

Anthropometry, carbohydrate and lipid metabolism in the East Flanders Prospective Twin Survey: heritabilities

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Abstract

Aims/hypothesis We determined the genetic contribution of 18 anthropometric and metabolic risk factors of type 2 diabetes using a young healthy twin population.

Methods Traits were measured in 240 monozygotic (MZ) and 138 dizygotic (DZ) twin pairs aged 18 to 34 years. Twins were recruited from the Belgian population-based East Flanders Prospective Twin Survey, which is characterised by its accurate zygosity determination and extensive collection of perinatal and placental data, including information on chorionicity. Heritability was estimated using structural equation modelling implemented in the Mx software package.

Results Intra-pair correlations of the anthropometric and metabolic characteristics did not differ between MZ monozygotic and MZ dichorionic pairs; consequently heritabilities were estimated using the classical twin approach. For body mass, BMI and fat mass, quantitative sex differences were observed; genetic variance explained 84, 85 and 81% of the total variation in men and 74, 75 and 70% in women, respectively. Heritability estimates of the waist-to-hip ratio, sum of four skinfold thicknesses and lean body mass were 70, 74 and 81%, respectively. The heritability estimates of fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance and beta cell function, as well as insulin-like growth factor binding protein-1 levels were 67, 49, 48, 62 and 47%, in

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that order. Finally, for total cholesterol, LDL-cholesterol, HDL-cholesterol, total cholesterol:HDL-cholesterol ratio, triacylglycerol, NEFA and leptin levels, genetic factors explained 75, 78, 76, 79, 58, 37 and 53% of the total variation, respectively.

Conclusions/interpretation Genetic factors explain the greater part of the variation in traits related to obesity, glucose intolerance/insulin resistance and dyslipidaemia.

Keywords Anthropometry · Carbohydrate metabolism · Chorionicity · East Flanders Prospective Twin Survey · Heritability · Lipid metabolism · Metabolic risk factors · Structural equation modelling · Twin study · Type 2 diabetes

Abbreviations

A	additive genetic effects
C	common environmental effects
D	non-additive genetic effects
DC	dichorionic
DZ	dizygotic
DZF	dizygotic women
DZM	dizygotic men
DZOS	dizygotic opposite sex
E	unique environmental effects
EFPTS	East Flanders Prospective Twin Survey
IGFBP-1	insulin-like growth factor binding protein-1
MC	monochorionic
MZ	monozygotic
MZF	monozygotic women
MZM	monozygotic men
S4SF	sum of four skinfold thicknesses

Introduction

Type 2 diabetes is a heterogeneous disease that involves both genetic and environmental factors. Its incidence is rising rapidly worldwide and consequently much research is focused on the genetic components of the disorder, in order to get a better understanding of the pathogenesis and eventually to achieve better, more personalised diagnostics, treatment and prevention [1].

The starting point in the search for genes is to estimate the degree of heritability of intermediate traits leading to the disease in the studied population. Heritability is the proportion of phenotypic variation of a trait that can be attributed to genetic variation [2]. The degree of heritability is an important determinant of the power to detect and localise disease-related genes [3]. Although heritabilities can in principle be estimated from all kinds of related individuals, twin studies allow the variation to be split up into genetic, shared environmental and unique environmen-

tal components, thus offering one of the most valid estimations [2].

A variety of studies estimating heritabilities of risk factors of type 2 diabetes in adult twins have been carried out. A summary of the larger studies (>200 twin pairs) on the risk factors obesity, glucose intolerance/insulin resistance and dyslipidaemia is provided in Electronic Supplementary Material (ESM) Table 1. The heritability of BMI, the most frequently used measure of obesity, has been estimated extensively and ranges between 40 and 90% [4–21]. For all the other traits, fewer studies have been performed; the total variation of fasting glucose and fasting insulin is explained by genetic factors for 12 to 50% [22, 23] and 14 to 54% [7, 18, 22–24], respectively; for total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerol heritability estimates range between 0 and 98% [7, 10, 13, 17, 21, 25–34]. In addition to sample numbers and statistical methodologies used, the large variation in heritability estimates may also be caused by the genetic background of the studied populations and the environmental exposures experienced (see ESM Table 1). For the majority of intermediate traits related to type 2 diabetes, the number of larger studies performed is quite small and studies in which heritabilities of several risk factors of type 2 diabetes were estimated in the same population are even scarcer (ESM Table 1).

To determine the genetic contribution to type 2 diabetes, we used variance components modelling to estimate the heritabilities of 18 anthropometric and metabolic risk factors of the disease, including parameters quantifying obesity, glucose intolerance/insulin resistance and dyslipidaemia. In addition, we also determined the heritabilities of the hormones insulin-like growth factor binding protein-1 (IGFBP-1), which has been shown to be related to several cardiovascular risk factors, and leptin, which plays a role as satiety signal regulating body composition and energy expenditure [35]. The characteristics were measured in 756 healthy twins, divided into 240 monozygotic (MZ) and 138 dizygotic (DZ) twin pairs in the age range of 18 to 34 years, recruited from the East Flanders Prospective Twin Survey (EFPTS).

Methods

Participants The EFPTS is a population-based twin register that started in 1964 and has recorded all multiple births in the Belgian Province of East Flanders until the present day. A detailed description of the EFPTS has been published [36]. Zygosity was determined using sequential analysis based on sex, fetal membranes, umbilical cord blood groups, placental alkaline phosphatase and DNA marker analysis. Between July 1964 and May 1982, the Twin

Survey had registered 2,141 twin pairs who met the World Health Organization criteria for live born infants (birthweight ≥ 500 g or gestational age ≥ 22 weeks if birthweight unknown). Pairs of whom one or both members were stillborn, died in neonatal or later life or suffered from major congenital malformations were excluded. We randomly contacted 803 pairs using an envelope system. To assure equally distributed groups, we stratified for birth year and zygosity. Since the twin survey was originally set up to determine chorionicity, the number of MZ twin pairs was over sampled. Eventually, 424 twin pairs (52.7%) volunteered to participate in the study. Sex, gestational age and birthweight did not differ between participants and non-participants (i.e. those who were eligible, but refused to participate or had not been contacted) ($p > 0.05$), although participants were slightly older than the non-participants and the proportion of monozygotic (MC) twins was higher in the group of participants ($p < 0.05$). A detailed description of the methods used to measure the anthropometric and metabolic characteristics of the twins is provided in the [Electronic Supplementary Material](#). For the present analysis participants suffering from type 1 diabetes were excluded and only twin pairs of whom both members participated were incorporated. Consequently, phenotypic data were available for 378 complete pairs, consisting of 113 monozygotic men (MZM), 127 monozygotic women (MZF), 46 DZ men (DZM), 49 DZ women (DZF) and 43 DZ opposite sex (DZOS) pairs. Additionally, for the analysis of lipid and carbohydrate parameters, participants taking drugs with potential effects on lipid or carbohydrate metabolism were excluded ($n = 11$). Twins were randomly assigned to be the first or the second member of a pair. The Ethics Committee of the Faculty of Medicine of the Katholieke Universiteit Leuven approved the project and all participants gave informed consent.

Descriptive statistical analysis Anthropometric and metabolic characteristics are expressed as mean \pm SD according to chorion type, for men and women separately. BMI, sum of four skinfold thicknesses (S4SF), fat mass, IGFBP-1, fasting insulin, insulin resistance, beta cell function, leptin, total cholesterol:HDL-cholesterol ratio and triacylglycerol values had a skewed distribution. After transforming these data into natural logarithms a normal distribution was obtained and the transformed data were used when performing statistical tests. Differences in means between MZ MC, MZ dichorionic (DC) and DZ twins were calculated using the PROC MIXED method implemented in SAS version 9.1 (SAS Institute, Cary, NC, USA). A random intercept model was used, where the intercept of each twin pair was modelled as a function of the population intercept plus a unique contribution of the twin pair. In addition, we allowed the variance–covariance structure of

the random intercept to differ between MZ MC, MZ DC and DZ pairs. Differences in means were considered significant if the 2 *df* *F* test indicated $p < 0.05$.

For all traits, effects of potential covariates were also checked using the random intercept model of PROC MIXED, where the variance–covariance structure of the random intercept was allowed to differ between MZ and DZ pairs. The variables BMI, WHR and S4SF were checked for the effect of potential confounding by sex and age; body mass, fat mass and lean body mass were checked for the effect of sex, age and height; the blood parameters were checked for the effect of sex, age and fat (BMI, WHR or S4SF). Covariates were considered significant if $p < 0.05$. Intra-pair correlation coefficients were calculated for MZ MC and MZ DC twin pairs and for each of the five sex by zygosity groups (MZM, MZF, DZM, DZF and DZOS) before and after adjustment for significant covariates using the Mx software package [37]. In addition, we used a linear regression analysis to test whether twin correlations differed between MZ MC and MZ DC twin pairs before and after adjusting for covariates.

Twin model fitting Twin methodology makes use of the fact that MZ twins are genetically identical, whereas DZ twins share 50% of their segregating genes. Assuming that MZ and DZ twins share their common environment to the same extent, a higher concordance rate in MZ twins than in DZ twins reflects genetic influences. To estimate the genetic and environmental components of variance of the traits, twin model fitting of raw data was implemented using the statistical package Mx [37]. Scripts were downloaded from the GenomEUtwin Mx-script library (<http://www.psy.vu.nl/mxbib/>). Univariate twin analyses were performed, where the phenotypic variance can be decomposed into additive genetic (A, additive effects of genes on multiple loci), non-additive genetic (D, interactions between alleles at the same locus [dominance] or on different loci [epistasis]), common environmental (C, environmental effects shared by twins reared in the same family) and unique environmental effects (E, environmental effects unique to the individual). MZ twins are assumed to share the same A and D genetic variance; DZ pairs are assumed to share one-half of the A variance and one-quarter of the D variance. The C variance is assumed to be the same for both MZ and DZ twin pairs. The broad sense heritability (H^2), which estimates the extent to which variation of a trait in a population can be explained by genetic variation, is defined as the proportion of genetic variance to total phenotypic variance. As non-additive genetic (D) and shared environmental effects (C) cannot be identified simultaneously in data from twins reared together, ACE and ADE models were fitted separately. The significance of variance components A, C

or D in the model was tested by dropping these parameters and comparing the fit of the models [5].

To test whether genetic and environmental factors influence a trait to the same degree in men and women, we compared a quantitative heterogeneity model (variance components free to differ across sexes) with a homogeneity model (variance components equal for both sexes). In addition, we verified whether the distribution of a trait differed among men and women by testing a scalar model, which assumes that the female variance components are common multiples of the male variance components. In this model the variance components were constrained to be equal for both sexes, but total variances were allowed to differ between men and women (Fig. 1) [5].

If for a certain trait the correlation of the opposite-sex pairs is smaller than the correlations for the like-sexed DZ pairs, it is suggested that the correlation between additive genetic factors in opposite-sex pairs is smaller than 0.5. This indicates that different genetic factors influence this trait in men and women. To test this, a full heterogeneity model (variance components free to differ across sexes, plus the correlation between the additive genetic factors in opposite-sex pairs free to be estimated between 0 and 0.5) was compared with a quantitative heterogeneity model.

All variance components were estimated both unadjusted and adjusted for the covariates. In the models, phenotypic means were adjusted for significant covariates by modelling them as definition variables in the means model. The difference in fit between the nested models was evaluated with the likelihood ratio χ^2 test, which uses the difference between the $-2\log$ likelihood of the full and the restricted model. This difference is distributed as χ^2 . The *df* of the test were calculated as the difference in *df* between the models. When the χ^2 was not statistically significant ($p < 0.05$), the most parsimonious model was selected, i.e. the model with the best fit given the number of *df*. When comparing the fit of non-nested models (e.g. ACE with ADE), the model with the lowest Akaike's information criterion was preferred.

Results

Descriptive statistical analysis The mean values of the anthropometric and metabolic characteristics of the twins are presented in Table 1 according to chorion type for men and women separately. In men, fasting glucose levels were lower in DZ twins than in MZ MC and MZ DC twins. In women, MZ DC twins were younger and had lower total cholesterol and LDL-cholesterol levels than MZ MC and DZ twins (Table 1).

The covariates adjusted for, the intra-pair correlations of MZ MC and MZ DC pairs, and the intra-pair correlations of

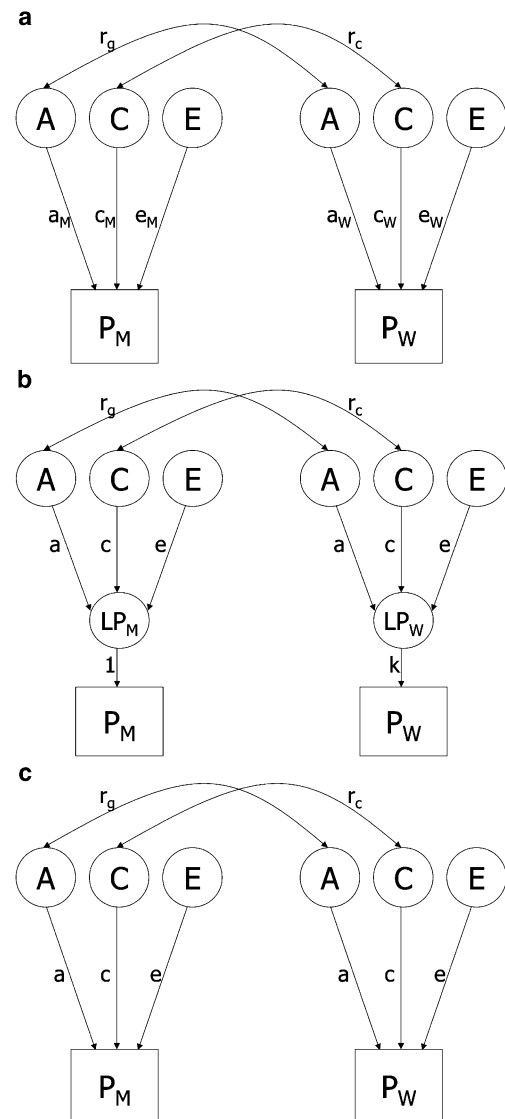


Fig. 1 Path diagram for a univariate quantitative heterogeneity (a), scalar (b) and homogeneity (c) model only presenting opposite sex pairs. Observed phenotypes (P_M and P_W) for the male and female twins are shown in rectangles, while latent factors (A, C and E) and latent phenotypes (LP_M and LP_W) are shown in circles. Path coefficients of observed variables on the different latent factors are shown in lower case: a, additive genetic effect; c, common environmental effect; e, unique environmental effect; k, scalar factor. The genetic correlation is represented by r_g (1 for MZ and 0.5 for DZ twins) and the common environmental correlation is represented by r_c (1 for MZ and DZ twins)

each sex by zygosity group before and after adjustment are summarised in Table 2. Intra-pair correlations of the anthropometric and metabolic characteristics did not differ between MZ MC and MZ DC pairs ($p > 0.05$). Adjusting for covariates strongly reduced the correlations of the leptin concentrations. The correlations of the other traits were only minimally affected by adjustment. Twin correlations for total cholesterol and LDL-cholesterol were high in both

Table 1 Anthropometric and metabolic characteristics of the twin population according to chorion type for men and women separately in the EFPTS

Characteristic	Men				Women			
	MZ MC	MZ DC	DZ	<i>p</i> value	MZ MC	MZ DC	DZ	<i>p</i> value
<i>n</i>	134	92	135		142	112	141	
Age (years) ^a	25.3±4.5	25.0±4.9	25.7±4.7	0.33	25.9±4.4	24.0±4.8	25.7±4.6	0.02
Body height (cm)	178.0±5.7	178.3±7.2	178.4±6.5	0.92	165.0±6.3	165.6±6.1	166.2±6.4	0.59
Body mass (kg)	70.7±10.5	69.6±8.4	70.9±10.2	0.67	60.5±9.7	60.5±10.6	60.8±10.2	0.98
BMI (kg/m ²)	22.1±1.1	21.8±1.1	22.1±1.2	0.73	22.0±1.2	21.8±1.2	21.8±1.2	0.86
WHR (%)	83.4±5.4	83.4±5.8	82.8±5.5	0.84	73.4±4.5	73.3±4.6	72.5±4.3	0.30
S4SF (mm) ^b	35.9±1.5	34.6±1.5	34.8±1.5	0.89	57.1±1.4	55.6±1.5	53.6±1.4	0.50
Fat mass (kg) ^b	11.9±1.6	11.2±1.5	12.2±1.5	0.33	16.9±1.3	16.7±1.4	16.8±1.3	0.95
Lean body mass (kg)	57.7±6.9	57.6±6.0	57.7±6.6	0.99	42.9±5.1	43.1±5.6	43.4±6.0	0.89
IGFBP-1 (ng/ml) ^b	11.0±1.9	10.3±1.8	11.5±1.9	0.46	16.8±2.0	16.6±2.2	18.4±2.3	0.58
Fasting insulin (pmol/l) ^b	33.5±1.5	32.7±1.6	32.7±1.6	0.94	38.6±1.5	35.8±1.6	40.1±1.5	0.24
Insulin resistance (HOMA) ^b	1.2±1.6	1.2±1.6	1.2±1.6	0.69	1.3±1.5	1.2±1.7	1.4±1.5	0.26
Fasting glucose (mmol/l)	5.0±0.5	4.9±0.4	4.8±0.4	0.01	4.5±0.3	4.6±0.4	4.6±0.4	0.23
Beta cell function (HOMA) ^b	77.2±1.5	78.2±1.6	86.5±1.7	0.10	135.2±1.7	115.7±1.6	126.7±1.6	0.17
Leptin (ng/ml) ^b	1.7±3.0	1.4±2.9	1.7±3.0	0.42	11.5±2.1	11.2±2.2	11.5±1.9	0.92
Total cholesterol (mmol/l)	4.8±0.9	4.7±1.0	4.9±1.1	0.31	5.3±0.9	4.9±0.8	5.3±1.0	0.006
LDL-cholesterol (mmol/l)	3.0±0.9	2.9±0.9	3.1±1.0	0.35	3.1±0.8	2.7±0.8	2.9±0.8	0.012
HDL-cholesterol (mmol/l)	1.3±0.3	1.3±0.3	1.4±0.4	0.94	1.8±0.4	1.8±0.4	1.9±0.4	0.39
Total cholesterol:HDL-cholesterol ratio ^b	3.6±1.4	3.5±1.3	3.7±1.4	0.67	3.0±1.3	2.8±1.3	2.9±1.3	0.34
Triacylglycerol (mmol/l) ^b	0.9±1.6	0.9±1.5	0.9±1.5	0.78	0.9±1.5	0.9±1.5	0.9±1.5	0.83
NEFA (mmol/l)	0.5±0.2	0.5±0.2	0.5±0.2	0.05	0.7±0.2	0.7±0.3	0.7±0.2	0.81

Data are expressed as mean±SD

HOMA, homeostasis model assessment

^a*p* value calculated using standard linear regression, because convergence criteria could not be met using a random intercept model

^bGeometric mean±SD

MZ and DZ twin pairs, indicating a common environmental effect. The DZ correlations of IGFBP-1, fasting insulin and insulin resistance were less than one-half of the MZ correlations, suggesting that non-additive genetic effects might be important. Correlations of the other traits were in agreement with a model containing additive genetic and unique environmental influences (Table 2).

Twin model fitting The variance components and 95% CIs of the best fitting models before and after adjustment for covariates are presented in Table 3. The best fitting model for lean body mass and the obesity parameters body mass, BMI, WHR, S4SF and fat mass was an AE model containing a major genetic component. For total cholesterol and LDL-cholesterol, the ACE model was the best fitting model. However, after adjusting for covariates, the AE model became the best fitting model. For IGFBP-1, fasting insulin and insulin resistance, a DE model containing a non-additive genetic and unique environmental component had the best fit. The variation of the remaining blood parameters, including fasting glucose, beta cell function, leptin, HDL-cholesterol, total cholesterol:HDL-cholesterol

ratio, triacylglycerol and NEFA levels were best explained by an AE model (Table 3).

Quantitative sex differences were present in body mass, BMI, WHR, S4SF, fat mass, lean body mass, leptin and total cholesterol:HDL-cholesterol ratio, because variance components estimates were significantly different between men and women (Table 3). The influences of additive genetic factors were larger in men than in women. For some traits, scalar sex differences were observed, implying that although variance components are equal across sexes, the total variances differ. As a result, total variance of IGFBP-1, HDL-cholesterol and NEFA levels in women was larger than in men, but smaller for fasting glucose and HDL-cholesterol levels (Table 3).

After adjusting for covariates, quantitative sex differences remained significant only for body mass, BMI and fat mass (Table 3). In addition, scalar sex differences were significant for WHR, S4SF, lean body mass, IGFBP-1, fasting glucose, leptin, HDL-cholesterol, total cholesterol:HDL-cholesterol ratio and NEFA levels. Total variance of IGFBP-1, HDL-cholesterol and NEFA levels was larger in

Table 2 Intra-pair correlations of MZ MC and MZ DC pairs, and of each sex by zygosity group before and after adjusting for covariates in the EFPTS

Characteristic	Monozygotic		Monozygotic		Dizygotic			Covariates
	MC	DC	Men	Women	Men	Women	OS	
<i>n</i> (of pairs)	138	102	113	127	46	49	43	
Body mass	0.85/0.79	0.84/0.76	0.86/0.82	0.76/0.73	0.38/0.28	0.58/0.57	0.26/0.35	Sex, age, height
BMI	0.80/0.78	0.81/0.77	0.86/0.83	0.77/0.74	0.46/0.31	0.53/0.56	0.47/0.46	Age
WHR	0.87/0.69	0.88/0.71	0.79/0.74	0.70/0.66	0.39/0.28	0.44/0.48	0.31/0.15	Sex, age
S4SF	0.82/0.73	0.84/0.75	0.81/0.79	0.72/0.68	0.46/0.37	0.64/0.63	0.36/0.31	Sex, age
Fat mass	0.85/0.78	0.85/0.75	0.85/0.82	0.73/0.69	0.46/0.36	0.43/0.46	0.42/0.35	Sex, age
Lean body mass	0.93/0.81	0.93/0.79	0.86/0.82	0.79/0.78	0.43/0.39	0.65/0.58	0.25/0.39	Sex, age, height
IGFBP-1	0.49/0.39	0.60/0.53	0.55/0.51	0.45/0.43	0.31/0.21	0.08/0.06	0.12/−0.05	Sex, age, BMI
Fasting insulin	0.57/0.48	0.52/0.50	0.49/0.45	0.58/0.52	0.07/0.13	0.18/0.19	0.07/−0.01	Age, S4SF
Insulin resistance	0.54/0.47	0.53/0.50	0.49/0.46	0.57/0.51	0.03/0.08	0.14/0.17	0.04/−0.05	Sex, age, S4SF
Fasting glucose	0.74/0.66	0.73/0.67	0.65/0.65	0.70/0.69	0.28/0.24	0.57/0.60	0.31/0.32	Sex, BMI
Beta cell function	0.71/0.58	0.66/0.60	0.52/0.50	0.68/0.66	0.32/0.40	0.47/0.46	0.37/0.33	Sex, age, S4SF
Leptin	0.85/0.53	0.85/0.57	0.70/0.58	0.64/0.52	0.35/0.02	0.66/0.31	0.38/0.37	Sex, age, S4SF
Total cholesterol	0.76/0.74	0.77/0.72	0.78/0.74	0.73/0.73	0.52/0.51	0.51/0.44	0.63/0.51	Age, S4SF
LDL-cholesterol	0.78/0.79	0.81/0.74	0.81/0.79	0.77/0.75	0.52/0.51	0.59/0.52	0.68/0.58	Sex, age, S4SF
HDL-cholesterol	0.78/0.71	0.84/0.77	0.75/0.76	0.74/0.74	0.31/0.30	0.44/0.44	0.52/0.52	Sex, S4SF
Total cholesterol:HDL-cholesterol ratio	0.81/0.78	0.86/0.81	0.84/0.82	0.78/0.76	0.50/0.41	0.50/0.49	0.65/0.54	Age, WHR
Triacylglycerol	0.58/0.56	0.67/0.60	0.59/0.54	0.63/0.60	0.29/0.41	0.34/0.34	0.16/0.17	Sex, S4SF
NEFA	0.49/0.35	0.43/0.39	0.39/0.38	0.34/0.37	0.10/0.14	0.20/0.18	0.25/0.26	Sex, S4SF

Values are unadjusted intra-pair correlation/adjusted intra-pair correlation.
OS, opposite sex

women than in men, but smaller for WHR, S4SF, lean body mass, fasting glucose, leptin and total cholesterol:HDL-cholesterol ratio (Table 3).

The adjusted correlations of WHR, fasting insulin and triacylglycerol levels for opposite-sex pairs were smaller than the correlations for like-sexed DZ pairs, suggesting that different genetic factors influence this trait in men and women (Table 2). However, the correlations between the additive genetic factors in opposite-sex pairs were not significantly smaller than 0.5 ($p > 0.05$).

Broad-sense heritability estimates (encompassing both additive and non-additive genetic effects) were slightly lower after adjusting for covariates, with the exception of total cholesterol and LDL-cholesterol, which had a higher heritability after adjustment (Table 3).

In summary, heritability estimates of body mass, BMI and fat mass were 84, 85 and 81% for men and 74, 75 and 70% for women, respectively. WHR, S4SF and lean body mass had heritability estimates of 70, 74 and 81%, correspondingly. For fasting glucose, fasting insulin, insulin resistance, beta cell function and IGFBP-1 levels, genetic factors explained 67, 49, 48, 62 and 47% of the total variation, respectively. Finally, heritability estimates of total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol, total cholesterol:HDL-cholesterol ratio, NEFA and leptin levels were 75, 58, 76, 78, 79, 37 and 53%, respectively (Table 3).

Discussion

To determine the genetic contribution to type 2 diabetes, we estimated the heritabilities of 18 anthropometric and metabolic characteristics related to this disease, including parameters quantifying obesity, glucose intolerance/insulin resistance and dyslipidaemia. The traits were measured in 138 DZ and 240 MZ young healthy twin pairs recruited from the population-based EFPTS [36].

Obesity, defined as excess accumulation of adipose tissue, is a complicated trait to measure and numerous methods exist for its determination [38]. In the present study several parameters defining obesity, including body mass, BMI, S4SF, WHR and fat mass were used and heritability estimates ranged from 70 to 85%. According to ESM Table 1, the heritabilities identified by us for BMI and body mass are in agreement with those reported in other twin studies, but the heritability identified by us for WHR is somewhat higher [7, 15, 18, 39]. Heritability estimates for S4SF and fat mass are not often reported and therefore we were not able to make a valid comparison. Despite the variety of measurements used to quantify obesity, the heritabilities of the different measurements were consistently in a high range, indicating that the contribution of genetic factors to total phenotypic variation in obesity is of great importance in the studied twin sample.

Table 3 Variance components estimates and 95% CIs of best-fitting models expressed in percentages

Characteristic	Unadjusted						Adjusted for covariates							
	Model	Sex	a ² (H ²)	c ²	d ² (H ²)	e ²	S	Model	Sex	a ² (H ²)	c ²	d ² (H ²)	e ²	S
	Body mass	AE	M	90 (86–93)	–	–	10 (7–14)	–	AE	M	84 (78–88)	–	–	16 (12–22)
	AE	W	80 (73–85)	–	–	20 (15–27)	–	AE	W	74 (66–80)	–	–	26 (20–34)	–
BMI	AE	M	87 (82–90)	–	–	13 (10–18)	–	AE	M	85 (79–89)	–	–	15 (11–21)	–
	AE	W	76 (68–82)	–	–	24 (18–32)	–	AE	W	75 (67–81)	–	–	25 (19–33)	–
WHR	AE	M	94 (92–96)	–	–	6 (4–8)	–	AE	W	70 (63–75)	–	–	30 (25–37)	M>W
	AE	W	70 (59–77)	–	–	30 (23–41)	–	AE	W	74 (68–79)	–	–	26 (21–32)	M>W
S4SF	AE	M	88 (83–91)	–	–	12 (9–17)	–	AE	M	74 (68–79)	–	–	26 (21–32)	M>W
	AE	W	75 (67–81)	–	–	25 (19–33)	–	AE	W	81 (74–86)	–	–	19 (14–26)	–
Fat mass	AE	M	88 (84–92)	–	–	12 (8–16)	–	AE	M	70 (60–77)	–	–	30 (23–40)	–
	AE	W	74 (65–80)	–	–	26 (20–35)	–	AE	W	81 (76–84)	–	–	19 (16–24)	M>W
Lean body mass	AE	M	97 (96–98)	–	–	3 (2–4)	–	AE	M	–	–	–	–	–
	AE	W	82 (76–87)	–	–	18 (13–24)	–	AE	W	–	–	–	–	–
IGFBP-1	DE	–	–	–	56 (47–64)	44 (36–53)	W>M	DE	–	–	–	47 (36–56)	53 (44–64)	W>M
Fasting insulin	DE	–	–	–	54 (45–62)	46 (38–55)	–	DE	–	–	–	49 (39–58)	51 (42–61)	–
Insulin resistance	DE	–	–	–	52 (42–60)	48 (40–58)	–	DE	–	–	–	48 (38–57)	52 (43–62)	–
Fasting glucose	AE	–	72 (66–77)	–	–	28 (23–34)	M>W	AE	–	67 (60–73)	–	–	33 (27–40)	M>W
Beta cell function	AE	–	69 (63–75)	–	–	31 (25–37)	–	AE	–	62 (54–69)	–	–	38 (31–46)	–
Leptin	AE	M	92 (88–94)	–	–	8 (6–12)	–	AE	M	53 (44–61)	–	–	47 (39–56)	M>W
	AE	W	63 (52–72)	–	–	37 (28–48)	–	AE	W	75 (69–79)	–	–	25 (21–31)	–
Total cholesterol	ACE	–	49 (28–74)	29 (4–48)	–	22 (18–28)	–	AE	–	78 (73–82)	–	–	22 (18–27)	–
LDL-cholesterol	ACE	–	43 (24–67)	37 (14–54)	–	20 (16–25)	M>W	AE	–	76 (70–81)	–	–	24 (19–30)	W>M
HDL-cholesterol	AE	–	82 (78–86)	–	–	18 (14–22)	W>M	AE	–	79 (74–83)	–	–	21 (17–26)	M>W
Total cholesterol:HDL-cholesterol ratio	AE	M	88 (83–91)	–	–	12 (9–17)	–	AE	M	–	–	–	–	–
	AE	W	79 (72–85)	–	–	21 (15–28)	–	AE	W	58 (49–66)	–	–	42 (34–51)	–
Triacylglycerol	AE	–	61 (52–68)	–	–	39 (32–48)	–	AE	–	37 (25–47)	–	–	63 (53–75)	W>M
NEFA	AE	–	46 (36–54)	–	–	54 (46–64)	W>M	AE	–	–	–	–	–	–

Values are variance components estimates (95% CI)

M, men; W, women; a², additive genetic variance; c², common environmental variance; d², non-additive genetic variance; e², unique environmental variance; S, scalar effect; H², broad heritability

Quantitative sex differences were significant for body mass, BMI and fat mass, with heritability estimates of 84, 85 and 81% in men and 74, 75 and 70% in women, respectively. Our results confirm those from other twin studies reporting quantitative sex differences in the heritability of body mass and BMI [4–6, 9, 20, 40]. However, in other twin studies the highest heritability estimates were not always observed in men (ESM Table 1), indicating that the influence of environmental factors in the total variation of these traits according to sex may vary in different populations.

In the present study, 49% of the total variation of fasting insulin was explained by genetic factors, which is in agreement with other twin studies (ESM Table 1) [41, 42]. The heritability of fasting glucose ($H^2=67\%$) was higher than those observed in other twin studies, where it ranged between 12 and 58% (ESM Table 1) [42, 43]. The heritabilities of beta cell function ($H^2=62\%$) and insulin resistance ($H^2=48\%$) was not higher than those of fasting glucose ($H^2=67\%$) and fasting insulin levels ($H^2=49\%$). This indicates that no additional power was obtained by calculating these alternative phenotypic markers with the homeostasis model assessment.

The variation of total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol and total cholesterol:HDL-cholesterol ratio was primarily explained by additive genetic factors, accounting for 75, 58, 76, 78 and 79% of the total variance, respectively. No evidence was observed for quantitative sex differences in heritability estimates for these traits. The percentages are slightly higher than the heritability estimates reported in the literature, but are in the same high range (ESM Table 1). To the best of our knowledge, ours is the first large twin study reporting the heritability of NEFA levels, which was 37%. This is somewhat surprising, since excessive circulating fatty acids represent a major contributor to the development of insulin resistance [44]. The heritability determined for NEFA levels in the current study was low compared with those reported for the other blood lipid parameters, indicating that environmental factors, e.g. nutrition, may be more important in determining the variation of NEFA levels than genetic factors.

Additive genetic variance explained 53% of the variation of total leptin concentration. In other western adult twin studies, heritabilities of total leptin concentrations ranged from 34 to 55% [45, 46], which is in agreement with our findings. Compared with other traits, the correlations and heritability estimates of leptin were strongly reduced after adjusting for covariates, which is probably caused by the adjustment for fat (using S4SF). This is not very surprising, since leptin is synthesised in and secreted from adipose tissue and plasma leptin levels are increased in obese humans in direct proportion to body fat mass [35]. The heritability of IGFBP-1 ($H^2=47\%$) levels determined in our

study was also relatively high, since the heritability in two other twin studies ranged from 0 to 36% [41, 47]. Interestingly, the heritabilities of IGFBP-1 levels in the latter studies were determined in older twins (mean age 63 years). This might suggest that the heritability of IGFBP-1 decreases with advancing age. Longitudinal twin studies need to be carried out to verify this observation.

We are the first to report that a model containing non-additive instead of additive genetic factors is the best-fitting model for insulin resistance, fasting insulin and IGFBP-1 levels [7, 18, 22, 24, 41–43]. In addition to the twin model fitting procedure, twin correlations can also give an impression of whether non-additive genetic effects might be involved, i.e. a DZ correlation of less than one-half of the MZ correlation suggests a contribution of non-additive genetic effects [18, 41–43]. The smaller sample size of some studies could be responsible for their not observing a DE model. Furthermore, the presence of non-additive genetic effects has not always been tested. However, if non-additive genetic effects are observed, this implies that a part of the variance is explained by interactions between alleles at the same locus or on different loci. Although this observation is likely to be the result of lack of power, we cannot exclude real non-additive genetic effects in the absence of additive genetic effects, as these three traits are closely related to or are a direct gene product.

MZ MC twins have a more adverse intra-uterine environment than MZ DC and DZ twins, resulting in a significantly lower weight at birth and higher perinatal mortality and morbidity [36]. Hence, it has been hypothesised that for disorders like type 2 diabetes, in the development of which prenatal environment plays a role, the classical twin study might be an unreliable method of estimating heritabilities [48, 49]. In the current twin sample, intra-pair correlations did not differ between MZ MC and MZ DC twins, suggesting that the chorion type of MZ twins did not bias the heritability estimates of the studied traits.

With the exception of NEFA levels, the genetic contribution to the traits studied in our twin sample was high. When comparing the heritability values found by us with those reported in other twin studies (Electronic Supplementary Material Table 1), our heritability estimates are in a slightly higher range. An important aspect affecting heritability estimates is the accuracy of the zygosity determination used in the present study via sequential analysis based on sex, fetal membranes, umbilical cord blood groups, placental alkaline phosphatase and DNA marker analysis. In other twin studies, zygosity was often determined using questionnaires [4], which sometimes leads to misclassification and consequently to underestimation of heritability [50]. Our high heritability estimates might also be a consequence of the homogeneous composition of the studied sample. All twins included were born

in the Belgian province of East Flanders, which is a relatively small but rather densely populated area [36]. Another factor possibly contributing to the high heritability estimates is age, as shown by the fact that several large twin studies have reported a decrease in the heritability of obesity (BMI) in adults with increasing age [5, 12, 16]. Our twin sample is young (mean=25 years) and the age range is small (18–34 years). As the power to detect quantitative trait loci is positively correlated with the size of the genetic effect [3], our study suggests that the search for candidate genes might be more efficient in a young homogeneous population.

In conclusion, this study is the first large study to give a comprehensive overview of the heritabilities of multiple risk factors related to type 2 diabetes within the same twin sample. The variation of traits related to obesity, glucose intolerance/insulin resistance and dyslipidaemia in this Belgian twin sample is in general highly explained by genetic factors. These high heritabilities are very promising for follow-up to this research, in which, in order to explain some of the genetic variance observed, sib-pair linkage analyses will be performed using microsatellite markers located in the close vicinity of selected candidate genes.

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