Obesity and Food Reward Regulation by the Brain

Doornweerd, S.

2018

document version
Publisher's PDF, also known as Version of record

Environmental factors and food intake regulation

PART 2

Physical activity and dietary intake in BMI discordant identical twins

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Obesity (Silver Spring). 2016 Jun;24(6):1349-55

ABSTRACT

OBJECTIVE

Despite the latest discovery of obesity associated genes, the rapid rise in global obesity suggests a major role for environmental factors. We investigated the influence of environmental factors on physical activity and dietary intake independent of genetic effects.

METHODS

We studied sixteen female monozygotic twins aged 48.8 ± 9.8 years (range 37-70) with a mean BMI discordance of 3.96 ± 2.1 kg/m^2 (range 0.7-8.2). We determined physical activity using 7-day accelerometry, and dietary intake using 3-day 24-hour recalls.

RESULTS

Heavier co-twins were generally less physically active (mean activity counts x 1000 per day ± SD; 505.5 ± 155.1 vs. 579.6 ± 185.4, P=0.047) and tended to spend 6.1 min/day less in moderate to vigorous physical activity than leaner co-twins (P=0.09). Energy intake did not significantly differ within pairs. Total fat intake (E%; P=0.03), in specific monounsaturated fat (P<0.01) and polyunsaturated fat (P=0.08), was higher in the heavier co-twins.

CONCLUSIONS

After eliminating genetic effects, higher BMI is associated with lower overall and moderate to vigorous physical activity and higher intake of total fat, although the direction of causality cannot be determined. Future identification of the environmental factors responsible for these findings might contribute to developing new strategies in managing obesity.

INTRODUCTION

The global rise in overweight and obesity is a major health concern because of the increased risk for chronic diseases like type 2 diabetes, cardiovascular disease (CVD) and cancer [1]. Causes of the obesity epidemic are suggested to be of both environmental and genetic origin. Currently, genes are being identified that predispose to the development of obesity [2]. However, since genetic variation has not changed substantially in the past 30 years, genes alone cannot explain the recent increase in obesity rates, suggesting a major role for a changing environment.

At the individual level, body weight increases if energy intake exceeds energy expenditure. Although dietary intake and physical activity are known as lifestyle factors, exposure to these factors has shown to be under genetic and environmental control. Studies show that an individual's genotype influences the level of exposure to a certain lifestyle factor (gene-environment correlation) [3-5], but also the way an individual responds to this lifestyle factor in terms of body weight gain (gene-environment interaction) [6,7].

In addition to these genetic effects, lifestyle behaviours are also influenced by environmental factors. Identifying environmental factors that influence lifestyle and are amenable for intervention offers a possibility to develop strategies for the prevention and treatment of obesity. To disentangle the effects of the environment from the effects of genes and reduce the impact of gene-environment interaction and gene-environment correlation, we eliminated the influence of genetic factors by using the special design of 'clonal controls', rare monozygotic twins discordant for body mass index (BMI) [8,9]. Monozygotic twins are identical in their genomic sequence, therefore differences in BMI between co-twins must arise from differences in individual-specific environmental factors. Our aim is to investigate whether lifestyle factors such as physical activity, sedentary behaviour and dietary intake are associated with BMI when genetic factors are eliminated.

METHODS

SUBJECTS

All participants are registered with the Netherlands Twin Register (NTR) [10], which comprises twins and their family members who took part in longitudinal survey studies between 1991-2009 and/or in the NTR biobank project between 2004-2008 [11,12]. BMI data were available for 2775 monozygotic twin pairs [13]. The selection of twins is shown in Figure 1. Twin pairs were selected for the current study if measured BMI difference was ≥ 3 kg/m^2 between co-twins at the biobank project. If subjects also had available survey data, twin pairs were selected if BMI difference ≥ 3 kg/m^2 at ≥ 1 survey (most recent), if BMI difference ≥

2 kg/m² at ≥ 3 surveys, or if BMI difference ≥ 2 kg/m² at the most recent survey. Only female pairs were included for homogeneity purposes.

Fifty-four pairs met the first selection criteria, were invited by letter and contacted by telephone to check further eligibility. Fourteen pairs (26%) were unwilling to participate mostly because of lack of time. Twenty-one pairs (39%) were excluded because of pregnancy (n=2), history of eating disorder (n=4), presence of diabetes mellitus (n=1), serious heart disease (n=1), neurological illness (n=3) or reported BMI difference < 2 kg/m² due to recent weight change (n=7). Because the subjects also participated in an MRI study, another 3 twin pairs were excluded because of MRI contra-indications. Finally, 2 pairs could not be contacted due to loss of follow-up. Thus, 16 female, weight-stable (< 5% weight change in previous 3 months) monozygotic pairs (31%) were included in this study. Zygosity of the twins was determined as described previously [12]. One pair was part of a monozygotic triplet. All twins lived apart from their co-twin, except for one pair that had lived in the same household since birth. The study was approved by the ethics committee of the VU University Medical Centre and was performed in accordance with the Helsinki Declaration. All subjects provided written informed consent.

CLINICAL AND BIOCHEMICAL ASSESSMENTS

All measurements were done by an experienced research physician and dietician during a 5-hour test visit in our research clinic and during the month following this visit. Subjects arrived after a12-hour overnight fast. Information on socio-demographics and health status was collected by a short oral and standardized interview. Weight, height and waist and hip circumferences were measured without shoes and wearing light clothing only. For the assessment of body composition, bio-electrical impedance analysis (Maltron BF-906 body fat analyser, Maltron Ltd, Essex, UK) was used. Blood pressure was measured in supine position (Dinamap Pro 100, GE Medical Systems Information Technologies, Inc., Milwaukee, Wisconsin). Heart rate was measured using a 3-lead electrocardiogram (VU University ambulatory monitoring system, VU-AMS) [14]. Venous blood samples were drawn for the assessment of glucose, HbA1c, total cholesterol, high-density lipoprotein cholesterol and triglycerides. Low-density lipoprotein cholesterol was calculated from the Friedewald formula. All biochemical assessments were done at the clinical chemistry laboratory of the VU University Medical Centre. Indirect calorimetry was used to estimate resting energy expenditure, while participants remained in supine position with eyes closed for 15 minutes (Vmax Encore n29; Viasys Healthcare, Houten, Netherlands). Participants completed the 36-item Short Form Health Survey to estimate overall mental and physical health status [15] and the Centre for Epidemiologic Studies-Depression (CES-D) questionnaire to screen for depressive symptoms. A CES-D score of 16 or greater identified subjects at risk for clinical depression [16].

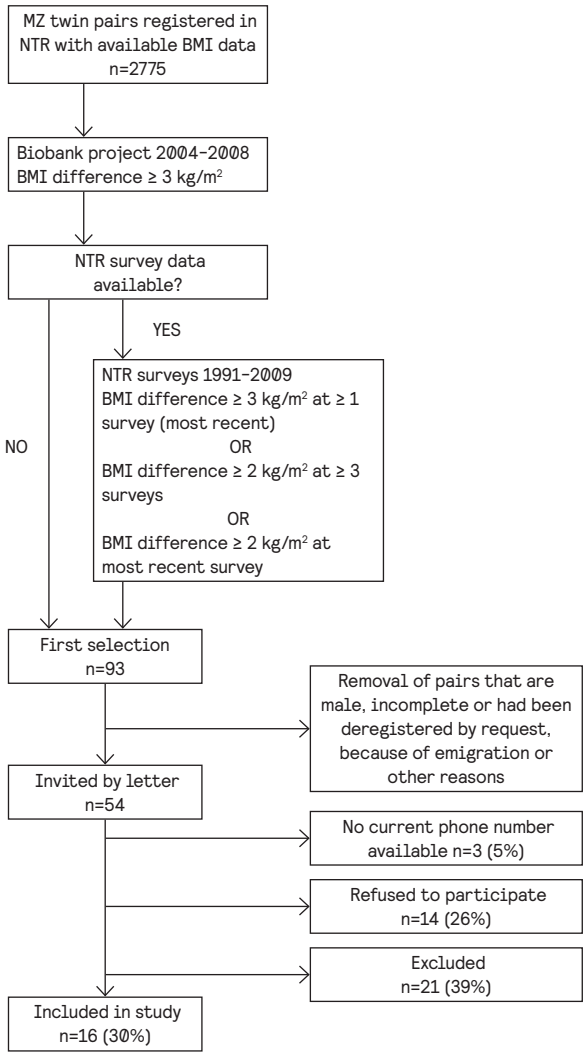

FIGURE 1
Flow chart of the study population. All numbers in the figure represent numbers of monozygotic twin pairs.

PHYSICAL ACTIVITY

Physical activity was measured using two methods.

Accelerometry Subjects received an Actigraph GT3X+ accelerometer (Actigraph LLC, Pensacola, FL, US) [17], and wore the accelerometer attached to an elastic belt on the right hip for all waking hours during a 7-day period, except during water-based activities. Every participant started wearing the device on a Saturday. Recorded data were 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. 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].

Questionnaire Following the 7-day wear period for the accelerometer, participants completed the short version of the International Physical Activity Questionnaire (IPAQ-SF) [19]. The IPAQ-SF assesses three types of physical activity over the previous week, including vigorous activity, moderate activity and walking. According to the IPAQ-SF scoring manual [19] outliers (i.e. cases in which the sum of walking, moderate and vigorous was greater than 960 min) were excluded. Also, each intensity domain (walking, moderate, vigorous) exceeding 180 min per day was truncated at a duration of 180 min per day. Total physical activity was calculated by multiplying time spent in each intensity domain by their estimated intensity in METs. One MET represents the energy expended while sitting quietly at rest. The MET intensities used were vigorous (8 METs), moderate (4 METs) and walking (3.3 METs).

DIETARY INTAKE

Dietary intake data was collected through 24-hour recalls on 2 weekdays and 1 Sunday by unannounced telephone calls during the month following the test visit [20], using the validated Unites States Department of Agriculture (USDA) five-step multiple-pass method [21]. A food portion size photo book, a table scale type KERN FCE 6 K2 ® and extensive tableware were used for portion-size estimation. All recalls were conducted by one research physician who was instructed by an experienced nutritionist. Food items were coded with the corresponding NEVO-code (Dutch Food Composition Table) [22] by a dietician blinded to BMI status of the subjects. Portion sizes were entered in gram weights. Food consumption and nutrient intake were determined using the NEVO database [22] and included mean intake of total energy (kcal); total fat, saturated fatty acids, mono- and polyunsaturated fatty acids, protein, carbohydrate, alcohol (E%) and dietary fibre (g/1000 kcal). We also determined mean intake of micronutrients calcium, iron, vitamin A, folate, vitamin B12, C and D.

STATISTICAL ANALYSIS

All data preparation and analysis were conducted using IBM SPSS Statistics for Windows (version 20, IBM Corp., 2011, Armonk, NY). Results are expressed as mean ± SD for data with a normal distribution. Differences between the leaner and heavier co-twins were tested with paired t-tests for continuous variables [23], McNemar tests for dichotomous variables and Wilcoxon signed-ranks tests for ordinal data. Since IPAQ-SF data are non-normally distributed results are expressed as median and interquartile ranges [19], and differences were tested with Wilcoxon signed-ranks test. In the total group of twins (n=32) linear

regression analysis was used to examine whether BMI and body fatness were associated with physical activity and dietary intake. To account for non-independence of family members, these analyses were done in Stata 13, including family ID as a cluster variable.

RESULTS

CLINICAL CHARACTERISTICS

Clinical and biochemical characteristics of the leaner and heavier co-twins are presented in Table 1. Selected twins had a mean age of 48.8 ± 9.8 years (range 37-70). As a result of the selection criteria, co-twins differed significantly in weight, BMI, waist-hip ratio and body fat percentage. Mean BMI difference was 3.96 kg/m^2 (range 0.7-8.2) during the test visit.

Resting energy expenditure was higher in the heavier than in the leaner co-twins. However, relative to lean body mass, resting energy expenditure was similar. Without exception the cardiovascular and metabolic risk factors were less favourable in the heavier than in the leaner co-twins, but, except for HDL-cholesterol and total/HDL-cholesterol ratio, these differences were not statistically significant. There were no differences between the co-twins in socio-demographic variables, including smoking, marital status, menopausal status, general physical and mental health and symptoms of depression (Table 2).

TABLE 1
Clinical and biochemical characteristics of leaner and heavier co-twins

	Leaner co-twins (n=16)	**Heavier co-twins (n=16)**	***P*-value**
Age (y)	49.8 ± 9.8	49.8 ± 9.8	-
Height (m)	1.68 ± 0.04	1.68 ± 0.05	0.6
Weight (kg)	68.9 ± 9.2	80.5 ± 11.0	< 0.001
BMI (kg/m²)	24.4 ± 3.1	28.4 ± 3.5	< 0.001
Waist-hip ratio	0.80 ± 0.1	0.84 ± 0.1	0.02
Body fat (%)	32.0 ± 6.1	37.8 ± 6.1	< 0.001
Systolic RR (mmHg) supine	119.4 ± 21.5	126.5 ± 20.6	0.09
Diastolic RR (mmHg) supine	66.6 ± 12.3	70.3 ± 6.1	0.3
Heart rate at rest (bpm)	59.7 ± 7.3	62.7 ± 8.2	0.3
REE (kcal/day)	1564.1 ± 144.3	1701.9 ± 236.3	0.01
REE/LBM (kcal/kg)	33.8 ± 2.8	34.3 ± 2.8	0.5
Glucose (mmol/L)	4.7 ± 0.3	4.8 ± 0.3	0.5
HbA1c (mmol/mol)	36.3 ± 2.6	36.7 ± 2.6	0.3
Total cholesterol (mmol/L)	5.2 ± 1.1	5.3 ± 1.2	0.8
HDL cholesterol (mmol/L)	2.0 ± 0.4	1.7 ± 0.4	0.05
LDL cholesterol (mmol/L)	2.9 ± 1.0	3.2 ± 1.2	0.3
Ratio total / HDL cholesterol	2.7 ± 0.6	3.2 ± 1.0	0.01
Triglycerides (mmol/L)	0.8 ± 0.2	0.9 ± 0.3	0.1

Mean ± SD; REE, resting energy expenditure; REE/LBM, resting energy expenditure divided by lean body mass; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein

TABLE 2
Socio-demographic characteristics of leaner and heavier co-twins

	Leaner co-twins (n=16)	Heavier co-twins (n=16)	*P*-value
Education			0.3
Only secondary (%)	5 (31.2)	5 (31.2)	
Vocational (%)	7 (43.8)	10 (62.5)	
Higher or academic (%)	4 (25.0)	1 (6.2)	
Work			0.3
Employed (%)	14 (87.5)	12 (75.0)	
Unemployed (%)	2 (12.5)	2 (12.5)	
Retired (%)	0 (0)	2 (12.5)	
Marital status			0.3
Unmarried (%)	4 (25.0)	1 (6.2)	
Married (%)	11 (68.8)	14 (87.5)	
Divorced or widower (%)	1 (6.2)	1 (6.2)	
Smoking status			0.3
Current smoker (%)	4 (25)	3 (18.8)	
Non-smoker (%)	12 (75)	13 (81.2)	
Menopausal status			0.7
Premenopausal (%)	7 (43.8)	6 (37.5)	
Postmenopausal (%)	6 (37.5)	5 (31.2)	
Unknown (%)	3 (18.8)	5 (31.2)	
Symptoms of depression			
CES-D score mean ± SD	7.1 ± 7.7	6.4 ± 7.1	0.8
CES-D score > 16 (%)	2 (12.5)	3 (18.8)	0.5
Health status			
SF-36 Physical Health	50.7 ± 8.8	51.7 ± 7.2	0.6
SF-36 Mental Health	52.6 ± 6.1	54.7 ± 7.2	0.3

N (%); Mean ± SD; CES-D, Centre for Epidemiologic Studies-Depression; SF-36, Short Form 36-item Health Survey

PHYSICAL ACTIVITY

Median duration of accelerometer monitoring was 7 days with mean duration of 14.9 hours (SD ± 0.9) per day. All participants had acceptable wear time duration and no differences existed in wear time between leaner and heavier co-twins (15.0 ± 0.89 vs. 14.7 ± 0.9; P=0.2). Heavier co-twins had 74100 lower overall activity counts (P<0.05) and 957 fewer step counts (P=0.05) per day than their leaner co-twins (Table 3). Linear regression analyses in the total group of twins (n=32) showed that activity counts correlated negatively with BMI (r=-0.2, P=0.07), fat percentage (r=-0.3, P=0.03) and fat mass (r=-0.3, P=0.03). Step counts correlated negatively with BMI (r=-0.3, P=0.055), fat percentage (r=-0.4, P<0.05) and fat mass (r=-0.4, P<0.05). In total, fifteen out of 16 (93.7%) leaner co-twins and eleven out of 16 (68.7%) heavier co-twins carried out at least 150 minutes per week of moderate to vigorous physical activity (MVPA) (P=0.2). Mean time spent in MVPA was 6.1 minutes per day less in heavier co-twins as compared to leaner co-twins (P=0.09).

Different results were obtained with self-reported physical activity measurements. Completed IPAQ-SF's from five leaner co-twins had to be excluded from the analyses because of outliers, following the IPAQ-SF scoring manual. Median (interquartile range, IQR) total physical activity was 4638 (IQR, 2719-6497) MET/min/week in leaner co-twins and 2853 (IQR, 2234-4788) MET/min/week in heavier co-twins (*P*=0.6). Also, no significant differences were found in median walking, moderate activity or vigorous activity as measured with IPAQ-SF between heavier and leaner co-twins (walking 1386 (IQR, 421-1386) vs. 2657 (IQR, 792-8316) MET/min/week, *P*=0.5; moderate activity 460 (IQR, 210-760) vs. 480 (IQR, 0-1920) MET/min/week, *P*=0.1; vigorous activity 0 (IQR, 0-1060) vs. 480 (IQR, 0-1920) MET/min/week, *P*=0.09).

TABLE 3
Physical activity of leaner and heavier co-twins measured by 7 day accelerometry

	Leaner co-twins (n=16)	Heavier co-twins (n=16)	*P*-value
Sedentary (min/day)	643.8 ± 37.5	656.2 ± 58.6	0.3
Light activity (min/day)	217.5 ± 54.8	195.8 ± 47.7	0.1
MVPA (min/day)	38.5 ± 17.4	32.4 ± 16.7	0.09
Sedentary (%)	71.8 ± 5.8	74.3 ± 6.0	0.1
Light activity (%)	24.0 ± 5.0	22.1 ± 4.9	0.2
MVPA activity (%)	4.2 ± 1.7	3.6 ± 1.8	0.09
Activity count (x 1000 per day)	579.6 ± 185.4	505.5 ± 155.1	< 0.05
Steps (steps/per day)	8294.8 ± 2708.7	7338.3 ± 2573.7	0.05

Mean ± SD; MVPA, moderate to vigorous physical activity

DIETARY INTAKE

Mean daily energy intake did not differ between the co-twins (Table 4). However, mean total fat intake was 4.4 E% higher in the heavier than in the leaner co-twins (*P*=0.03). More specific, monounsaturated fat intake was higher (*P*<0.01) and polyunsaturated fat intake tended to be higher (*P*=0.08) in heavier than leaner co-twins, while no differences were found for saturated fat intake. Leaner co-twins had a higher alcohol intake than heavier counterparts. Micronutrient analyses showed that leaner relative to heavier co-twins had a higher intake of iron, in specific, non-heme iron. Heavier co-twins had a higher intake of fats, oils and savoury sauces as compared to leaner co-twins. Linear regression analyses in the total group of twins (n=32) showed that energy intake was not associated with BMI. However, total fat intake was positively associated with BMI (r=0.3, *P*<0.05) and fat mass (r=0.3, *P*<0.05).

TABLE 4
Dietary intake for leaner and heavier co-twins as estimated by 3 day 24-hour recalls

	Leaner co-twins (n=16)	Heavier co-twins (n=16)	*P*-value
Total energy (kcal)	1971.4 ± 496.8	1912.8 ± 443.6	0.7
Macronutrients			
Carbohydrates (E%)	47.0 ± 5.0	45.5 ± 5.7	0.4
Protein (E%)	15.3 ± 3.1	15.2 ± 2.9	0.9
Total fat (E%)	31.2 ± 4.1	35.6 ± 6.7	0.03
Saturated fatty acids (E%)	12.3 ± 1.7	13.1 ± 2.8	0.4
Monounsaturated fatty acids (E%)	10.0 ± 1.7	12.1 ± 2.9	< 0.01
Polyunsaturated fatty acids (E%)	5.9 ± 2.1	7.1 ± 2.1	0.08
Dietary fibre (g/1000 kcal)	9.8 ± 1.6	9.2 ± 2.9	0.5
Alcohol (E%)[a]	3.1 (0.2 – 6.4)	0.1 (0 – 3.1)	< 0.01
Micronutrients			
Calcium (mg)	1110.9 ± 349.9	957.5 ± 419.2	0.1
Total iron (mg)	10.6 ± 3.0	8.6 ± 2.3	< 0.01
Iron heme (mg)	0.6 ± 0.5	0.7 ± 0.4	0.8
Iron non-heme (mg)	9.9 ± 3.1	7.9 ± 2.3	< 0.01
Vitamin A (µg)	1866.3 ± 1912.7	1745.6 ± 1158.4	0.8
Folate (µg)	212.5 ± 61.7	201.5 ± 60.0	0.4
Vitamin B12 (µg)	3.6 ± 1.7	4.1 ± 1.7	0.5
Vitamin C (mg)	85.3 ± 44.1	87.4 ± 54.0	0.8
Vitamin D (µg)	2.4 ± 1.3	2.4 ± 1.2	0.9
Food groups			
Fruits and vegetables (g)	319.6 ± 93.3	305.4 ± 155.4	0.7
Dairy products and cheese (g)	436.8 ± 265.1	327.2 ± 184.0	0.1
Bread, potato and grain products (g)	317.5 ± 130.4	363.3 ± 121.4	0.2
Meat (g)	95.5 ± 59.2	91.7 ± 33.8	0.8
Fish (g)[a]	0 (0 – 87.8)	0 (0 – 75.9)	0.4
Nuts, seeds, crisps and snacks (g)[a]	36.8 (0 – 72.0)	50.3 (19.4 – 104.8)	0.2
Sugar, sweets and pastries (g)	81.8 ± 38.6	83.9 ± 48.9	0.9
Fats, oils and savoury sauces (g)	34.8 ± 20.4	58.1 ± 29.9	< 0.05

Mean ± SD unless otherwise specified; E%, percentage of total energy intake. [a]Median and interquartile range

DISCUSSION

We found that, within rare monozygotic twins discordant for BMI, heavier co-twins had a lower level of total physical activity and a trend towards less time spent in moderate to vigorous (MVPA) than their leaner co-twins. There were no differences in energy intake, but heavier co-twins had a higher intake of fats, oils and savoury sauces resulting in higher macronutrient intake of total fat, in specific, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), as compared to their leaner co-twins. Leaner co-twins had an increased intake of alcohol and total iron than their heavier counterparts. These analyses in monozygotic twins with identical genetic backgrounds allow the elimination of confounding by genetic factors and strongly reduce confounding by gene-environment interaction and correlation.

Thus, the differences we observed must have emerged from differences in exposure to environmental factors.

Our observations are in line with previous studies investigating lifestyle factors in monozygotic twins discordant for obesity [24, 25]. A preference for fatty foods was found in obese versus lean co-twins after qualitative recall of food consumption patterns [24]. Apparently, this acquired preference was already present at adolescence before onset of BMI discordance, suggesting a causal role in the development of obesity [24]. Due to the qualitative nature of the data, however, the fatty foods preferred by the obese co-twins in the previous study could not be subdivided into proportions of saturated and unsaturated fats. In our more quantitative data analyses we showed that the intake of, in specific, MUFA was higher in heavier versus leaner co-twins. The effect of MUFA on CVD risk is controversial, as previous meta-analyses of cohort studies showed inconsistent results [26, 27]. However, subgroup analyses found a significant beneficial effect on CVD of MUFA derived from vegetable oils rather than animal products [28]. Olive oil is suggested to be the driver of the beneficial health effects of the Mediterranean diet [29]. We were unable to identify the exact food sources of MUFA in our study. However, the higher intake of fats, oils and savoury sauces might give a part of the explanation, since this food group mainly contains fat products derived from plants (e.g. margarine, oils and table sauces). Also the concomitant higher intake of PUFA in the heavier co-twins suggests that the MUFA are supplied by vegetables rather than animal products.

Our results are in contrast to a previous study that observed lower rather than higher MUFA and PUFA intakes in the obese compared to non-obese monozygotic co-twins [30]. An explanation for this discrepancy might be that in the previous study fat intake was avoided more actively in obese subjects, while in our study participants had primarily declared not to be on weight loss diets. Another option is that relative to 24-h recalls, food diaries as used in the previous study are more susceptible to underreporting of unhealthy foods, such as fats, since participants may influence their food intake when they are aware all consumed foods must be recorded.

Similar to our study, no differences were found in total energy intake between lean and obese co-twins in a study using food diaries in monozygotic discordant twins [30]. It has been suggested that overweight and obese subjects underreport their energy intake during dietary surveys [31]. The previous twin study confirmed this underreporting by comparing data from food diaries with doubly labelled water assessments as a measure for true total energy expenditure [30]. The USDA five-step multiple-pass method we used in our study is a valuable method for quantitative dietary intake assessments as compared to, for example, food frequency questionnaires and food diaries [20]. Nevertheless, we cannot exclude the possibility that obesity-related underreporting affected our results.

Current physical activity guidelines recommend at least 150 minutes per week of MVPA to reduce risk for many chronic diseases [32]. Our data show that 93.7% of leaner and 68.7% of heavier co-twins get sufficient physical activity to meet this requirement, which are similar proportions as in the general population where 7 of 10 individuals reach this demand [33]. The clinical relevance of our observations in MVPA remains open to question since the influence of MVPA on body weight is controversial [34]. Several accelerometer studies showed inverse associations between MVPA and risk of obesity [35, 36], while other longitudinal studies failed to detect an association between self-reported leisure time exercise behaviour and BMI [37, 38]. The discrepancies in these observations might be a result of different definitions of physical activity and different test methods being used. Accelerometers measure motion without distinguishing between voluntary leisure time exercise behaviour and other aspects of physical activity such as household activities and walking or cycling to work. Thus, the higher MVPA we observed in the leaner versus heavier co-twins in our study represents activity performed in all domains of activity during a day rather than just exercise behaviour.

The lower total physical activity and time spent in MVPA in heavier versus leaner co-twins we observed is in line with two previous studies investigating monozygotic twins discordant for BMI [25, 30]. Similar to our results, obese versus lean co-twins showed lower accelerometer activity counts [25] and less reported high-intensity activity [30]. Another study failed to detect differences in physical activity among BMI discordant monozygotic co-twins [39]. This study, however, used retrospective interviews rather than objective assessment tools such as accelerometry. Taken together, the results of two previous studies [25, 30] in combination with our results demonstrate that the origin of the association between lower physical activity and higher BMI lies (at least in part) in the exposure to unique environmental factors, independent of genotype.

It could be hypothesized that these unique environmental factors could act through the genome by causing epigenetic differences [40]. Alterations in DNA methylation and histone modification before and after birth may influence the development of disease without changing the DNA sequence. A recently published study, however, found no significant differences in gene expression of BMI-associated loci between BMI discordant monozygotic twins [13]. However, the assessments were performed in peripheral blood only, leaving role for an epigenetic influence on regulatory pathways underlying BMI discordance through other tissues.

Because of the cross-sectional nature of our study no conclusion can be drawn whether the reduced physical activity resulted in higher BMI or whether the increased weight led to decreased physical activity. However, a previous retrospective monozygotic twin study on leisure time activity observed that the physically inactive co-twin at adolescence had a higher risk of becoming obese compared to their

physically active co-twin in adulthood [25], suggesting a causal role for physical inactivity in the development of obesity.

Our final study sample comprised 2 twin pairs that were not strictly BMI discordant during the clinical assessments (BMI differences of 0.71 and 1.02 kg/m^2). This is possibly because subjects guessed their BMI incorrectly at the moment of screening. Post hoc analyses after excluding these 2 pairs did not influence the results in terms of effect sizes, although the decrease in power obviously resulted in less statistical significance of our findings.

We acknowledge that a sample of 16 twin pairs may seem relatively small. However, since body weight is a highly heritable trait, a mean BMI discordance of almost 4 kg/m^2 as seen in our study sample between monozygotic twins is very rare [13]. The sample size should be appreciated in light of this design, which involves monozygotic twins discordant for BMI, but perfectly matched with respect to age, gender and genetic background. This design in combination with the accuracy of the phenotypic measures is optimal with respect to power.

Strengths of our study are the use of accelerometers to objectively measure physical activity and the use of the USDA five-step multiple-pass method to assess dietary intake. Although underreporting remains a subject of concern as in all dietary surveys, the 24-hour recall method, in our opinion, is the next best thing by not interfering with actual dietary behaviour and keeping respondent burden low.

In summary, we demonstrated that higher BMI is associated with a lower total physical activity and a higher intake of total fat, in specific MUFA and PUFA. Our finding that these associations were observed within monozygotic twin pairs with an identical genetic background implicates that these associations are independent of genetic factors. Thus, exposure to unique environmental factors is responsible for the more health-compromising lifestyle factors observed in individuals with a higher BMI. However, it cannot be determined whether the lower physical activity and higher fat intake are a cause or a consequence of the increased BMI. Future identification of the underlying unshared environmental factors responsible for our findings, for instance by qualitative in-depth interviews of BMI discordant monozygotic pairs, may provide starting points towards developing new strategies in the management of obesity.

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