Obesity and Food Reward Regulation by the Brain

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Genetic factors and food intake regulation

PART 3

Physical activity and food intake in females with low and high genetic risk to obesity

VII

Stieneke Doornweerd, Richard G IJzerman,
Gonneke Willemsen, Dorret I Boomsma, Nathalie P van der Eijk,
Hidde P van der Ploeg, Eco J de Geus

ABSTRACT

OBJECTIVE

Observational studies cannot determine whether deviant physical activity, dietary intake and eating behaviour are a cause or consequence of obesity.

METHODS

In an attempt to seperate cause and effect, we selected 60 women (age 45.8 ± 6.9 years, BMI range 17.4 – 43.9 kg/m^2) with either a low or high genetic risk score (GRS) for obesity and either a low or high BMI from 11495 people in the Netherlands Twin Register. GRS was based on 77 obesity-associated single nucleotide polymorphisms. We measured physical activity, dietary intake and eating behaviour using 7-day accelerometry, 3-day 24-hour recalls and questionnaires.

RESULTS

Women with high BMI had fewer step counts, more sedentary behaviour and more emotional and restrained eating than women with low BMI, irrespective of GRS. Women with high GRS and high BMI had a higher intake of (animal) protein than women with high GRS but low BMI. In women with low GRS protein intake of low and high BMI groups was similar.

CONCLUSIONS

Unfavourable changes in the balance between sedentary behaviour and light intensity physical activity, emotional and restrained eating are likely secondary to increased BMI. In contrast, higher intake of (animal) protein may be a component of the genetic predisposition to obesity.

INTRODUCTION

The on-going rise in obesity prevalence has been attributed to an increased availability of high calorie energy dense food and a decreased physical activity level [1]. Experimental studies manipulating energy intake or physical activity have confirmed the existence of robust causal effects of each of these factors on body mass index (BMI) [2,3], yet it remains difficult to establish the relative contribution of dietary intake and physical activity patterns in the development of obesity in the population. Observational studies in population based samples can establish the extent of the association between physical activity, diet and BMI but they cannot rule out that BMI itself is causative of changes in physical activity level or diet [4].

In an attempt to separate cause and effect, the so-called four corners epidemiological model has been proposed [5-7]. In this model, participants with either a high or low genetic susceptibility to a trait of interest are selected to have either high or low observed levels of that trait. For instance, offspring from hypertensive parents could be selected that have either low or high blood pressure levels themselves, and a similar selection of either low or high blood pressure can be made in offspring of normotensive parents [5]. The expectation is that variables that are associated with blood pressure in offspring irrespective of the genetic risk for blood pressure are secondary to high blood pressure or largely influenced by environmental determinants that operate independent of the familial risk for blood pressure. In contrast, variables that are associated with high blood pressure *only* in the hypertensive offspring of hypertensive parents, but not in the hypertensive offspring of normotensive parents, could be part of a causal pathway leading from genetic risk to high blood pressure. This four corners model has for instance been applied to show that defective microvascular function may be a causal factor leading to high blood pressure and is not simply a mere consequence of it [6].

An important assumption in the original four corners design is that parents expressing a high level of a risk factor do so because of an underlying heritable factor. When applied to parents with high BMI this need not be the case. Shared environmental factors related to socioeconomic position or cultural and neighbourhood characteristics could also lead to high BMI in both parents. This would interfere with the primary reasoning in the four corners design. Fortunately, thanks to the recent progress in gene discovery based on genome wide association studies (GWAS) [8,9], we can now express the genetic risk for BMI independent of the family environmental background by using genetic risk scores (GRS) based on known markers of causal alleles. We therefore propose an adapted four corners design that selects individuals with either high or low BMI values, and, within each group, either low or high GRS based on recently established single nucleotide polymorphisms (SNPs) [8,9].

The aims of this study were to investigate whether physical activity, sedentary behaviour, dietary intake and eating behaviour are associated with GRS for obesity and, thus exert a direct effect on BMI or, rather, reflect a consequence of increased BMI itself.

METHODS

PARTICIPANTS

All participants eligible for inclusion in this study are registered in the Netherlands Twin Register (NTR) [10], which consists of twins and their family members taking part in longitudinal survey studies every 2-3 years. The sampling frame for the current study were all participants who had undergone BMI measurements as part of earlier studies [10, 11] and genotyping as part of GWAS projects [8, 12]. DNA was extracted from either blood or buccal cell samples using the QIAamp DNA Blood Maxi (QIAGEN, Dusseldorf, Germany). Genotyping of single nucleotide polymorphisms (SNPs) was done as described in more detail previously [10]. For each participant a genetic risk score (GRS) for obesity was calculated using 77 known obesity SNPs from the most recent meta-analysis of GWAS in adults [9]. The 77 loci were used that reached genome-wide significance for BMI ($P<5 \times 10\text{-}8$) in the European analysis [9]. A weighted GRS was calculated by summing the BMI-increasing alleles after weighting the alleles by the effect size reported for that locus (Supplementary Table 1).

The total sample with both BMI and GRS data available was 11495 participants (Figure 1). Before running a second selection phase, individuals were excluded if they 1) had no weight and/or height recorded during the latest NTR biobank study [11], necessary for calculating recent BMI, 2) were part of monozygotic twins or triplets, or 3) were part of the same family as another individual in the sample. The removal of all but one member of a same family was done to create a sample of genetically unrelated individuals. To this end, spouses and parents of registered twins were first selected followed by randomly chosen siblings or members of twin pairs. Figure 2 displays the distributions of GRS and BMI of the population at this point during the selection phase. Then, a scatter diagram was constructed by plotting BMI on one axis and GRS on the other axis (Figure 3). Four corners were created by using cut-off points that corresponded to the lower and upper 25% of the distribution of BMI (i.e. ≤ 22 kg/m^2 and ≥ 27 kg/m^2) and the lower and upper 20% of the distribution of GRS (i.e. ≤ 0.90 and ≥ 1.03). The creation of four corners resulted in women with low GRS low BMI, low GRS high BMI, high GRS low BMI and high GRS high BMI. Finally, because of earlier reported gender differences in responses to food cues, with females showing higher activations than men [13] we excluded all male participants from this final sample.

In total 248 women received an invitation letter and were contact-

ed by telephone to check further eligibility for participation and provide more information when needed (Figure 1). Hundred and thirteen women were unwilling to participate because of lack of time or travel distance being too long. Of the remaining, 15 women with recent body weight change (>5% change in previous 3 months) were excluded. Another 31 women were excluded because of comorbidities or use of weight-affecting medication (Figure 1). Because the subjects also participated in an MRI study, 4 women were excluded because of MRI contra-indications. Thus, a final sample of 60 women was included in this study, in specific: women with low GRS/low BMI (n=16), low GRS/high BMI (n=12), high GRS/low BMI (n=15) and high GRS/high BMI (n=17). The study was approved by the VU University Medical Centre ethics committee and performed in accordance with the Helsinki Declaration. All women provided written informed consent.

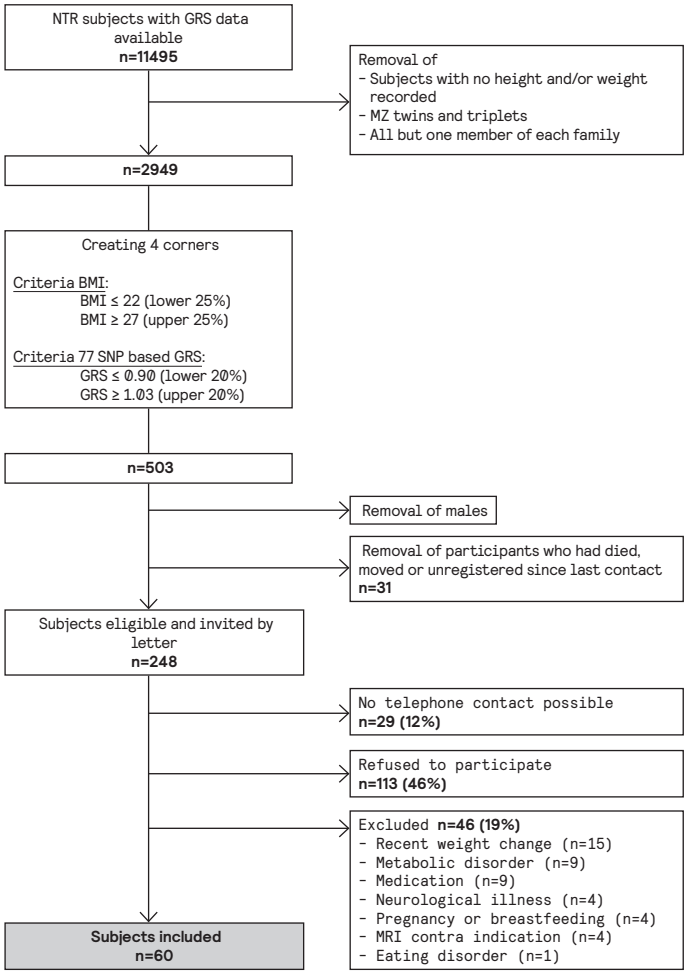

FIGURE 1
Flow chart of the study population. NTR, Netherlands Twin Registry; BMI, body mass index; GRS, genetic risk score; MZ, monozygotic; MRI, magnetic resonance imaging.

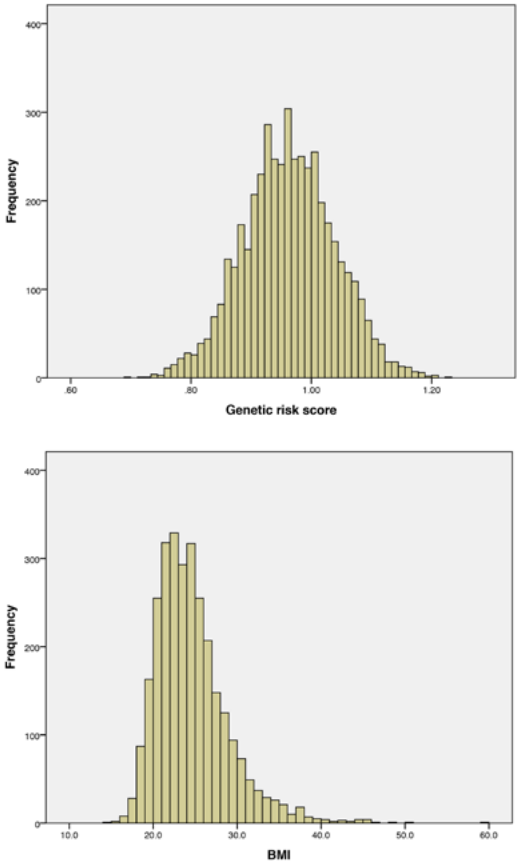

FIGURE 2
Histograms showing the distributions of genetic risk score (A) and BMI (B) of the larger population before the four corners were created.

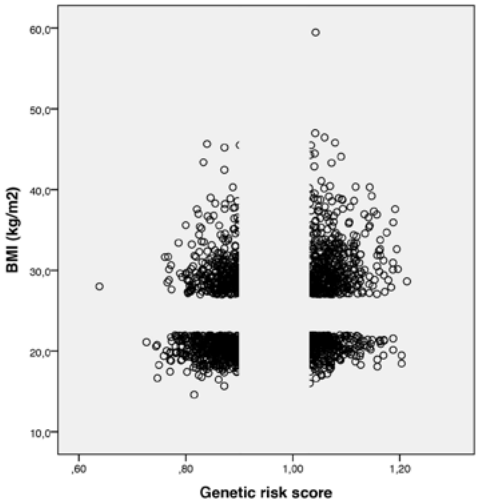

FIGURE 3
Representation of a scatter diagram of women with genetic risk score (GRS) on the horizontal axis and measured BMI on the vertical axis. The creation of four corners is based on the lower and upper 25% of BMI distribution and lower and upper 20% of GRS distribution.

MEASUREMENTS

Clinical and biochemical measurements All measurements were done during a visit in our research clinic and during the month following this visit, as described in more detail previously [14]. Participants arrived between 8 and 10 AM at the clinic after a 12-hour overnight fast. Information on health and socio-demographics was collected by a short oral and standardized interview and using two questionnaires on physical and mental health [15, 16]. Anthropometrics were measured with participants wearing no shoes and light clothes only. Body composition was measured using bio-electrical impedance analysis (Maltron BF-906 body fat analyser, Maltron Ltd, Essex, UK). Blood samples were drawn and analysed in the clinical chemistry laboratory of the VU University Medical Centre for the assessment of glucose and lipid spectrum. We used indirect calorimetry for the assessment of resting energy expenditure, as assessed during 15 minutes while participants were lying at rest with their eyes closed.

Physical activity, dietary intake and eating behaviour Physical activity was measured by 7-day accelerometry using Actigraph GT3X+ devices during all waking hours in the week following the test visit [17]. All participants started wearing the device on a Saturday. Recorded data was analysed using Actilife software (version 6.10.2). Non-wear time was defined and excluded if there were 60 consecutive minutes with zero counts, with allowance of 2 minutes with counts between 0-100 counts/min. The amount of wear time was considered acceptable when there was a minimum of 4 days of at least 10 hours of wear time per day. Existing cut-points were used to define sedentary (<100 counts/minute), light (100-2019 counts/minute), moderate (2020-5998 counts/minute) and vigorous (>5999 counts/minute) intensity activity [18].

Dietary intake was estimated in two ways. First, at the end of the visit participants were offered a test meal of which they could eat as much as they wanted [19]. The meal comprised white and multigrain bread, a mixed green salad, orange juice, Dutch cheese, fresh meats, margarine, mayonnaise, peanut butter, jam, cake, a chocolate muffin, a banana and an apple. Participants were unaware that their food consumption was monitored afterwards. Further, to estimate dietary intake of participants in their natural environment we used 24-hour recalls during unannounced telephone calls on two weekdays and one Sunday in the weeks following the visit [20]. Food items were coded by a skilled nutritionist who was blinded for BMI and GRS of the participant. Intake of total energy (kcal) and macronutrients (E%) during the test meal and recall assessments were analysed using the Dutch Food Composition Database (NEVO) [21].

Eating behaviour was assessed with the Dutch Eating Behaviour Questionnaire (DEBQ), a 33-item standardized and validated tool to measure eating behaviour on the scales emotional (13 items), external (10 items) and restrained eating (10 items) [22]. Answers ranged from

one (never) to five (often). Twelve questions had the extra option 'not applicable', in which case this question was excluded from the analysis. Finally, The Eating Disorder Inventory (EDI) was administered to screen for symptoms of eating disorders [23].

STATISTICAL ANALYSIS

Results of continuous variables with a normal distribution are presented as mean ± SD. Differences in these variables among the four corners were tested using one-way ANOVA and, if the overall ANOVA was $P<0.05$, Tukey's post hoc analyses, similar to previous studies using a four corners design [6,7,24,25]. Not normally distributed data are expressed as median and interquartile ranges, and group differences were tested using the non-parametric Kruskal-Wallis test. Chi-square test was used for analyses of categorical variables. All analyses were conducted using IBM SPSS Statistics (version 20, IBM Corp., 2011, Armonk, NY).

To illustrate the interpretation of the data in the four corners approach [5], the expected patterns are presented in Figure 4. In a first pattern (Figure 4A) participants with high BMI have higher values on the variable of interest than participants with low BMI, irrespective of GRS. In that case, the variable of interest likely reflects a consequence of high BMI rather than being a causal intermediate between the genetic risk for obesity and increased BMI. In a second pattern (Figure 4B) participants with high BMI have higher outcomes than participants with low BMI when GRS is high. When GRS is low, differences are much smaller or absent. This pattern likely reflects a causal pathway where the genetic predisposition to obesity acted on BMI in part through an effect on the variable of interest.

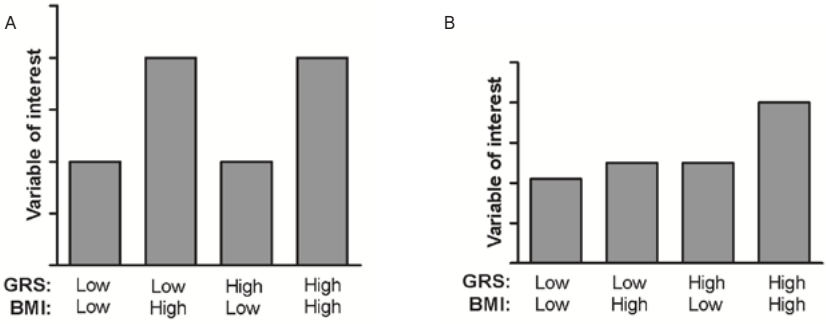

FIGURE 4
Possible patterns of data by the four corners model. Figure A shows the pattern in which participants with high BMI have higher levels of a variable than participants with low BMI, irrespective of GRS. This pattern likely reflects a consequence of high BMI. In figure B participants with high BMI have higher levels of a variable *only* when GRS is high. When GRS is low, the difference between participants with low BMI and participants with high BMI is absent (or, at least, much smaller). This pattern reflects a feature of genetic predisposition to increased BMI.

RESULTS

CLINICAL CHARACTERISTICS

There were no differences between the four groups in social-demographic variables (Table 1). The selection of four corners resulted in expected differences among groups in GRS and BMI (Table 2). Body weight, waist-to-hip ratio and body fat percentage were significantly higher in women with high BMI, irrespective of GRS. Resting energy expenditure was similar among groups after accounting for differences in body mass. In keeping with a causal effect of BMI on these risk factors, women with high BMI had more unfavourable outcomes on blood pressure, HDL cholesterol and total/HDL cholesterol ratio irrespective of GRS, although for blood pressure this difference was not statistically significant.

PHYSICAL ACTIVITY

All but one woman had acceptable wear time duration of the accelerometer and could be included in the analysis. Wear time duration was similar among the four groups (Table 3). Women with high BMI had fewer step counts than women with low BMI, irrespective of GRS. For total activity counts, this pattern was similar (P=0.05). Specification of accelerometer data showed that women with high BMI spent more time in sedentary behaviour (Figure 5A) and less time in light activity as compared to women with low BMI, irrespective of GRS.

DIETARY INTAKE

Dietary intake during the ad libitum test meal was not different between the groups (data not shown). The 24-hour recall assessments showed no statistically significant group differences in intake of total energy or fat (Table 3). However, when GRS was high, women with high BMI had higher intake of protein, in specific animal protein, than women with low BMI (P<0.05) (Figure 5B). When GRS was low, this difference was not present.

EATING BEHAVIOUR

Women with high BMI scored significantly higher on the DEBQ on the items emotional eating and restrained eating, irrespective of GRS (Figure 5C). The EDI revealed that high BMI was associated with a higher drive for thinness, more frequent symptoms of bulimia and more experienced body dissatisfaction (Table 3). These higher scores were also irrespective of GRS.

TABLE 1
Socio-demographic characteristics

	Low GRS Low BMI	Low GRS High BMI	High GRS Low BMI	High GRS High BMI	*P*-value
	n=16	n=12	n=15	n=17	
Food vs. non-food pictures					
Only secondary (%)	2 (12.5)	2 (16.7)	2 (13.3)	2 (11.8)	1.0
Vocational (%)	8 (50)	6 (50)	6 (40)	9 (52.9)	
Higher or academic (%)	6 (12.5)	4 (33.3)	7 (46.7)	6 (35.3)	
Work					
Employed (%)	15 (93.8)	11 (91.7)	14 (93.3)	17 (100)	0.7
Unemployed (%)	1 (6.2)	1 (8.3)	1 (6.7)	0 (0)	
Marital status					
Single (%)	1 (6.2)	1 (8.3)	1 (6.7)	2 (11.8)	0.8
Married (%)	12 (75)	11 (91.7)	13 (86.7)	13 (76.5)	
Divorced or widower (%)	3 (18.8)	0 (0)	1 (6.7)	2 (11.8)	
Smoking status					
Current smoker (%)	4 (25)	1 (8.3)	2 (13.3)	0 (0)	0.4
Previous smoker (%)	4 (25)	5 (41.7)	5 (33.3)	5 (29.4)	
No smoker (%)	8 (50)	6 (50)	8 (53.3)	12 (70.6)	
Menopausal status					
Premenopausal (%)	11 (68.8)	7 (58.3)	11 (73.3)	9 (52.9)	0.4
Postmenopausal (%)	2 (12.5)	2 (16.7)	3 (20)	5 (29.4)	
Unknown (%)	3 (18.8)	3 (25)	1 (6.7)	3 (17.6)	
Symptoms of depression					
CES-D score	5.9 ± 6.2	6.2 ± 4.1	5.0 ± 7.3	5.9 ± 5.5	1.0
CES-D score > 16 (%)	1 (6.3)	0 (0)	2 (13.3)	1 (5.9)	0.6
Health status					
SF-36 Physical Health	56.3 ± 2.8	52.2 ± 9.9	51.7 ± 10.2	53.8 ± 4.6	0.3
SF-36 Mental Health	52.8 ± 7.6	51.8 ± 9.1	53.6 ± 7.7	52.8 ± 4.1	0.9
Medication use					
Oral contraceptives	3 (18.8)	4 (33.3)	1 (6.7)	2 (11.8)	0.3
Antihypertensive medication	1 (6.3)	1 (8.3)	0 (0)	2 (11.8)	0.6
Statins	0 (0)	0 (0)	1 (6.7)	1 (6.7)	0.6
Alcohol use					
Not at all	1 (6.2)	3 (25)	4 (26.7)	5 (29.4)	0.2
Not daily	12 (75)	6 (50)	11 (73.3)	11 (64.7)	
Daily	3 (18.8)	3 (25)	0 (0)	1 (5.9)	

N (%) or mean ± SD; GRS, genetic risk score; CES-D, Centre for Epidemiologic Studies-Depression scale; SF-36, Short Form 36-item Health Survey

TABLE 2
Clinical and biochemical characteristics

	Low GRS Low BMI (1)	Low GRS High BMI (2)	High GRS Low BMI (3)	High GRS High BMI (4)	*P*-value
	n=16	n=12	n=15	n=17	
Age (y)	44.8 ± 6.3	47.0 ± 7.3	44.3 ± 7.4	47.1 ± 6.9	0.6
GRS	0.87 ± 0.05	0.87 ± 0.05	1.06 ± 0.05	1.05 ± 0.06	<0.001*
Weight (kg)	61.2 ± 6.6	89.7 ± 10.9	60.5 ± 4.8	92.7 ± 13.2	<0.001†
BMI (kg/m²)	20.9 ± 0.9	31.6 ± 3.7	20.4 ± 1.3	32.8 ± 4.8	<0.001†
Waist circumference (cm)	73.9 ± 4.7	97.3 ± 8.6	73.0 ± 3.0	102.8 ± 14.0	<0.001†
Waist-hip ratio	0.78 ± 0.04	0.87 ± 0.05	0.78 ± 0.03	0.87 ± 0.06	<0.001†
Body fat (%)	27.8 ± 3.1	42.7 ± 2.9	27.1 ± 3.4	44.6 ± 4.7	<0.001†
Systolic RR (mmHg)	116.3 ± 14.7	129.1 ± 16.9	116.0 ± 12.1	123.2 ± 17.7	0.1
REE (kcal/day)	1466.8 ± 217.8	1626.2 ± 193.7	1445.7 ± 129.8	1687.0 ± 256.0	0.004‡
REE/LBM (kcal/kg)	33.3 ± 3.8	29.9 ± 4.9	32.9 ± 3.0	32.8 ± 5.9	0.2
Glucose (mmol/L)	4.8 ± 0.4	4.8 ± 0.5	5.0 ± 0.4	5.1 ± 0.6	0.1
Total chol (mmol/L)	4.9 ± 0.8	5.2 ± 0.8	4.9 ± 1.2	5.2 ± 1.3	0.9
HDL chol (mmol/L)	1.9 ± 0.3	1.4 ± 0.5	1.9 ± 0.3	1.5 ± 0.6	0.008§
LDL chol (mmol/L)	2.8 ± 0.6	3.5 ± 0.9	2.7 ± 1.0	3.1 ± 1.0	0.1
Ratio total / HDL chol	2.8 ± 0.8	4.0 ± 1.0	2.6 ± 0.9	4.0 ± 1.9	0.003‖
Triglycerides (mmol/L)	1.0 ± 0.5	1.1 ± 0.5	0.9 ± 0.6	1.2 ± 0.6	0.4

Mean ± SD; GRS, genetic risk score; REE, resting energy expenditure; REE/LBM, resting energy expenditure divided by lean body mass; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*$P<0.001$ for comparing corner 3>1; 4>1; 3>2; 4>2; †$P<0.001$ for comparing corner 2>1; 2>3; 4>1; 4>3; ‡$P<0.05$ for comparing corner 4>1; 4>3; §$P<0.05$ for comparing corner 3>2; ‖$P<0.05$ for comparing corner 2>3; 4>1; 4>3.

TABLE 3
Physical activity, dietary intake and eating behaviour

	Low GRS Low BMI (1)	Low GRS High BMI (2)	High GRS Low BMI (3)	High GRS High BMI (4)	*P*-value
Physical activity					
Wear time (h/day)	14.7 ± 1.3	14.8 ± 1.1	14.4 ± 1.2	14.9 ± 0.8	0.6
Activity counts (x 1000/day)	561.8 ± 99.3	437.4 ± 128.6	592.1 ± 165.0	530.0 ± 158.9	0.05
Steps (steps/day)	7327.3 ± 1194.2	5935.0 ± 2153.9	8719.3 ± 2318.3	7448.8 ± 2471.8	<0.05*
Sedentary (min/day)	636.0 ± 87.7	682.3 ± 44.2	600.7 ± 67.5	653.7 ± 52.8	<0.05*
Light activity (min/day)	209.7 ± 38.3	176.6 ± 40.4	221.9 ± 48.9	201.2 ± 39.7	0.06
MVPA (min/day)	36.9 ± 12.4	28.8 ± 13.0	40.5 ± 15.9	36.6 ± 20.0	0.3
Sedentary (%)	71.9 ± 5.1	77.0 ± 4.6	69.6 ± 5.8	73.4 ± 5.0	<0.01*
Light activity (%)	23.9 ± 4.6	19.8 ± 3.6	25.7 ± 5.0	22.6 ± 4.2	0.01*
MVPA activity (%)	4.3 ± 1.7	3.2 ± 1.3	4.7 ± 1.6	4.1 ± 2.1	0.2
Dietary intake					
Energy (kcal)	2067 ± 529	1762 ± 447	1837 ± 484	1830 ± 438	0.3
Carbohydrates (E%)	44.6 ± 6.3	46.2 ± 6.8	44.2 ± 7.0	43.2 ± 6.0	0.7
Protein (E%)	14.4 ± 3.2	15.7 ± 2.9	15.4 ± 2.1	18.2 ± 3.4	<0.01†
Vegetable protein (E%)	6.0 ± 1.6	6.0 ± 1.0	6.7 ± 1.6	6.3 1.2	0.4
Animal protein (E%)	8.4 ± 3.0	9.8 ± 2.8	8.6 ± 3.0	11.9 ± 3.6	<0.01†
Total fat (E%)	36.7 ± 5.0	33.3 ± 5.7	36.0 ± 5.7	34.5 ± 6.3	0.4
Saturated fat (E%)	13.8 ± 3.1	13.0 ± 2.9	13.6 ± 3.6	13.0 ± 3.1	0.9
Monounsaturated fat (E%)	12.9 ± 2.5	11.2 ± 2.0	12.3 ± 2.0	11.7 ± 2.8	0.3
Polyunsaturated fat (E%)	6.5 ± 1.5	5.9 ± 1.4	6.9 ± 2.0	6.4 ± 2.1	0.6
Eating Behaviour					
Emotional eating	1.9 ± 0.7	2.6 ± 0.5	1.8 ± 0.5	2.4 ± 0.8	<0.01‡
External eating	2.9 ± 0.6	2.9 ± 0.3	2.8 ± 0.4	3.1 ± 0.7	0.6
Restrained eating	1.8 ± 0.9	3.1 ± 1.0	2.3 ± 0.9	3.2 ± 0.8	<0.001§
Symptoms of Eating Disorder					
Drive for Thinness	11.9 ± 2.9	21.2 ± 7.8	12.5 ± 4.1	20.9 ± 5.3	<0.001‖
Bulimia	8.9 ± 2.2	11.8 ± 3.1	8.1 ± 1.2	12.4 ± 4.0	<0.001#
Body Dissatisfaction	19.9 ± 7.0	35.3 ± 12.0	20.4 ± 6.8	38.6 ± 8.7	<0.001‖

Mean ± SD; GRS, genetic risk score; MVPA, moderate to vigorous physical activity; E%, percentage of total energy intake. *P<0.05 for comparing corner 3>2; †P<0.05 for comparing corner 4>1; 4>3; ‡P<0.05 for comparing corner 2>3; 4>3; §P<0.05 for comparing corner 2>1; 4>1: 4>3; ‖P<0.001 for comparing corner 2>1; 4>1; 2>3; 4>3; #P<0.01 for comparing corner 4>1; 2>3; 4>3.

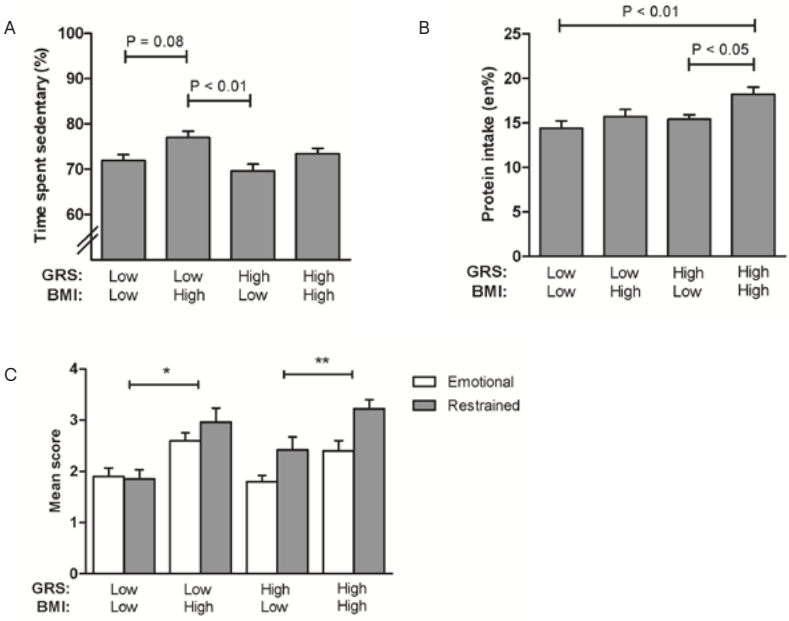

FIGURE 5
Differences between the women from the four corners in time spent in sedentary behaviour (A), intake of protein (B) and emotional and restrained eating scores on the Dutch Eating Behaviour Questionnaire (C). Data represent means ± standard error of mean. E%, percentage of total energy intake. *P<0.01 for restrained eating. **P<0.05 for emotional and restrained eating.

DISCUSSION

We used a special, previously established four corners study design in an attempt to investigate whether deviant physical activity, sedentary behaviour, dietary intake and eating behaviour exert a causal effect on BMI or merely reflect a consequence of increased BMI itself [5-7]. We improved the original four corners design by selecting participants not on parental characteristics but on their observed genetic risk score (GRS), thus preventing confounding by shared environmental factors inherent in the original parent-offspring four corners model.

Our accelerometer data showed that women with higher BMI had lower overall step counts than women with low BMI, irrespective of GRS for obesity. Specification of the data revealed that this difference was explained by an unfavourable imbalance of increased sedentary behaviour and decreased light intensity physical activity, rather than lower engagement in moderate to vigorous physical activity (MVPA), since differences in the latter were not observed. Our finding that this imbalance between sedentary behaviour and light intensity physical activity was observed regardless of GRS for obesity, suggests that these deviant behaviours have no causal contribution to the genetic predisposition to obesity but, rather, reflect a consequence of increased BMI itself. This clue for a reverse causal relation is in line with a recent study using Mendelian randomization to demonstrate that adiposity causes an increase in sedentary behaviour in children [26]. However, neither study can rule out the possibility of bidirectional causality, i.e. a vicious circle of increased physical inactivity due to obesity that in turn further advances adiposity by negatively impacting on the energy balance.

The results of our study provide support for an important role of environmental factors in kick-starting this vicious circle. In our design, women in the low GRS/high BMI corner are selected to be at an above-average environmental risk for high BMI. This environmental risk is clearly transferred to their daily engagements in light intensity physical activity and sedentary behaviour. This is in accordance with results from twin studies in which we [14] and others [27, 28] demonstrated that lower physical activity levels in overweight individuals are explained by adverse environmental factors, although these observations mostly concerned changes in (moderate to vigorous) physical activity rather than sedentary behaviour. Together, these studies suggest that a small change in weight, induced by environmental factors, might subsequently initiate a cycle of physical inactivity resulting in further weight gain that further increases physical inactivity, and so on.

During the test meal and 24-hour recall assessments, we did not observe differences in intake of total energy or fat between the groups. Since underreporting is a well-known issue in self-reported dietary surveys, especially in women with higher BMI [29], we cannot exclude the possibility that true differences in these variables remained undetected in our study. However, using 24-hour dietary recalls, we did observe a

higher intake of protein, in specific animal protein, in women with high BMI, *only* if GRS for obesity was high. This pattern would not be expected if increased animal protein intake was secondary to high BMI but, instead, suggests that the intake of animal protein contributes to the genetic predisposition to obesity. This conflicts with existing practices of high-protein diets being used as a tool to lose body weight after obesity has already developed [30]. However, this beneficial effect of protein consumption has been debated, especially when the protein sources are considered [31, 32]. Whereas vegetable proteins have been suggested to lower risk of obesity, the intake of animal protein appears to have a deleterious effect on BMI [31, 33]. Possible mechanisms are higher levels of insulin and insulin-like growth factor 1 (IGF-1), and a concomitant higher intake of fat and total calories, inherent to the consumption of meat products [34].

Since the GRS we used in our study is based on SNPs of multiple genes, it is uncertain which specific gene is responsible for the higher protein intake we observed in high BMI women. Genes suggested to influence appetite and food intake are those involved in leptin-melanocortin signalling and include *POMC, MC4R, BDNF* and *FTO* [35], although *FTO* has also been suggested to function beyond the regulation of food intake [36]. More studies are needed to investigate which genes are responsible for specific preferences in macronutrient intake.

The higher emotional and restraint eating seen in individuals with high BMI are consistent with previous literature [37]. Emotional eating refers to eating in response to emotional states such as sadness or anxiety, whereas restrained eating refers to a tendency to cognitively restrict food intake in order to avoid weight gain. Paradoxically, dietary restraint could cause weight gain through periods of disinhibition and bingeing, and often coincides with emotional eating [38]. The pattern of our data, i.e. higher emotional and restraint eating in women with high BMI, *irrespective* of GRS, suggests that abnormal eating behaviour occurs *as a result* of increased BMI, rather than being a genetically etiological component of it. This finding is in contrast to a previous study which observed an association between a 32 SNP based GRS for obesity and emotional eating, thereby suggesting a mediating link between genetic variation and obesity [39]. However, this study , in contrast to our current study, could not rule out the influence of confounding by BMI itself.

A weakness of our study was a relatively modest sample size, which may have caused our study to have insufficient statistical power to detect small differences in e.g. MVPA and emotional eating between individuals with low GRS and individuals with high GRS. Also, the upper BMI cut-off point of ≥ 27 kg/m^2 resulted in the inclusion of overweight and not just obese individuals, which might have affected the sensitivity to detect differences between the groups with low BMI and high BMI. For these reasons we used a lenient nominal alpha of 0.05 throughout. Unfortunately, this does not just increase power but also

increases the risk of type 1 errors. Our findings, therefore, must be considered exploratory and clearly need confirmation in independent studies. On the other hand, the power of our study should be evaluated with respect to the study design, i.e. a unique selection of individuals with extreme genotypes and phenotypes from a cohort of >11 thousand individuals. Using a GRS rather than parental history information to index genetic predisposition enabled us to remove confounding by shared environmental factors from the original four corner design. Further, relative to previous studies using risk scores based on single SNPs or smaller amounts of genetic variants, we enhanced power to detect GRS-related effects by using a weighted GRS based on 77 recently detected obesity loci [9]. Nevertheless, future research may benefit from larger sample sizes and risk scores comprising more obesity-related SNPs, to optimize power of detecting genetic effects on behavioural traits underlying obesity.

Although we cannot exclude the possibility that our data are biased due to underreporting or under-eating for the duration of data collection [29], misreporting is an obstacle in all nutritional surveys and the 24-hour recall method is not more susceptible to this issue than other methods. Rather, the 24-hour recall method has shown to be valid in estimating the 'usual diet' while providing a low participant burden [40]. A final limitation is that our study sample was restricted to women, which makes it difficult to generalize our findings to the male population.

In summary, using a special four corners approach, we suggested that higher levels of sedentary behaviour and emotional and restrained eating likely occur after the development of overweight, which could favour the initiation of a vicious circle of more weight gain and physical inactivity and abnormal eating behaviour. In contrast, a higher intake of (animal) protein might be a causal component of the genetic predisposition to obesity. This study provides ground to call for further interventional studies characterizing the effects of (animal) protein intake on BMI.

SUPPLEMENTARY TABLE 1
SNPs included in the polygenic risk score

Chr	Nearest gene	SNP name	Alleles Effect / Other	Effect allele frequency	GWAS β	Variance explained
16	*FTO*	rs1558902	A/T	0.415	0.082	0.325%
18	*MC4R*	rs6567160	C/T	0.236	0.056	0.111%
2	*TMEM18*	rs13021737	G/A	0.828	0.060	0.103%
4	*GNPDA2*	rs10938397	G/A	0.434	0.040	0.079%
1	*SEC16B*	rs543874	G/A	0.193	0.048	0.072%
6	*TFAP2B*	rs2207139	G/A	0.177	0.045	0.058%
11	*BDNF*	rs11030104	A/G	0.792	0.041	0.056%
1	*NEGR1*	rs3101336	C/T	0.613	0.033	0.053%
12	*BCDIN3D*	rs7138803	A/G	0.384	0.032	0.047%
2	*ADCY3*	rs10182181	G/A	0.462	0.031	0.047%
16	*ATP2A1*	rs3888190	A/C	0.403	0.031	0.046%
3	*ETV5*	rs1516725	C/T	0.872	0.045	0.045%
19	*QPCTL*	rs2287019	C/T	0.804	0.036	0.041%
16	*GPRC5B*	rs12446632	G/A	0.865	0.040	0.038%
19	*ZC3H4*	rs3810291	A/G	0.666	0.028	0.036%
11	*MTCH2*	rs3817334	T/C	0.407	0.026	0.033%
1	*GNAT2*	rs17024393	C/T	0.040	0.066	0.033%
15	*MAP2K5*	rs16951275	T/C	0.784	0.031	0.033%
5	*POC5*	rs2112347	T/G	0.629	0.026	0.032%
4	*SLC39A8*	rs13107325	T/C	0.072	0.048	0.030%
7	*PMS2L11*	rs2245368	C/T	0.180	0.032	0.030%
1	*FPGT-TNNI3K*	rs12566985	G/A	0.446	0.024	0.029%
13	*MTIF3*	rs12016871	T/C	0.203	0.030	0.029%
3	*RASA2*	rs16851483	T/G	0.066	0.048	0.029%
3	*CADM2*	rs13078960	G/T	0.196	0.030	0.028%
14	*NRXN3*	rs7141420	T/C	0.527	0.024	0.028%
9	*LINGO2*	rs10968576	G/A	0.320	0.025	0.027%
13	*OLFM4*	rs12429545	A/G	0.133	0.033	0.026%
1	*AGBL4*	rs657452	A/G	0.394	0.023	0.025%
4	*SCARB2*	rs17001654	G/C	0.153	0.031	0.024%
11	*CADM1*	rs12286929	G/A	0.523	0.022	0.023%
1	*PTBP2*	rs11165643	T/C	0.583	0.022	0.023%
14	*STXBP6*	rs10132280	C/A	0.682	0.023	0.023%
10	*TCF7L2*	rs7903146	C/T	0.713	0.023	0.022%
2	*FLJ30838*	rs1016287	T/C	0.287	0.023	0.021%
8	*HNF4G*	rs17405819	T/C	0.700	0.022	0.021%
4	*HHIP*	rs11727676	T/C	0.910	0.036	0.021%
10	*HIF1AN*	rs17094222	C/T	0.211	0.025	0.021%
1	*FUBP1*	rs12401738	A/G	0.352	0.021	0.020%
7	*HIP1*	rs1167827	G/A	0.553	0.020	0.020%
11	*TRIM66*	rs4256980	G/C	0.646	0.021	0.020%
16	*NLRC3*	rs758747	T/C	0.265	0.023	0.020%
14	*PRKD1*	rs12885454	C/A	0.642	0.021	0.020%
14	*PRKD1*	rs11847697	T/C	0.042	0.049	0.019%
3	*FHIT*	rs2365389	C/T	0.582	0.020	0.019%
6	*C6orf106*	rs205262	G/A	0.273	0.022	0.019%
2	*ERBB4*	rs7599312	G/A	0.724	0.022	0.019%
1	*NAV1*	rs2820292	C/A	0.555	0.020	0.019%
16	*SBK1*	rs2650492	A/G	0.303	0.021	0.018%

9	*TLR4*	rs1928295	T/C	0.548	0.019	0.018%
2	*KCNK3*	rs11126666	A/G	0.283	0.021	0.017%
16	*KAT8*	rs9925964	A/G	0.620	0.019	0.017%
19	*TOMM40*	rs2075650	A/G	0.848	0.026	0.017%
12	*CLIP1*	rs11057405	G/A	0.901	0.031	0.017%
3	*RARB*	rs6804842	G/A	0.575	0.019	0.017%
6	*PARK2*	rs13191362	A/G	0.879	0.028	0.016%
3	*GBE1*	rs3849570	A/C	0.359	0.019	0.016%
17	*RPTOR*	rs12940622	G/A	0.575	0.018	0.016%
17	*RABEP1*	rs1000940	G/A	0.320	0.019	0.016%
9	*C9orf93*	rs4740619	T/C	0.542	0.018	0.016%
2	*LRP1B*	rs2121279	T/C	0.152	0.025	0.015%
10	*NT5C2*	rs11191560	C/T	0.089	0.031	0.015%
15	*DMXL2*	rs3736485	A/G	0.454	0.018	0.015%
10	*GRID1*	rs7899106	G/A	0.052	0.040	0.015%
6	*FOXO3*	rs9400239	C/T	0.688	0.019	0.015%
9	*LMX1B*	rs10733682	A/G	0.478	0.017	0.015%
1	*ELAVL4*	rs11583200	C/T	0.396	0.018	0.015%
6	*TDRG1*	rs2033529	G/A	0.293	0.019	0.015%
2	*EHBP1*	rs11688816	G/A	0.525	0.017	0.015%
2	*UBE2E3*	rs1528435	T/C	0.631	0.018	0.015%
11	*HSD17B12*	rs2176598	T/C	0.251	0.020	0.015%
19	*KCTD15*	rs29941	G/A	0.669	0.018	0.015%
18	*GRP*	rs7243357	T/G	0.812	0.022	0.014%
19	*PGPEP1*	rs17724992	A/G	0.746	0.019	0.014%
9	*EPB41L4B*	rs6477694	C/T	0.365	0.017	0.014%
8	*RALYL*	rs2033732	C/T	0.747	0.019	0.014%
18	*C18orf8*	rs1808579	C/T	0.534	0.017	0.014%

Chr, chromosome; SNP, single nucleotide polymorphism. Alleles, effect allele frequency, effect size and explained variance are values from meta-analysis in European males and females by Locke et al.

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