711	664	155	0.32	1
BRHS	1,631	1,540	405	0.33	0.15
BWHHS	1,591	1,625	399	0.34	0.59
Caerphilly	523	512	120	0.33	0.75
CHDS	286	273	55	0.31	0.37
CoLaus	1,778	1,980	547	0.36	0.91
Dan-MONICA	993	1,000	252	0.33	0.83
EFSOCH	568	523	124	0.32	0.82
ELSA	2,265	2,169	544	0.33	0.47
Finrisk	9,251	8,979	2,138	0.32	0.59
GEMINAKAR	530	477	113	0.32	0.43
Generation Scotland	3,261	3,251	782	0.33	0.52
GOYA males	443	473	99	0.34	0.2
GOYA females	338	329	98	0.33	0.09
HBCS	699	746	181	0.34	0.39
Health 2006	1,436	1,429	346	0.33	0.77
Health 2008	291	269	64	0.32	0.87
HUNT	24,621	24,579	6,276	0.33	0.23
Inter 99	2,364	2,423	612	0.34	0.63
Midspan	953	931	215	0.33	0.87
NFBC 1966	1,711	1,612	406	0.33	0.38
NFBC 1986	554	521	96	0.3	0.08
NHANES	864	928	253	0.35	0.88

NSHD	827	760	164	0.32	0.36
NTR	1,727	1,607	384	0.32	0.72
PROSPER	2,403	2,244	498	0.31	0.56
Whitehall II (phase 3)	1,276	1,261	299	0.33	0.50
Whitehall II (phase 7)	1,285	1,317	319	0.33	0.50

Table S3. Per allele percentage increases in measures of regional adiposity measures (fat mass, fat free mass, leptin, adiponectin, arm circumference, triceps skinfold, subscapular skinfold) among never, former and current smokers, before and after adjustment for body mass index

		ADJUSTED FOR AGE				ADJUSTED FOR AGE AND BMI *			
		Never smokers	Former smokers	Current smokers		Never smokers	Former smokers	Current smokers	
					p for interaction +				*p for interaction +*
Fat mass	*% increase*	0.42	-0.33	-0.43		0.85	-0.31	-0.43	
	95%CI	(-0.09, 0.94)	(-0.93, 0.27)	(-1.13, 0.28)		(0.25, 1.45)	(-0.97, 0.36)	(-1.13, 0.28)	
	p	0.11	0.28	0.24	0.08	0.005	0.37	0.42	0.015
	N	15,249	11,381	6,914					
	I^2	45%	20%	6%		21%	19%	5%	
Fat free mass	*% increase*	0.36	-0.03	0.03		0.44	-0.08	-0.08	
	95%CI	(0.09, 0.63)	(-0.33, 0.28)	(-0.35, 0.41)		(0.22, 0.67)	(-0.34, 0.19)	(-0.40, 0.25)	
	p	0.008	0.86	0.89	0.13	1.19×10^{-4}	0.57	0.64	3.95×10^{-3}
	N	15,543	11,511	7,011					
	I^2	19%	0%	36%		13%	0%	24%	
Leptin	*% increase*	-0.97	0.03	-3.48		-0.66	-0.38	-1.36	
	95%CI	(-3.34, 1.45)	(-2.32, 2.43)	(-6.42, -0.44)		(-2.53, 1.26)	(-2.24, 1.52)	(-3.64, 0.98)	
	p	0.43	0.98	0.025	0.2	0.5	0.69	0.25	0.81
	N	8,840	8,472	6,073					

	I^2	15%	0%	5%		0%	23%	0%	
Adiponectin	*% increase*	-0.04	-2.96	-0.31		-0.23	-2.88	-1.14	
	95%CI	(-2.17, 2.13)	(-5.35, -0.51)	(-3.07, 2.54)		(-2.30, 1.89)	(-5.22, -0.48)	(-3.81, 1.60)	
	p	0.97	0.18	0.83	0.18	0.83	0.019	0.41	0.26
	N	8,840	8,472	6,073					
	I^2	16%	18%	2%		18%	21%	18%	
Arm circumference	*% increase*	0.11	-0.17	-0.4		-0.08	-0.03	0.06	
	95%CI	(-0.05, 0.27)	(-0.36, 0.02)	(-0.60, -0.20)		(-0.17, 0.01)	(-0.14, 0.08)	(-0.05, 0.17)	
	p	0.17	0.08	8.40×10^{-5}	3.29×10^{-4}	0.09	0.6	0.25	0.14
	N	32,413	20,063	20,061					
	I^2	0%	0%	0%		0%	46%	0%	
Triceps skinfold	*% increase*	0.86	1.98	-2.14		-0.64	1.98	-1.6	
	95%CI	(-1.05, 2.81)	(-0.18, 4.18)	(-5.31, 1.13)		(-2.17, 0.90)	(0.12, 3.87)	(-3.92, 0.79)	
	p	0.38	0.072	0.2	0.12	0.41	0.037	0.19	0.034
	N	3,234	3,064	1,460					
	I^2	71%	0%	0%		42%	0%	0%	
Subscapular skinfold	*% increase*	-0.16	-0.93	-2.29		0.14	-0.41	-1.23	
	95%CI	(-2.14, 1.87)	(-2.83, 1.01)	(-5.54, 1.06)		(-1.01, 1.31)	(-1.89, 1.10)	(-3.29, 0.87)	
	p	0.88	0.34	0.18	0.55	0.81	0.59	0.25	0.55

N	3,234	3,064	1,460			
I^2	56%	0%	8%	0%	0%	3%

*adjustment only made for age and height for fat mass and fat free mass

+Interaction assessed by assessing heterogeneity between effect estimates according to smoking status, with fixed effects model

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **[Within the title page 1 and design section of the abstract page 6]**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **[See results section of abstract page 6]**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **[Introduction on page 8]**
Objectives	3	State specific objectives, including any prespecified hypotheses **[See page 8]**
Methods		
Study design	4	Present key elements of study design early in the paper **[Abstract page 6, end of introduction page 8, methods pages 9-10]**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **[See Supplementary material]**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **[Top of methods section page 9, but mainly in supplementary material]**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **[See pages 9-10]**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **[See Supplementary material]**
Bias	9	Describe any efforts to address potential sources of bias **[last paragraph on page 10, page 11, penultimate paragraph page 14]**
Study size	10	Explain how the study size was arrived at **[N/A]**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **[page 10 under "Statistical Analysis"]**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **[See pages 10-11]**
		(*b*) Describe any methods used to examine subgroups and interactions **[See pages 10-11]**
		(*c*) Explain how missing data were addressed **[N/A]**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was

		addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses **[bottom of page 10, page 11, penultimate para on page 14]**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **[Supp Table S1]**
		(b) Give reasons for non-participation at each stage **[N/A]**
		(c) Consider use of a flow diagram **[N/A]**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **[Supp Table S1]**
		(b) Indicate number of participants with missing data for each variable of interest **[Supp Table S1, S2]**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **[N/A]**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures **[See Supplementary material]**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **[See pages 12-13, Table 1, Table S3, Figures S1-S4]**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **[N/A]**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **[See page 13, last para]**
Discussion		
Key results	18	Summarise key results with reference to study objectives **[see page 14, first two paras]**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **[see page 14, last three paras.]**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **[see pages 14-15]**
Generalisability	21	Discuss the generalisability (external validity) of the study results **[see page 15]**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **[acknowledgements for each primary study, pages 16-20]**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

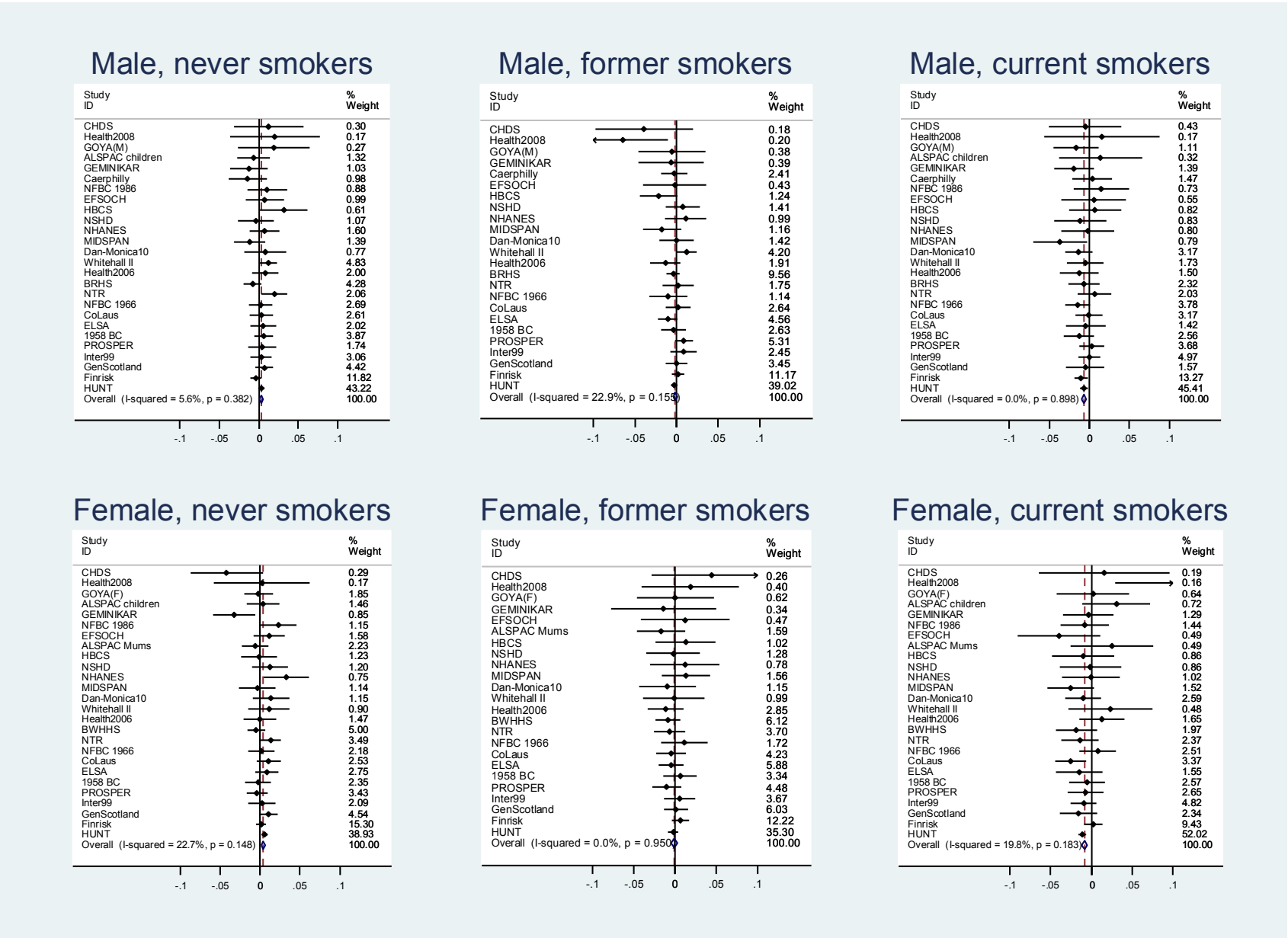
Supplementary Figure S1-S4. Associations of rs1051730/rs16969968 with adiposity phenotypes (fixed effects meta-analysis) in never-smokers, ex-smokers and current smokers. Results for male participants in upper panels, and for females in lower panels. Horizontal axis indicates difference in mean log(phenotype) per allele

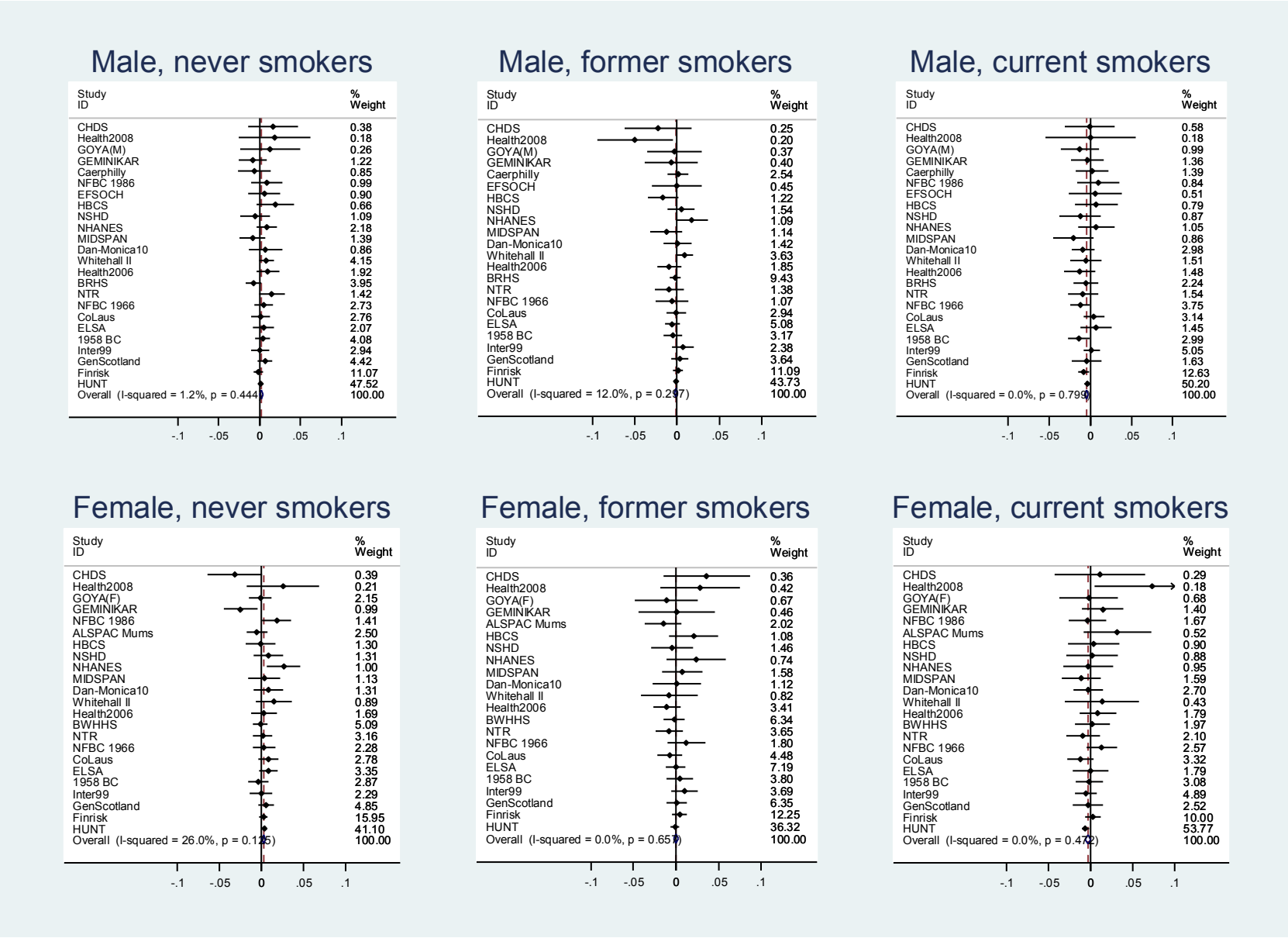
S1. BMI

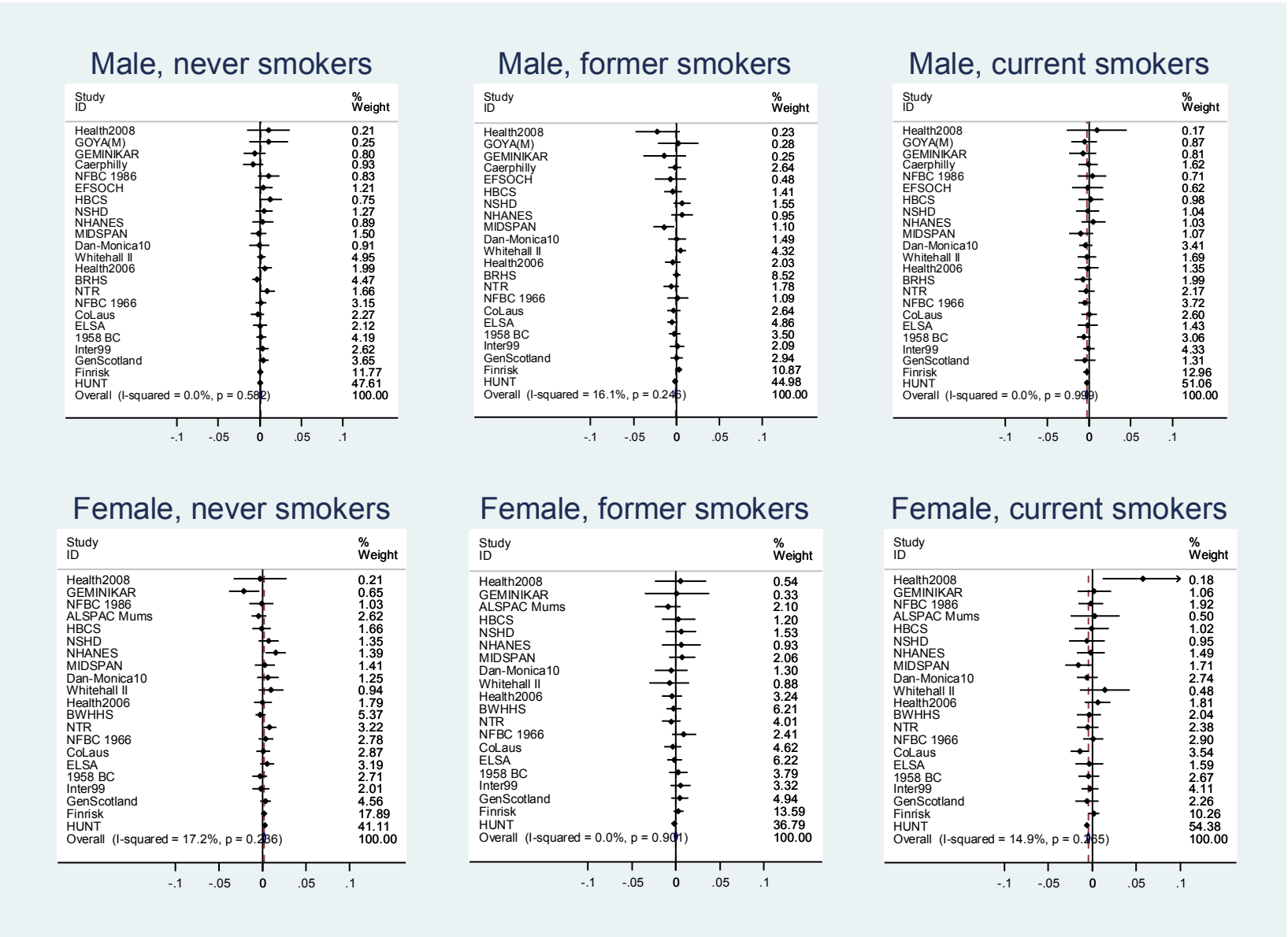
S2. Waist cirumference

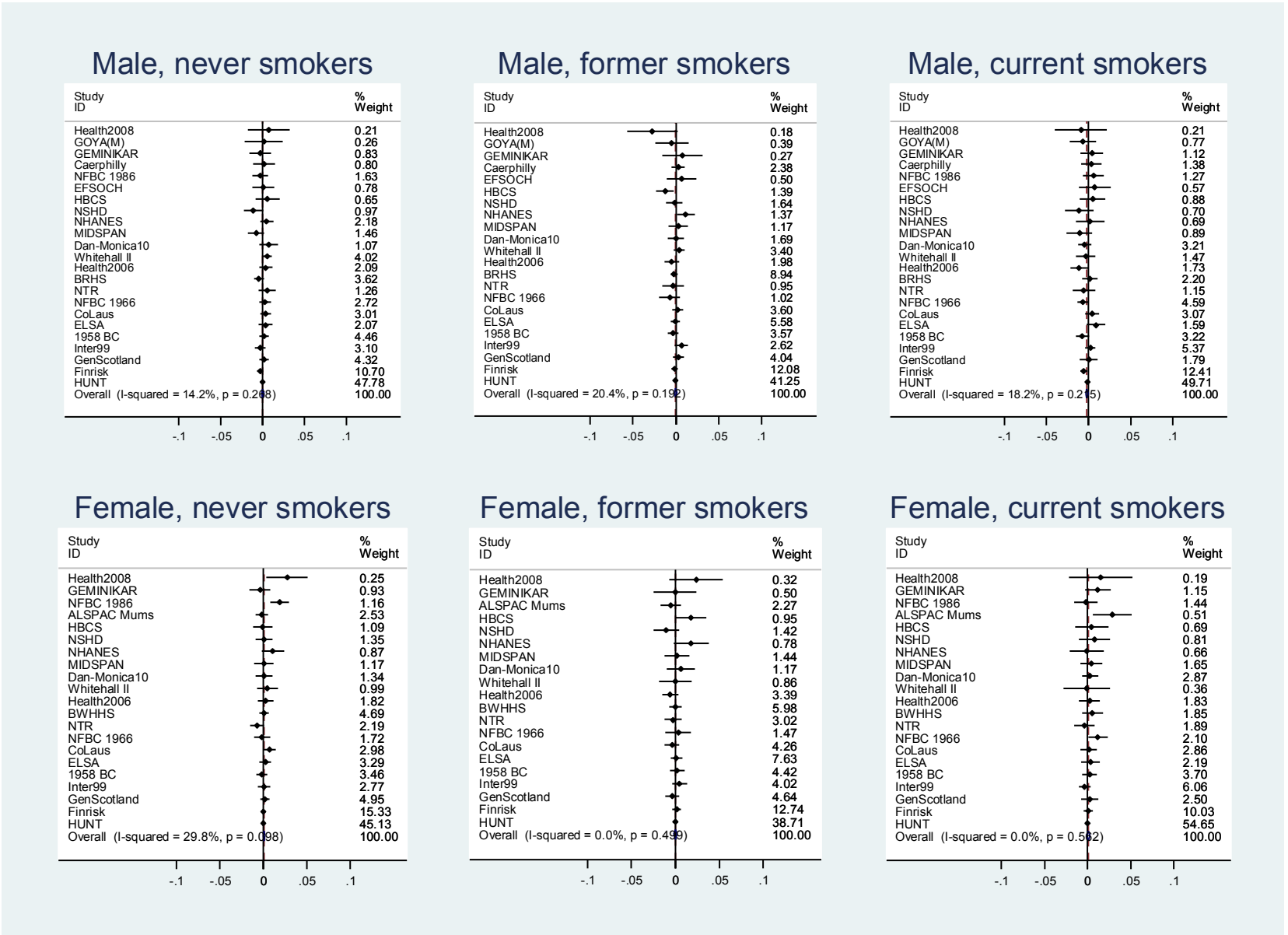
S3. Hip circumference

S4. Waist-hip ratio









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Primary Subject Heading:	Smoking and tobacco
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	EPIDEMIOLOGY, GENETICS, Substance misuse < PSYCHIATRY, General diabetes < DIABETES & ENDOCRINOLOGY

Heavier smoking may lead to relative increase in waist circumference: evidence for causal relationship from Mendelian Randomisation meta-analysis. The CARTA consortium

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Abstract

Objectives: To investigate, using a Mendelian Randomisation approach, whether heavier smoking is associated with a range of regional adiposity phenotypes, in particular those related to abdominal adiposity.

Design: Mendelian Randomisation meta-analyses using a genetic variant (rs16969968/rs1051730 in the *CHRNA5-CHRNA3-CHRNB4* gene region) as a proxy for smoking heaviness, of the associations of smoking heaviness with a range of adiposity phenotypes.

Participants: 148,731 current, former and never smokers of European ancestry aged ≥16 years from 29 studies in the consortium for Causal Analysis Research in Tobacco and Alcohol (CARTA).

Primary outcome measures: Waist and hip circumference, and waist-hip ratio.

Results: The data included up to 66,809 never smokers, 43,009 former smokers and 38,913 current daily cigarette smokers. Among current smokers, for each extra minor allele, the geometric mean was lower for waist circumference by -0.40% (95% confidence interval -0.57,-0.22), with effects on hip circumference, waist-hip ratio and body mass index (BMI) being -0.31% (95%CI -0.42,-0.19), -0.08% (-0.19,0.03) and -0.74% (-0.96,-0.51) respectively. By contrast, among never smokers, these effects were higher by 0.23% (0.09, 0.36), 0.17% (0.08, 0.26), 0.07% (-0.01, 0.15) and 0.35% (0.18, 0.52) respectively. When adjusting the three central adiposity measures for BMI, the effects among current smokers changed direction and were higher by 0.14% (0.05,0.22) for waist circumference, 0.02% (-0.05,0.08) for hip circumference and 0.10% (0.02,0.19) for waist-hip ratio, for each extra minor allele.

Conclusions: For a given BMI, a gene variant associated with increased cigarette consumption was associated with increased waist circumference. Smoking in an effort to control weight may lead to accumulation of central adiposity.

Strengths and limitations of this study

- This is a very large Mendelian randomisation study of the relationship between smoking and several anthropometric phenotypes relating to regional adiposity.
- Data included never, former and current smokers from a very wide spectrum of ages among 29 studies.
- By using a genetic variant associated with smoking heaviness as a proxy for smoking heaviness, bias from confounding is minimised and findings not affected by reverse causality.
- Data for direct measures of fat such as fat mass, and the biomarker leptin, were available for only about one fifth of the participants on whom weight, height, waist and hip were measured
- Participants were exclusively of self-reported European ancestry, and were mostly recruited in European countries.

Introduction

Tobacco is the single most important cause of preventable death globally: one in two young people taking up lifelong cigarette smoking will die of causes related to it [1]. Enormous efforts have gone into developing interventions for smoking cessation. Spontaneous cessation rates are low due to the high proportion of smokers that are dependent on nicotine, and effective treatments are still not widely available. One barrier to smoking cessation is the fear of weight gain. In a study of almost 2000 smokers in the USA, recruited into a trial of bupropion and/or nicotine inhalers to promote cessation, 50% of female and 26% of male smokers reported that gaining weight discouraged them from trying to quit [2], while among adults in Finland, daily smokers were found to report more weight concerns than former smokers or occasional smokers [3].

A genetic variant in the chromosome 15 *CHRNA5-CHRNA3-CHRNB4* gene region (rs16969968) codes for a functional amino acid change D398N in the nicotinic receptor alpha 5 subunit. This SNP and rs1051730, which is in perfect LD with rs16969968 in European populations, is associated with smoking quantity in smokers [4]. The minor allele of this variant is associated with an average increase in smoking amount of one cigarette per day in smokers and increases in cotinine (a metabolite of nicotine) levels [5 6]. It has also been found that the variant was associated with lower mean body mass index (BMI) [7-9], thus adding evidence that heavier smoking quantity leads to lower BMI. The latter study also noted lower waist and hip circumference among smokers with the variant [8]. However, prior observational evidence suggests that waist circumference and waist-hip ratio may be higher in smokers than in non-smokers after adjusting for BMI [10]. It has also been observed that smoking in adolescence predicts abdominal obesity in adulthood [11]. Moreover, heavy smokers exhibit greater central adiposity than light smokers, based on an analysis of middle aged smokers of European ancestry [12]. These studies suggest that smoking leads to a central fat accumulation at the expense of peripheral fat loss, particularly in women [13]. In addition, there are also suggestions that smoking may lead to loss of muscle mass as indicated by lower hip circumferences in smokers. This is of high public health relevance in view of the reported greater impact of increased central adiposity both on mortality [14 15], and on the development of diabetes especially among women [16 17]. and that smoking is associated with an increased risk of type 2 diabetes[18].

We previously used Mendelian Randomisation methods to investigate the effect of smoking quantity on BMI [7 9]. This method exploits Mendel's laws concerning the random assortment of alleles at the time of gamete formation so that individuals are allocated at random to having 0, 1 or 2 alleles in the rs1051730/rs16969968 genotype. The effect of this genotype on smoking quantity among smokers has been demonstrated [6], and thus the inverse relationship between allele count and BMI is not subject to effects of confounding and reverse causality. Using a substantial pool of studies in the consortium for Causal Analysis Research in Tobacco and Alcohol (CARTA), we have extended our use of Mendelian Randomisation methods to examine the effect of smoking quantity on a range of adiposity phenotypes. We test the hypotheses that (i) phenotypes representing central adiposity are affected by smoking quantity differentially from other phenotypes, and (ii) these effects are more marked among women than among men.

Methods

Study Populations

We used data on individuals (≥16 years) of self-reported European ancestry from 29 studies from the CARTA consortium (http://www.bris.ac.uk/expsych/research/brain/targ/research/collaborations/carta/): the 1958 Birth Cohort (1958BC) , the Avon Longitudinal Study of Parents and Children (ALSPAC, including both mothers and children), the British Regional Heart Study (BRHS), the British Women's Heart and Health Study (BWHHS), the Caerphilly Prospective Study (CaPS), the Christchurch Health and Development Study (CHDS), CoLaus, the Danish Monica study (Dan-MONICA), the Exeter Family Study of Child Health (EFSOCH), the English Longitudinal Study of Ageing (ELSA), the National FINRISK studies, GEMINAKAR, GS:SFHS (Generation Scotland: Scottish Family Health Study), the Genomics of Overweight Young Adults (GOYA) females, GOYA males, the Helsinki Birth Cohort Study (HBCS), Health2006, Health2008, the Nord-Trøndelag Health Study (HUNT), Inter99, MIDSPAN, the Northern Finland Birth Cohorts (NFBC 1966 and NFBC 1986), the National Health and Nutrition Examination Survey (NHANES), the MRC National Survey of Health & Development (NSHD), the Netherlands Twin Register (NTR), the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) and Whitehall II. All studies received ethics approval from local research ethics committees. Further details of these studies are provided in supplementary material.

Genotype

Within each study, individuals were genotyped for one of two single nucleotide polymorphisms (SNPs) in the *CHRNA5-A3-B4* nicotinic receptor subunit gene cluster, either rs16969968 or rs1051730. These single nucleotide polymorphisms are in perfect linkage disequilibrium with each other in Europeans (R^2 = 1.00 in HapMap 3, http://hapmap.ncbi.nlm.nih.gov/) and therefore represent the same genetic signal. Where studies had data available for both SNPs, we used the SNP that was genotyped in the largest number of individuals. Details of genotyping methods within each study are provided in supplementary material.

Adiposity measures

Direct physical measurements included weight, height, waist and hip circumference, arm circumference, triceps skinfold and subscapular skinfold thickness. Fat mass and fat free mass were available from bioimpedance measures, while leptin and adiponectin were the two biochemical markers related to fat mass.

Body mass index (weight/height^2) and waist-hip ratio (waist/hip) were calculated.

Waist circumference and waist-hip ratio were taken as key measures of central adiposity, while body mass index (BMI) acted as a non-specific measure of adiposity for purposes of adjustment in regression analysis.

Smoking status

Smoking status was self-reported (either by questionnaire or interview) at the same time as regional adiposity measures for all studies, with the exception of 1958 BC (see supplementary material). Individuals were classified as current, former, ever (i.e., current and former combined) or never cigarette smokers. Where information on pipe and cigar smoking was available, individuals reporting being current or former smokers of pipes or cigars but not cigarettes were excluded from all analyses.

For studies with adolescent populations (ALSPAC children and NFBC 1986), analyses were restricted to current daily smokers who reported smoking at least one cigarette per day (current smokers) and individuals who had never tried smoking (never smokers).

Statistical analysis

Analyses were conducted within each contributing study using Stata (Stata Corp, College Station, TX, USA) and R (R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org) software, following the same analysis plan. Analyses were restricted to individuals with full data on smoking status and rs1051730/rs16969968 genotype, and having data on at least one of the regional adiposity phenotypes.

Within each study, genotype frequencies were tested for deviation from Hardy Weinberg Equilibrium (HWE) using a chi-squared test. Mendelian randomisation analyses of the association between rs1051730/rs16969968 and each regional adiposity phenotype were performed using linear regression, stratified by smoking status (never, former and current) and sex, and adjusted for age. Apart from height, natural logarithmic transforms were taken of every anthropometric phenotype. An additive genetic model was assumed on log values, so that each effect size could be exponentiated to represent the percentage increase per minor (risk) allele. These analyses were presented separately for each smoking status category. All phenotypic measures were further adjusted for log (BMI) (apart from weight, height and BMI itself), thus assessing the effect of the particular adiposity measure after adjusting for this global weight measure. Log (weight) was adjusted for height instead of log (BMI). Since adjustment for ratio variables in anthropometric studies has been criticised [19], we further adjusted waist circumference for log(weight) and height. Finally we repeated analysis of waist circumference adjusted for BMI restricted to participants with BMI under 30 kg/m^2. 95% confidence intervals have been quoted for all effect sizes.

Meta analysis was also carried out of the relationship between reported daily cigarette consumption and rs1051730/rs16969968 genotype, among current smokers.

Although analyses were carried out for males and females separately, the estimates were combined where no evidence for separate sex effects was seen. For NHANES, which has a survey design, Taylor series linearization was implemented to estimate variances. For studies including related family members appropriate methods were used to adjust standard errors: in GEMINAKAR, twin pair identity was included as a cluster variable in the model, in MIDSPAN linear mixed effects regression models fitted using restricted maximum likelihood were used to account for related individuals, while in NTR, only unrelated individuals were included. ALSPAC mothers and children were analysed

as separate samples; as there are related individuals across these samples, sensitivity analyses were performed excluding each of these studies in turn.

Results from individual studies were meta-analysed in Stata (version 13) using the "metan" command from Stata. Where there was evidence of heterogeneity between studies ($I^2 > 50\%$), it was planned that both fixed and random effects analyses would be performed: however as this never occurred, results for fixed effects analysis only are shown. Meta-regression analysis, using the "metareg" command from Stata, was used to examine whether SNP effects varied by smoking status or by sex, or by a smoking by sex combination.

Results

Descriptive statistics

The maximum sample size available, with genotype recorded, was 148,731 for weight, height and BMI, over 29 studies. The data on individuals with weight, height, smoking status and genotype recorded, included 66,809 never smokers, 43,009 former smokers and 38,912 current smokers. Waist circumference was available in 28 studies (n=142,381), hip circumference and waist-hip ratio in 25 studies (n=139,667). Measures of fat mass and fat free mass were provided by 10 studies (n=28,231), arm circumference by nine studies (n=72,536), and the skinfolds by five studies (n=7,758). Finally leptin and adiponectin were measured in nine studies (n=23,630 and 19,191 respectively). Overall, 47% of the combined study population was male. The median age within the contributing studies ranged from 16-74 years. Descriptive statistics for each of the study populations are found in the supplementary material (Table S1).

Minor allele frequency for rs1051730/rs16969968 ranged between 0.31 and 0.36. There was no strong evidence for deviation from the Hardy-Weinberg Equilibrium in any of the studies (p-values all ≥ 0.09, Table S2).

Mendelian Randomisation analysis

Table 1 shows the per-allele increases in each phenotype, within each smoking status category. As previously shown [9], increase in BMI was positive in never smokers: +0.35% (95%CI 0.18, 0.52; $p=6.38*10^{-5}$), non-significant in former smokers: -0.14% (95%CI -0.34, +0.07; p=0.19) and significantly inverse in current smokers: -0.74% (95%CI -0.96, -0.51; $p=2*10^{-10}$). Full results for each contributing study are shown in Figure S1.

Waist circumference was higher per minor allele in never smokers: +0.23% (95%CI 0.09, 0.36; p=0.0012), non-significantly related in former smokers -0.07% (95%CI -0.24, 0.09; p=0.37), and lower in current smokers -0.37% (95%CI -0.55, -0.19; $p=1.69*10^{-5}$): differences among smoking groups were highly significant ($p=3.85*10^{-7}$), see Figure S2. The per-allele effect on waist circumference in current smokers was about half the magnitude of that seen for BMI. After adjustment for log(BMI), the minor allele of rs1051730-rs16969968 was not associated with waist circumference in either never smokers: +0.01% (95%CI -0.06, 0.08; p=0.72) or former smokers +0.06% (95%CI -0.02, 0.15; p=0.15). However in current smokers, the minor allele was associated with a 0.14% (95%CI 0.05, 0.22; p=0.003) higher waist circumference after adjustment for log(BMI). Very similar results were seen in all three smoking status categories after waist was adjusted for log(weight) and height instead of log(BMI). Effects of genotype on waist circumference were shown to differ between smoking status categories before adjustment ($p=3.85*10^{-7}$) but only weakly after adjustment for log(BMI) (p=0.102), and after adjustment for log(weight) and height (p=0.018). Little heterogeneity of study results was evident (I^2<=25% within all smoking groups). After restricting analysis to participants with BMI under 30 kg/m^2, we found that the percentage increases in waist circumference (after adjustment for log(BMI)) were 0.04% (95%CI -0.03, 0.12) for never smokers, 0.03% (95%CI -0.06, 0.13) for ex-smokers and 0.12% (95%CI 0.02, 0.21) for current smokers: however the test for difference in effects gave p=0.41.

Unadjusted results for hip circumference were very similar to that seen for waist, both in direction and magnitude, in all smoking status groups (Figure S3). However after adjustment for log(BMI), effects were not apparent in any of the three groups, and nor was the interaction of gene and smoking status.

Results for waist-hip ratio were similar to BMI, waist and hip circumference in direction but were smaller in magnitude: +0.07%, 0.00% and -0.08% increases in never-smokers, former smokers and current smokers respectively, (p=0.083 for differences between smoking categories), see Figure S4. After adjustment for log(BMI), increases remained non-significant for never smokers and former smokers (-0.01% and 0.04%) but increased significantly among current smokers (0.10%) (p=0.13 for differences among smoking groups).

For several other phenotypes, per-allele decreases were observed in current smokers that exceeded those seen either in former or never smokers (Table S4). However there was only statistical evidence for decreases among current smokers for arm circumference ($p=8.4*10^{-5}$) and leptin (p=0.025), while the difference between smoking groups was only significant for arm circumference ($p=3.29*10^{-4}$). Both effects became non-significant after adjustment for log(BMI). Fat mass and fat free mass, after adjustment by height, showed differences in effects by smoking group. These effects were more due to per-allele increases seen among never smokers than decreases among current smokers.

Meta-regression analyses showed no clear evidence for associations between genotype and each adiposity phenotype being modified by sex: p-values exceeded 0.1 for all phenotypes, adjusted or unadjusted, apart from hip circumference. The per-allele decreases in hip circumference among current smokers appeared more marked among women (p=0.067), but this effect was no longer apparent after adjusting for BMI (p=0.51).

The mean difference in daily cigarette consumption was 0.77 among current smokers (95%CI 0.67 to 0.88, I^2=17%).

Discussion

This meta-analysis of 29 studies comprising almost 150,000 participants with key adiposity phenotypes, has demonstrated firstly, that a variant associated with increased cigarette consumption was associated not only with lower BMI among current smokers, consistent with earlier findings [7 8], but also with lower waist and hip circumference. Secondly, the inverse association of the variant with lower waist circumference among current smokers changed direction after adjusting for BMI. The variant was positively associated with waist circumference but associated neither with hip circumference after BMI adjustment, nor waist-hip ratio. Our results suggest that for every copy of the minor allele associated with cigarette consumption (i.e. increasing cigarette per day consumption by approximately one cigarette), waist circumference will be increased by 0.14% if BMI were to remain constant. This suggests a preferential re-distribution towards central adiposity associated with higher cigarette consumption: this important finding is in keeping with our hypothesis and extends current observational data.

We also observed that none of the effects were modified by sex, contrary to our second hypothesis. Finally we have already noted among never-smokers an unexpected positive association of the gene variant with BMI [9] : the current analysis demonstrates this same association with waist and hip circumference. This occurred in the opposite direction to the inverse association of various adiposity measures with the gene variant seen in current smokers (before adjustment for BMI).

The analysis consisted of never, former and current smokers from a very wide spectrum of ages among the 29 studies. The sample size was very large for the primary phenotypes considered here. Participants were exclusively of self-reported European ancestry, and were mostly recruited in European countries. The data available for direct measures of fat such as fat mass, and the biomarker leptin, were available for only about one fifth of the participants on whom weight, height, waist and hip were measured. Effects according to genotype for these phenotypes showed broadly similar results for the three smoking categories to those seen for BMI.

Mendelian randomisation has proved a powerful tool for eliciting causal associations between phenotypic measures[20]. In the present analysis, Mendel's laws concerning random assignment of genotype should produce an unconfounded comparison between the genotype influencing smoking consumption and the outcomes of interest, namely anthropometric phenotypes. Furthermore, because this random assignment occurs at the very outset of life, the associations between genotype and anthropometric measures cannot be due to reverse causality. If the genotype only influences smoking consumption, and not the initiation of smoking, then the relationship between genotype and anthropometric outcomes would only be expected among smokers.

In fact, while the variant was associated with lower waist and hip circumference among current smokers, it was associated with greater waist and hip circumference among never-smokers. This suggests that the true effect among current smokers may be even greater than estimated. When we adjusted waist circumference for BMI, there was no association with the gene variant among never smokers. The relative proportions of ever-smokers and never-smokers was not clearly associated with genotype in the CARTA consortium, as reported elsewhere[9].

The reversal of the association between waist circumference and allele count from negative to positive among current smokers after adjustment for BMI may be consistent with alternative explanations. Firstly, heavy smokers may have less muscle mass; however no association between allele count and fat free mass could be detected in our analysis among smokers. Secondly, the test for interaction for smoking status and allele count on waist circumference after adjustment was of weak statistical significance. Thirdly, the adjustment of one measure of adiposity with another with which it is highly correlated may have caused a spurious association. We repeated our analysis for participants with BMI under 30 only, where the correlation was more modest, and obtained similar results albeit with reduced evidence for an effect.

Stratification of our analyses by smoking status, could in theory introduce bias by conditioning on a collider (rs1051730/rs16969968) [21]. This variant shows some evidence for association with smoking cessation (current vs former smoking) [22]. Whilst this is a possibility, no effect modifications of this variant with potential confounders by smoking status, were demonstrated among 56,625 participants in the HUNT study [8].

Cross sectional observational data from Switzerland has demonstrated that waist and hip circumference were more strongly related to number of cigarettes smoked per day than was BMI[13], while in Scotland being a smoker was associated with greater central adiposity among women[12] . In a Finnish longitudinal twin cohort study, smoking in adolescence predicted abdominal obesity in adulthood [11]. Observational data are however prone to confounding and reverse causality, and the present study adds some evidence that the associations reported are likely to be causal.

Some observational studies have noted that low fat free mass [23] and bone mineral density [24], were more common among smokers. The present analysis has not substantiated the association with fat free mass although our sample size was much more limited for this phenotype.

Our findings resonate with observational studies which have shown associations between smoking and risk of diabetes [17 18], especially as analysis of the British Women's Heart and Health Study showed that abdominal adiposity was a stronger predictor of diabetes than was BMI [16]. Waist circumference, and waist-to-hip ratio were strongly associated, independently of BMI, with the risk of death among 359,387 participants from nine countries in the European Prospective Investigation into Cancer and Nutrition [15]. Therefore the health hazards of smoking could well be enhanced, or partly mediated through increasing abdominal adiposity. In addition, the desire of many smokers to use smoking as a means of weight control [2] might be counterproductive, if a loss of weight is accompanied by a relative increase in waist circumference: this possibility could be used in counselling people seeking to quit smoking.

People who quit smoking appear to be at increased risk of acquiring diabetes in the short term and this was not explained by weight gain in a Japanese population [25]. The present study took place almost exclusively of white European participants, and replication of the findings among other ethnic populations would be of great value. This is especially urgent on a global scale since smoking levels are increasing among several non-white ethnic groups, and this is seen to be partly responsible for increases in coronary heart disease mortality in Beijing, China [26], in Syria [27], and in Tunisia among women [28]. In addition, increases in average waist circumferences have been observed even when average BMI levels have remained constant [29], and metabolic disorders especially

diabetes have increased in prevalence[30]. It is thus possible increased CHD mortality will be partly fuelled by increasing smoking levels.

Mendelian Randomisation studies have more potential than traditional observational epidemiological studies to establish causality for specific exposures [20], and they should now be used to investigate other impacts of smoking, in particular on pathways leading to Type 2 diabetes, as well as on Type 2 diabetes itself. The findings of the current study could now be further tested by assembling data from randomised trials of smoking cessation, where post intervention data on measures of central adiposity are available. If confirmed, a tendency for smokers to acquire an "apple shape" due to increasing central adiposity might provide a novel health promotion message to encourage smoking cessation, and appropriate new interventions should then be designed and evaluated as part of overall tobacco control policies in society.

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NHANES: The National Health and Nutrition Examination Survey (NHANES) (http://www.cdc.gov/nchs/nhanes.htm) is a program of health surveys run by the National Center for Health Statistics, part of the Centers for Disease Control and Prevention in the United States. Data collection for NHANES was approved by the NCHS Research Ethics Review Board. Analysis of deidentified data from the survey is exempt from the federal regulations for the protection of human research participants. Analysis of restricted data through the NCHS Research Data Center is also approved by the NCHS ERB.

The findings and conclusions in this paper are those of the author(s) and do not necessarily represent the views of the Research Data Center, the National Center for Health Statistics, or the Centers for Disease Control and Prevention.

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Infrastructure), VU University's Institutes for Health and Care Research and Neuroscience Campus Amsterdam. The NTR study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center, Amsterdam (IRB number IRB-2991 under Federalwide Assurance 3703; IRB/institute code 03-180), and all subjects provided written informed consent.

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Author Contributions

RWM, AET, TIAK, MRM and NS conceived the study and contributed to the writing of the manuscript. RWM conducted the final analyses. All other authors conducted individual study analyses and contributed to the writing of the manuscript.

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Table 1. Per allele percentage increases in measures of regional adiposity (BMI, weigh, waist circumference, hip circumference, waist-hip ratio) among never, ex and current smokers, before and after adjustment for body mass index

		ADJUSTED FOR AGE				ADJUSTED FOR AGE AND BMI			
		Never smokers	Former smokers	Current smokers	*p for interaction**	Never smokers	Former smokers	Current smokers	*p for interaction**
BMI (kg/m^2)	*% increase*	0.35	-0.14	-0.74		-			
	95%CI	(0.18,0.52)	(-0.34,0.07)	(-0.96,-0.51)					
	p	6.38×10^{-5}	0.19	2.00×10^{-10}	4.95×10^{-13}				
	N	66,809	43,009	38,912					
	I^2	14%	0%	0%					
Waist circumference (cm)	*% increase*	0.23	-0.07	-0.40		0.01	0.06	0.14	
	95%CI	(0.09,0.36)	(-0.24,0.09)	(-0.57,-0.22)		(-0.06,0.08)	(-0.02,0.15)	(0.05,0.22)	
	p	0.0012	0.37	1.69×10^{-5}	3.85×10^{-7}	0.72	0.15	0.003	0.087
	N	64,265	40,756	37,360					
	I^2	14%	0%	10%		0%	0%	13%	
Hip circumference (cm)	*% increase*	0.17	-0.07	-0.31		0.02	0.02	0.02	
	95%CI	(0.08,0.26)	(-0.17,0.04)	(-0.42,-0.19)		(-0.03,0.07)	(-0.04,0.08)	(-0.05,0.08)	
	p	2.95×10^{-4}	0.23	2.55×10^{-7}	1.79×10^{-9}	0.38	0.54	0.59	0.99
	N	62,323	40,512	36,833					
	I^2	7%	0%	0%		16%	0%	0%	

Waist-hip ratio	*% increase*	0.07	0	-0.08		-0.01	0.04	0.1	
	95%CI	(-0.01,0.15)	(-0.10,0.10)	(-0.19,0.03)		(-0.08,0.06)	(-0.04,0.13)	(0.02,0.19)	
	p	0.087	0.97	0.14	0.083	0.78	0.30	0.02	0.13
	N	62,322	40,512	36,833					
	I^2	21%	9%	15%		0%	0%	13%	

*Interaction assessed by assessing heterogeneity between effect estimates according to smoking status, with fixed effects model

Table S1. Distribution of smoking status, sex and age, and availability of regional adiposity phenotypes, in each of the 29 studies

Study	Total N	Never smokers	Ex smokers	Current smokers	% male	Median age	Weight	Height	Waist	Hip	Arm	TSF	SSF	Fat mass	Fat free mass	Leptin	Adiponectin
1958 BC	5,022	2,353	1,449	1,220	50	42	*	*	*	*	*						
ALSPAC children	1,664	1,336	0	328	48	17.8	*	*						*	*		
ALSPAC mothers	1,530	1,004	395	131	0	48	*	*	*	*	*						
BRHS	3,576	1,040	2,072	464	100	68	*	*	*	*	*	*	*	*	*	*	*
BWHHS	3,615	2,043	1,180	392	0	68	*	*	*	*	*					*	*
Caerphilly	1,155	226	592	337	100	62	*	*	*	*							
CHDS	614	313	136	165	50	30	*	*	*	*							
CoLaus	4,305	1,817	1,393	1,095	43	53	*	*	*	*				*	*	*	*
Dan-MONICA	2,245	642	575	1,028	51	54	*	*	*	*	*			*	*		
EFSOCH	1,214	749	228	238	44	32	*	*	*	*	*	*	*				
ELSA	4,978	1,726	2,551	701	46	65	*	*	*	*							
Finrisk	20,368	9,755	5,493	5,120	46	51	*	*	*	*							

GEMINAKAR	1,120	556	185	379	48	38	*	*	*	*		*	*			*	*
Generation Scotland	7,294	3,893	2,421	980	41	57	*	*	*	*		*	*				
GOYA males	765	759	108	148	0	45	*	*	*	*				*	*	*	*
GOYA females	1,015	172	213	380	100	38	*	*	*								
HBCS	1,626	703	551	372	43	61	*	*	*	*				*	*	*	*
Health 2006	3,211	1,382	1,078	751	44	50	*	*	*	*				*	*		
Health 2008	624	280	221	123	44	47	*	*	*	*				*	*		
HUNT	55,476	24,302	14,144	17,030	48	47	*	*	*	*	*						
Inter 99	5,399	1,986	1,427	1,986	49	45	*	*	*	*						*	*
Midspan	2,099	994	574	531	45	45	*	*	*	*							
NFBC 1966	3,729	1,763	577	1,389	50	31	*	*	*	*							
NFBC 1986	1,171	752	0	419	48	16	*	*	*	*							
NHANES	2,045	987	616	442	38[1]	43[1]	*	*	*	*	*	*	*	*			
NSHD	1,751	776	639	336	48	53	*	*	*	*	*					*	*

NTR	3,718	1,822	1,081	815	36	39	*	*	*	*						
PROSPER	5,145	1,761	2,022	1,362	48	74	*	*							*	*
Whitehall II (phase 3)	2,836	1,383	1,088	365	75	48	*	*	*	*						
Whitehall II (phase 7)	2,921	1,426	1,278	217	77	59							*	*		

1. Weighted values from survey data

Table S2. Distribution of genotypes for SNP rs1051730/rs16969968, minor allele frequency and p-value from test for Hardy-Weinberg equilibrium (HWE)

Study	Major homozygotes	Heterozygotes	Minor homozygotes	MAF	HWE p-value
1958 BC	2,178	2,230	614	0.34	0.4
ALSPAC children	740	752	172	0.33	0.37
ALSPAC mothers	711	664	155	0.32	1
BRHS	1,631	1,540	405	0.33	0.15
BWHHS	1,591	1,625	399	0.34	0.59
Caerphilly	523	512	120	0.33	0.75
CHDS	286	273	55	0.31	0.37
CoLaus	1,778	1,980	547	0.36	0.91
Dan-MONICA	993	1,000	252	0.33	0.83
EFSOCH	568	523	124	0.32	0.82
ELSA	2,265	2,169	544	0.33	0.47
Finrisk	9,251	8,979	2,138	0.32	0.59
GEMINAKAR	530	477	113	0.32	0.43
Generation Scotland	3,261	3,251	782	0.33	0.52
GOYA males	443	473	99	0.34	0.2
GOYA females	338	329	98	0.33	0.09
HBCS	699	746	181	0.34	0.39
Health 2006	1,436	1,429	346	0.33	0.77
Health 2008	291	269	64	0.32	0.87
HUNT	24,621	24,579	6,276	0.33	0.23
Inter 99	2,364	2,423	612	0.34	0.63
Midspan	953	931	215	0.33	0.87
NFBC 1966	1,711	1,612	406	0.33	0.38
NFBC 1986	554	521	96	0.3	0.08
NHANES	864	928	253	0.35	0.88

NSHD	827	760	164	0.32	0.36
NTR	1,727	1,607	384	0.32	0.72
PROSPER	2,403	2,244	498	0.31	0.56
Whitehall II (phase 3)	1,276	1,261	299	0.33	0.50
Whitehall II (phase 7)	1,285	1,317	319	0.33	0.50

Table S3. Per allele percentage increases in measures of regional adiposity measures (fat mass, fat free mass, leptin, adiponectin, arm circumference, triceps skinfold, subscapular skinfold) among never, former and current smokers, before and after adjustment for body mass index

		ADJUSTED FOR AGE				ADJUSTED FOR AGE AND BMI *			
		Never smokers	Former smokers	Current smokers		Never smokers	Former smokers	Current smokers	
					p for interaction +				*p for interaction +*
Fat mass	*% increase*	0.42	-0.33	-0.43		0.85	-0.31	-0.43	
	95%CI	(-0.09, 0.94)	(-0.93, 0.27)	(-1.13, 0.28)		(0.25, 1.45)	(-0.97, 0.36)	(-1.13, 0.28)	
	p	0.11	0.28	0.24	0.08	0.005	0.37	0.42	0.015
	N	15,249	11,381	6,914					
	I^2	45%	20%	6%		21%	19%	5%	
Fat free mass	*% increase*	0.36	-0.03	0.03		0.44	-0.08	-0.08	
	95%CI	(0.09, 0.63)	(-0.33, 0.28)	(-0.35, 0.41)		(0.22, 0.67)	(-0.34, 0.19)	(-0.40, 0.25)	
	p	0.008	0.86	0.89	0.13	1.19×10^{-4}	0.57	0.64	3.95×10^{-3}
	N	15,543	11,511	7,011					
	I^2	19%	0%	36%		13%	0%	24%	
Leptin	*% increase*	-0.97	0.03	-3.48		-0.66	-0.38	-1.36	
	95%CI	(-3.34, 1.45)	(-2.32, 2.43)	(-6.42, -0.44)		(-2.53, 1.26)	(-2.24, 1.52)	(-3.64, 0.98)	
	p	0.43	0.98	0.025	0.2	0.5	0.69	0.25	0.81
	N	8,840	8,472	6,073					

	I^2	15%	0%	5%		0%	23%	0%	
Adiponectin	*% increase*	-0.04	-2.96	-0.31		-0.23	-2.88	-1.14	
	95%CI	(-2.17, 2.13)	(-5.35, -0.51)	(-3.07, 2.54)		(-2.30, 1.89)	(-5.22, -0.48)	(-3.81, 1.60)	
	p	0.97	0.18	0.83	0.18	0.83	0.019	0.41	0.26
	N	8,840	8,472	6,073					
	I^2	16%	18%	2%		18%	21%	18%	
Arm circumference	*% increase*	0.11	-0.17	-0.4		-0.08	-0.03	0.06	
	95%CI	(-0.05, 0.27)	(-0.36, 0.02)	(-0.60, -0.20)		(-0.17, 0.01)	(-0.14, 0.08)	(-0.05, 0.17)	
	p	0.17	0.08	8.40×10^{-5}	3.29×10^{-4}	0.09	0.6	0.25	0.14
	N	32,413	20,063	20,061					
	I^2	0%	0%	0%		0%	46%	0%	
Triceps skinfold	*% increase*	0.86	1.98	-2.14		-0.64	1.98	-1.6	
	95%CI	(-1.05, 2.81)	(-0.18, 4.18)	(-5.31, 1.13)		(-2.17, 0.90)	(0.12, 3.87)	(-3.92, 0.79)	
	p	0.38	0.072	0.2	0.12	0.41	0.037	0.19	0.034
	N	3,234	3,064	1,460					
	I^2	71%	0%	0%		42%	0%	0%	
Subscapular skinfold	*% increase*	-0.16	-0.93	-2.29		0.14	-0.41	-1.23	
	95%CI	(-2.14, 1.87)	(-2.83, 1.01)	(-5.54, 1.06)		(-1.01, 1.31)	(-1.89, 1.10)	(-3.29, 0.87)	
	p	0.88	0.34	0.18	0.55	0.81	0.59	0.25	0.55

N	3,234	3,064	1,460			
I^2	56%	0%	8%	0%	0%	3%

*adjustment only made for age and height for fat mass and fat free mass

+Interaction assessed by assessing heterogeneity between effect estimates according to smoking status, with fixed effects model

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **[Within the title page 1 and design section of the abstract page 6]**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **[See results section of abstract page 6]**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **[Introduction on page 8]**
Objectives	3	State specific objectives, including any prespecified hypotheses **[See page 8]**
Methods		
Study design	4	Present key elements of study design early in the paper **[Abstract page 6, end of introduction page 8, methods pages 9-10]**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **[See Supplementary material]**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **[Top of methods section page 9, but mainly in supplementary material]**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **[See pages 9-10]**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **[See Supplementary material]**
Bias	9	Describe any efforts to address potential sources of bias **[last paragraph on page 10, page 11, penultimate paragraph page 14]**
Study size	10	Explain how the study size was arrived at **[N/A]**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **[page 10 under "Statistical Analysis"]**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **[See pages 10-11]**
		(*b*) Describe any methods used to examine subgroups and interactions **[See pages 10-11]**
		(*c*) Explain how missing data were addressed **[N/A]**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was

		addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses **[bottom of page 10, page 11, penultimate para on page 14]**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **[Supp Table S1]**
		(b) Give reasons for non-participation at each stage **[N/A]**
		(c) Consider use of a flow diagram **[N/A]**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **[Supp Table S1]**
		(b) Indicate number of participants with missing data for each variable of interest **[Supp Table S1, S2]**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **[N/A]**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures **[See Supplementary material]**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **[See pages 12-13, Table 1, Table S3, Figures S1-S4]**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **[N/A]**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **[See page 13, last para]**
Discussion		
Key results	18	Summarise key results with reference to study objectives **[see page 14, first two paras]**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **[see page 14, last three paras.]**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **[see pages 14-15]**
Generalisability	21	Discuss the generalisability (external validity) of the study results **[see page 15]**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **[acknowledgements for each primary study, pages 16-20]**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

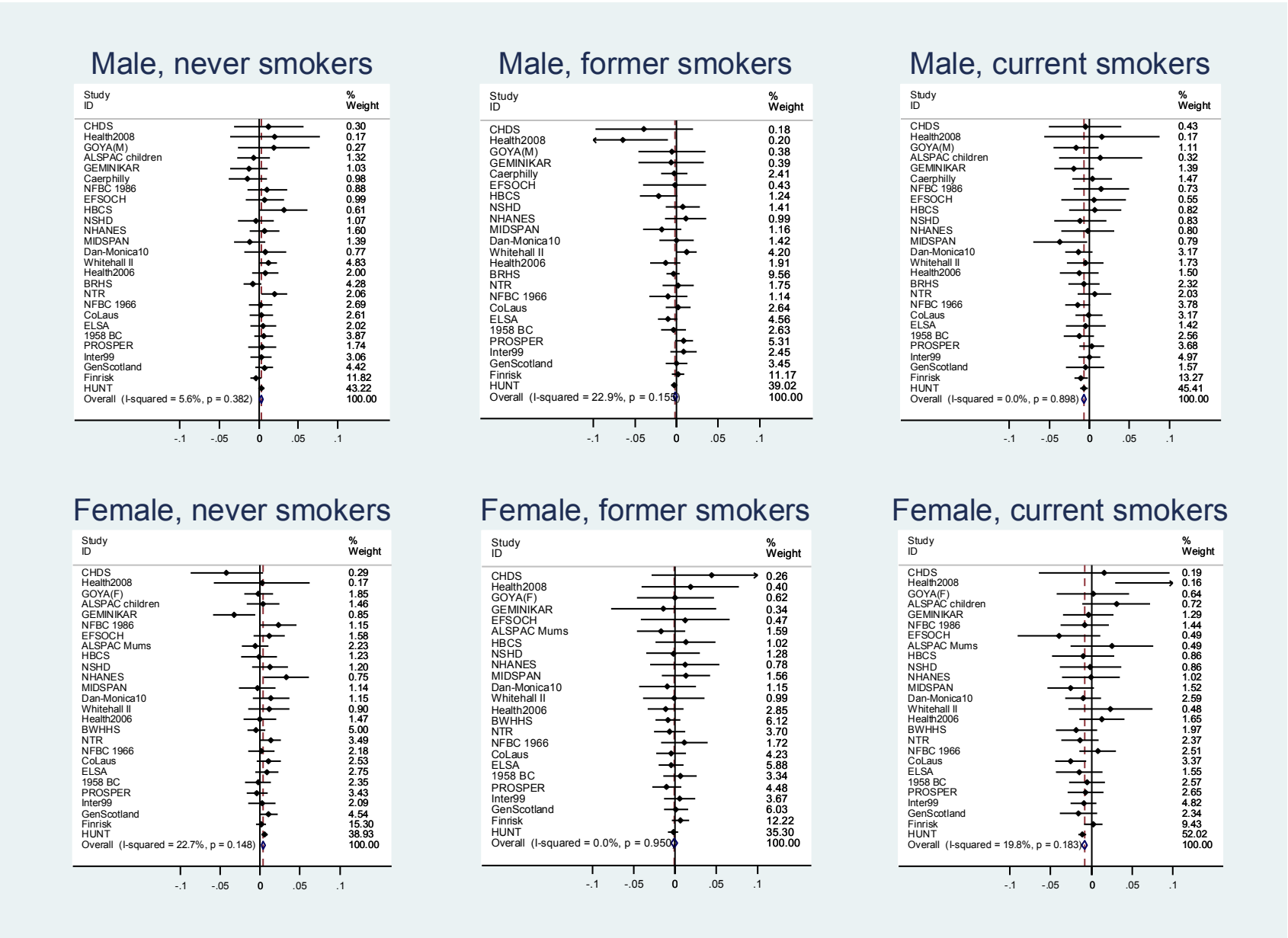
Supplementary Figure S1-S4. Associations of rs1051730/rs16969968 with adiposity phenotypes (fixed effects meta-analysis) in never-smokers, ex-smokers and current smokers. Results for male participants in upper panels, and for females in lower panels. Horizontal axis indicates difference in mean log(phenotype) per allele

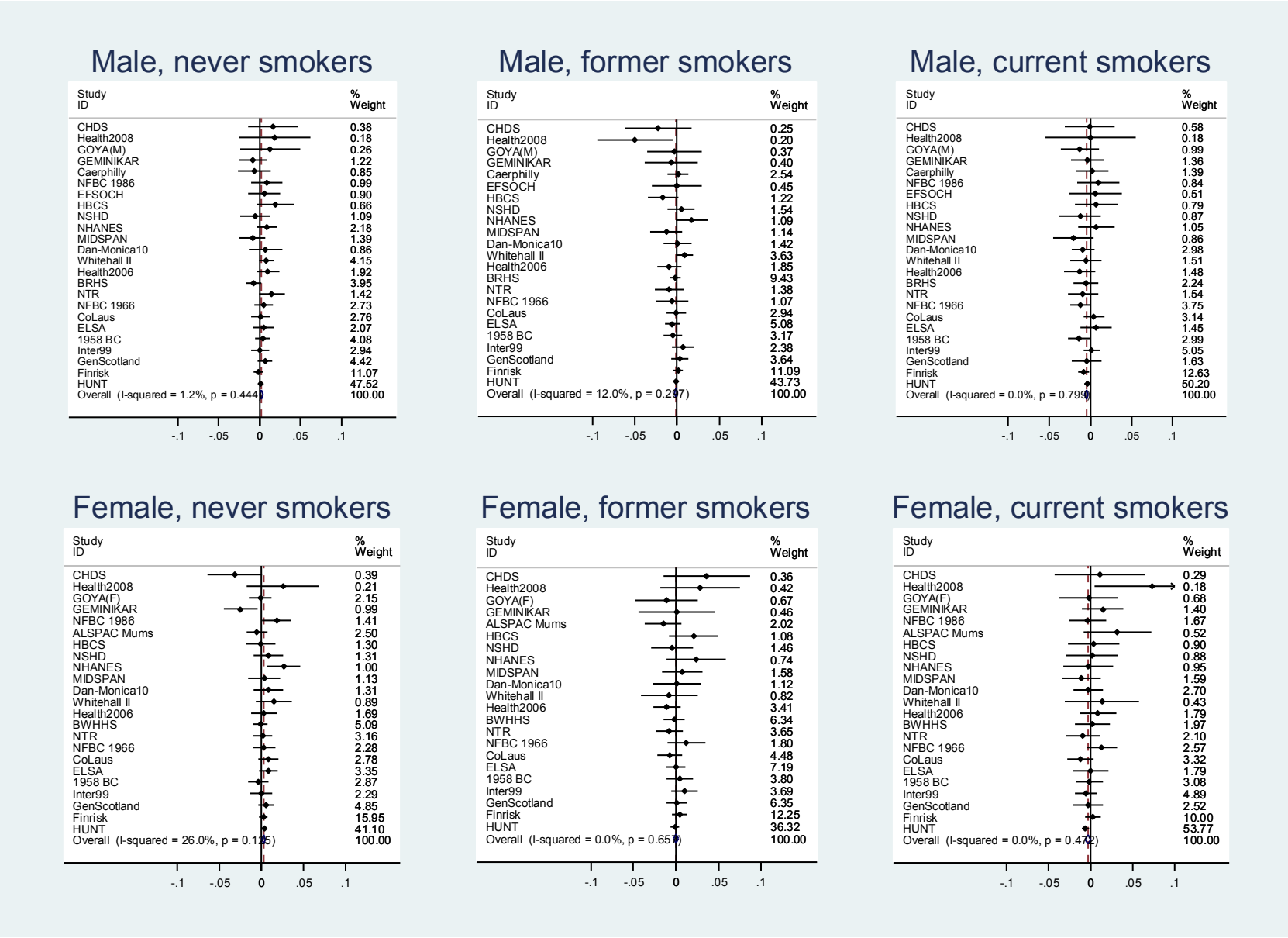
S1. BMI

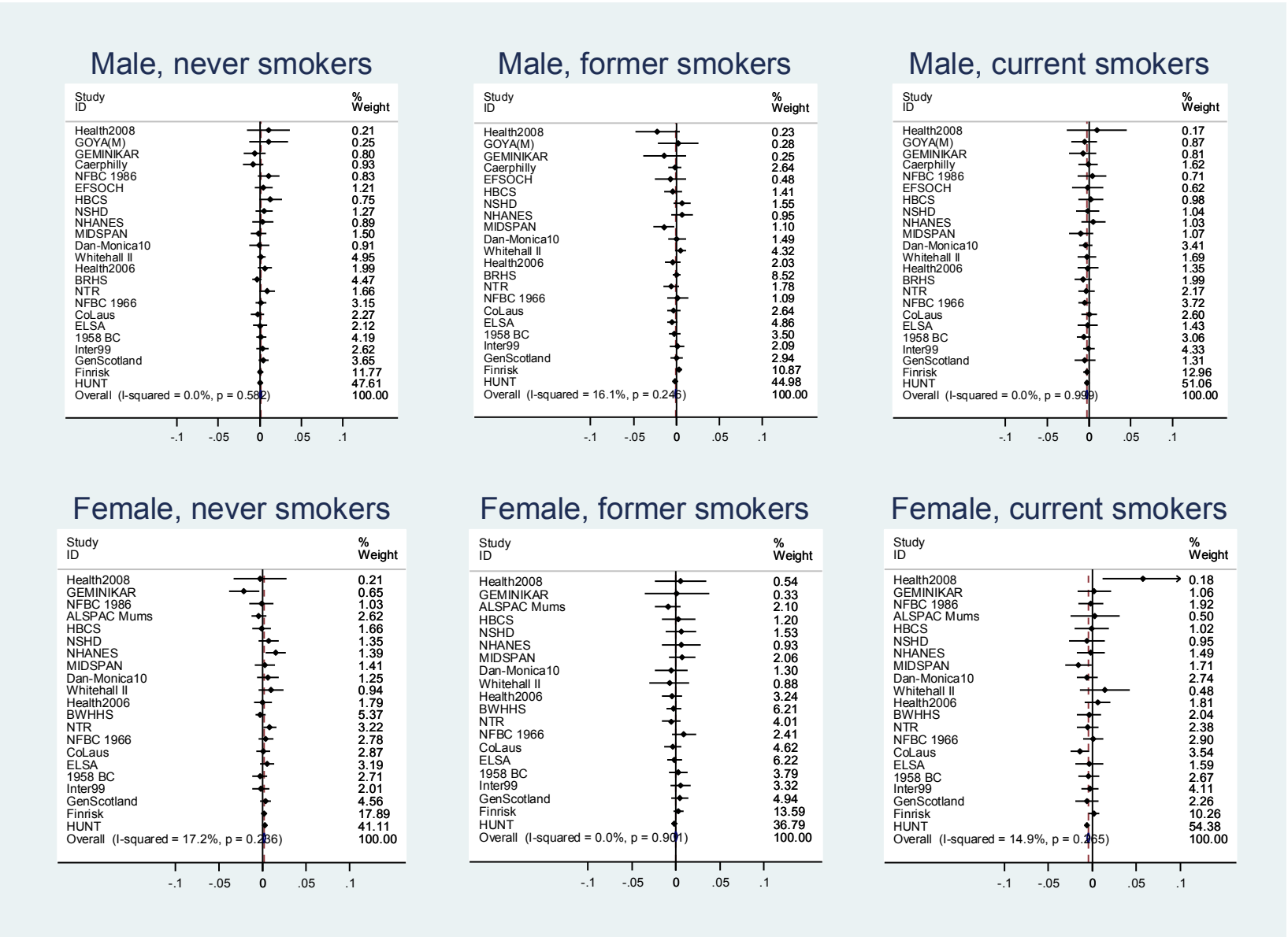
S2. Waist cirumference

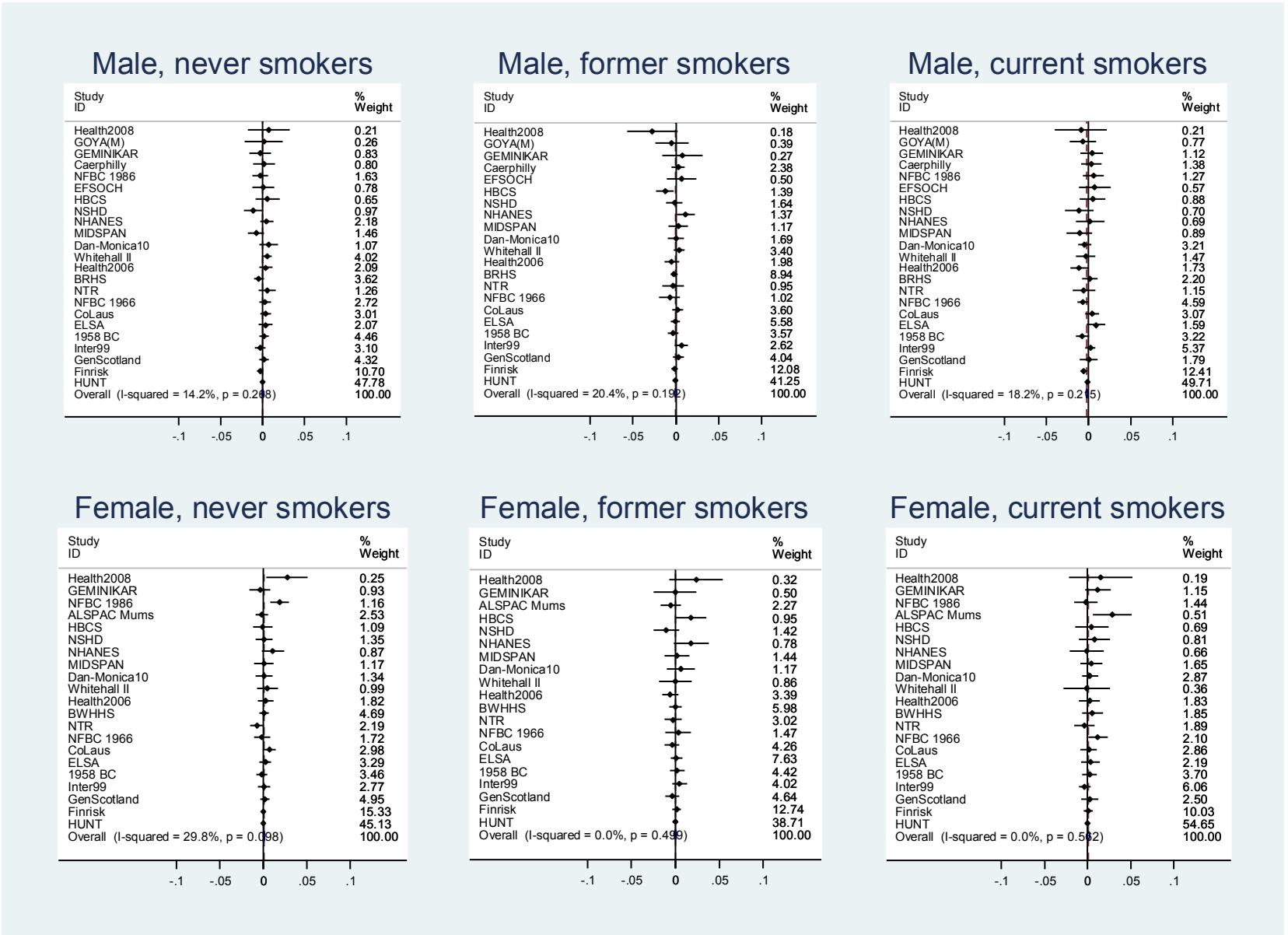
S3. Hip circumference

S4. Waist-hip ratio









Correction

Morris RW, Taylor AE, Fluharty ME, *et al*. Heavier smoking may lead to a relative increase in waist circumference: evidence for a causal relationship from a Mendelian randomisation meta-analysis. The CARTA consortium. *BMJ Open* 2015;5:e008808. The author name Tarun Veer Singh Ahluwalia should be spelt Tarunveer Singh Ahluwalia, and the abbreviation is Ahluwalia TS. Also, the surname of Maiken Elvestad Gabrielsen is 'Gabrielsen' only so should be abbreviated to Gabrielsen ME as opposed to Elvestad Gabrielsen M.

BMJ Open 2015;**5**:e008808. doi:10.1136/bmjopen-2015-008808corr1