

CONTRIBUTION OF GENES TO THE CHANGES ON BODY COMPOSITION COMPONENTS: A TWO-YEAR LONGITUDINAL STUDY IN A SMALL COHORT OF TWINS

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Abstract

Context. High heritability of body composition variables is well-known, however, longitudinal effect of genes is still unclear.

Objective. The aim of this study was to investigate the pattern of longitudinal changes in anthropometric variables in a small cohort of twins.

Design. Longitudinal classical twin study, performed in 2009 and in 2011 on the same twin pairs.

Subjects and Methods. Eighteen healthy adult Hungarian twin pairs (13 monozygotic [MZ], 5 dizygotic [DZ]; mean age 54.0 ± 15.2 years; average body mass index 24.4 ± 5.4 kg/m² in 2009 and 25.1 ± 4.7 kg/m² in 2011, respectively) recruited from the Hungarian Twin Registry underwent bioimpedance analysis (OMRON).

Results. Significant, higher positive intrapair correlations were detected in the longitudinal change in weight ($p < 0.05$), body fat mass ($p < 0.01$), non-fat mass ($p < 0.01$), and body mass index ($p < 0.01$) in MZ compared to DZ twins, suggesting the possibility of longitudinal genetic

determinants. Negative associations were observed with regard to the two-year change in waist and hip circumferences, suggesting the longitudinal role of environmental factors in these phenotypes.

Conclusions. The findings of the present human twin study suggest that weight, body fat mass, non-fat mass, and body mass index are determined genetically and longer exposure to pathologic environmental factors is necessary to elicit alterations in the regulation of these parameters. Longer-term confirmation in a larger sample is required to confirm these results.

Key words: genetics, body mass index, body fat mass, environment, heritability.

INTRODUCTION

Obesity is a complex condition of excessive fat accumulation linked to a variety of genetic and non-genetic risk factors. Obesity has reached epidemic proportions worldwide with more than

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one billion overweight adults, of which at least 300 million are clinically obese (1). Various epidemiological studies based on family, adoption, and twin relations indicate that genetic influences contribute substantially to variation in obesity (2, 3). Multiple twin studies have shown that a sizeable proportion of body weight, waist and hip circumference, and body mass index (BMI) variances are due to genetic variance, which usually ranges between 60% and 80% (4-7). Common obesity is polygenic, involving complex gene-gene and gene-environment interactions that contribute to multi-factorial obese phenotypes (8). Collectively, these findings suggest that gene-environment interactions in particular may increase the risk of obesity among those who are genetically predisposed to weight gain.

BMI is a simple but quantitative anthropometric measure of body fat based on height and weight that applies to adult men and women. A Danish study found that BMI is a good reflection of body fat (9). Several studies have investigated the interplay of genetic and environmental influences on anthropometric parameters related to obesity by studying twin cohorts. Two longitudinal studies indicated the stable heritability of BMI with follow-up times of 28 and 6 years, respectively (10, 11). However, some studies reported decreasing heritability across age (12), and yet others reported increasing values (13). To accept the theory of age-induced effects on heritability, one must assume that different genes become activated at different ages even if heritability does not change substantially. As recently reported, genetic effects may account for

a BMI heritability of 75% in men and 71% in women (14). A small German study consisting of 30 twins investigated several body composition traits by tetrapolar bioelectrical impedance analysis and found that genetic factors amounted to a variance of 65-82% (15). A large Danish study found a heritability of 58-63% for BMI, 59-63% for body fat percentage, 48-61% for waist circumference, and 52-58% for hip circumference (16).

Beyond BMI, little is known as to whether heritable factors may contribute to the pattern of change in body composition parameters during a lifetime. Therefore, the genetics of body composition change remains an important unresolved question (17). To the best of our knowledge, no longitudinal study has investigated the role of genetic effects on aging-related changes in body composition. Therefore, our aim was to investigate the pattern of longitudinal changes in the provided phenotypes of body composition components, assuming that not only the measured traits, but also their pattern of longitudinal change, could be genetically determined.

MATERIALS AND METHODS

Subjects and study design

Thirty-six healthy Hungarian adult twin subjects (18 twin pairs; 13 monozygotic [MZ] and 5 /four same-sex and one opposite-sex/ dizygotic [DZ] pairs; 3 males and 33 females; mean age 54.0 ± 15.2 years) who were participants of the International Twin Study 2009 were tested at the festival in 2009 and again two years later in 2011 recruited from the

Hungarian Twin Registry (18). In 2009, 160 Hungarian twin pairs were measured during two Hungarian twin festivals (Szigethalom, Ágfalva) or at two large hospitals in Budapest. The same 160 twin pairs were invited to participate in the follow-up study during the same twin festivals in Szigethalom and Ágfalva in July, 2011. Of the 160 twin pairs, only 19 twin pairs decided to participate again, mainly due to bad weather conditions on the days of the twin festivals. Exclusion criteria included pregnancy, foreseeable lack of compliance with test procedures, and patients having pacemakers. In the absence of genotyping and in order to maximize the accuracy of zygosity classification, we used a multiple-choice, self-reported questionnaire. Zygosity was assigned according to a seven-part, self-reported response (19). Participants were asked to fill out a questionnaire in order to report any clinical symptoms, a complete medical history of the previous two years, and any medication being taken for cardiovascular conditions. All study subjects gave informed consent prior to entering the study, which was conducted in full compliance with regulations of the Ethical Committee of Semmelweis University and thus meets the standards of the Declaration of Helsinki.

Assessment of body composition

Body composition was determined by a clinically validated, portable body consistency monitor (OMRON BF500, Omron Healthcare Ltd., Kyoto, Japan) using bioelectrical impedance analysis (20). The monitor sends a weak electrical current of 50 kHz and less than 500 μ A through the subject's body to determine the amount

of fat tissue. Muscles, blood vessels, and bones with high water content conduct electricity easily, while body fat has little electric conductivity. During the course of day, body water tends to gradually shift to the lower limbs making the ratio of water between the upper and lower body different in the morning and evening. Accordingly, the body's electrical impedance varies during the day. Use of electrodes for both hands and feet may reduce the influence of fluctuations on measured values. To further improve consistency, measurements were taken in the late morning hours and in the afternoon. To avoid electromagnetic interference, subjects with pacemakers were excluded from the study. Subjects were asked not to eat 1 hour, not to drink coffee and not to smoke for 3 hours, and not to drink alcohol for 10 hours before the exam.

Under the guidance of a trained assistant, current height, age, and gender information were entered into the monitor in order to generate results. The subjects were instructed to stand on the monitor barefooted with straight knees and back, looking straight ahead with horizontally raised arms and extended elbows. The extended arms were at a 90° angle to the subject's body until the BMI, total body fat, and fat-free mass were calculated. Body fat percentage refers to the amount of body fat mass as a fraction of total body weight, and was calculated as follows:

$$\text{Body fat percentage (\%)} = [\text{Body fat mass (kg)} / \text{Body weight (kg)}] \times 100$$

Fat-free mass was calculated as: 100% - body fat percentage (%).

Waist and hip circumferences were measured by placing a measuring tape in a horizontal plane mid-way between the top of the iliac bone and the bottom of the rib cage, and at hip level, respectively. The same device was used in both years by the same personnel.

Statistical analysis

A descriptive analysis (mean, standard deviation, and percentage for categorical variables) for the investigated body composition parameters was conducted by SPSS Statistics 17. P values less than 0.05 were considered significant between MZ and DZ twin groups using independent-sample t-tests.

First, the intrasubject differences of body composition parameters during the two-year follow-up period were calculated for each MZ and DZ twin subjects. Next, the intrapair changes during the two-year follow-up period were calculated for both zygosity groups

by extracting the intrasubject difference for twin B from the same value for twin A. The average values in MZ and DZ groups were calculated separately and compared by independent-sample t-test. If the change in two-year intrapair similarity for a phenotype is smaller in MZ than DZ pairs, there is evidence for genetic influence. Finally, the age-adjusted intraclass (partial) correlation of intrapair changes in body composition parameters during the two-year follow-up period was calculated for MZ and DZ twins. Higher intraclass correlation in MZ twins compared to DZ indicates a genetic effect.

RESULTS

Subject characteristics

Baseline characteristics of the study subjects according to zygosity are presented in Table 1. Regardless of

Table 1. Baseline characteristics of study subjects

	Total (n=36)*	Monozygotic (n=26)*	Dizygotic (n=10)*
Male:female (n)	3:33	2:24	1:9
Age (2009), years [‡]	54.0±15.2	50.0±15.0	64.4±10.3 [‡]
weight (2009), kg [‡]	66.8±17.1	64.1±17.6	73.7±14.5
weight (2011), kg [‡]	68.3±14.7	66.3±14.6	73.5±14.3
waist circumference (2009), cm [‡]	88.4±13.2	86.1±12.6	93.8±13.7 [§]
waist circumference (2011), cm [‡]	83.8±12.8	80.2±12.2	93.2±9.2
hip circumference (2009), cm [‡]	98.6±12.8	96.5±93.6	103.5±12.9
hip circumference (2011), cm [‡]	96.8±13.3	93.6±13.2	105.3±10.0 [§]
body fat mass (2009), % [‡]	30.6±11.1	28.2±10.6	36.4±10.2 [§]
body fat mass (2011), % [‡]	33.4±8.9	32.1±8.3	36.8±10.1
non-fat mass (2009), % [‡]	69.4±11.1	71.8±10.6	63.6±10.2 [§]
non-fat mass (2011), % [‡]	66.6±8.9	67.9±8.3	63.2±10.1
BMI (2009), kg/m ^{2‡}	24.4±5.4	23.2±5.1	27.4±5.2 [§]
BMI (2011), kg/m ^{2‡}	25.1±4.7	24.2±4.3	27.3±5.2

*number of twin pairs; ‡mean ± standard deviation; §p<0.05; †p<0.01, \$p<0.005
BMI, body mass index

zygosity, female gender dominated in both groups. Dizygotic participants were significantly older than MZ participants ($p < 0.001$). Some significant differences were observed between baseline anthropometric parameters of MZ and DZ twins ($p < 0.05$ or $p < 0.005$).

Changes in body composition parameters over time

No significant intrasubject changes were observed in body composition parameters of MZ and DZ twin subjects during the two-year follow-up period (Table 2). Borderline significance was observed regarding the change in body fat mass and non-fat mass between MZ and DZ cohorts ($p = 0.052$). In addition, changes in the

absolute values of the measured body composition parameters were calculated for each twin pair. No significant differences were observed in the average intrapair change in body composition parameters within MZ and DZ twin pairs during the two-year follow-up period (Table 3).

Concordance in the direction of intrapair change in body composition parameters

Concordance of intrapair change examined similarities in the direction of change among twin pairs. There were significantly more concordant pairs in the MZ *versus* DZ cohort with regards to the direction of change in weight, body mass, non-fat mass, and BMI.

Table 2. Intrasubject changes in body composition parameters during the two-year follow-up period

	Monozygotic* (n=26)‡	Dizygotic* (n=10)‡
weight, kg	2.23±5.5	-0.27±2.5
waist circumference, cm	-4.44±5.8	-0.60±8.4
hip circumference, cm	-1.29±8.1	1.8±3.9
body fat mass, %	4.98±6.9§	0.44±2.6
non-fat mass, %	-4.98±6.9§	-0.44±2.6
BMI, kg/m ²	1.03±2.7	-0.09±0.9

*mean ± standard deviation; ‡number of twin subjects; § $p = 0.052$
BMI, body mass index

Table 3. Intrapair changes in body composition parameters during the two-year follow-up period

	Monozygotic* (n=13)‡	Dizygotic* (n=5)‡
weight, kg	2.61±4.2	4.26±2.1
waist circumference, cm	5.29±3.0	4.80±4.5
hip circumference, cm	8.75±7.1	2.80±2.5
body fat mass, %	3.26±3.1	2.92±4.2
non-fat mass, %	3.26±3.1	2.92±4.2
BMI, kg/m ²	1.39±2.1	1.31±0.6

*mean ± standard deviation; ‡number of twin pairs
BMI, body mass index

There were also concordant MZ twin pairs with regards to changes in waist and hip circumferences; however, the relationship was not significant (Table 4). Finally, the age-adjusted intraclass (partial) correlation of intrainpair changes in body composition parameters (weight, body fat, non-fat mass and BMI) was higher in MZ twins compared to DZ twins during the two-year follow-up period, indicating a genetic effect (Table 5).

DISCUSSION

Genetic studies have demonstrated that the relative contribution of

genes to a phenotype usually decreases over time, resulting in lower estimates of heritability. This decrease is due, in part, to the accumulation of various environmental factors that tend to increase the total phenotypic variance while maintaining a constant genetic variance. On the other hand, different sets of genes could be contributing to the variance of a phenotype over time. The data obtained from twin studies represent an opportunity to investigate these hypotheses. By comparing identical twins (who share 100% of their genes) with non-identical twins (who share 50% of their genes), twin studies

Table 4. Concordance in the direction of intrainpair changes in body composition parameters during the two-year follow-up period

	Monozygotic (n=13)*	Dizygotic (n=5)*
weight, kg	7 (26.9) [‡]	0 (0.0)
waist circumference, cm	9 (34.6)	5 (50.0)
hip circumference, cm	7 (26.9)	5 (50.0)
body fat mass, %	10 (38.5) [§]	1 (10.0)
non-fat mass, %	10 (38.5) [§]	1 (10.0)
BMI, kg/m ²	9 (34.6) [§]	0 (0.0)

*number of twin pairs (% of concordant answers in parentheses); [‡]p<0.05; [§]p<0.01
BMI, body mass index

Table 5. Age-adjusted intraclass (partial) correlation of intrainpair changes in body composition parameters during the two-year follow-up period

	Monozygotic (n=13)*	Dizygotic (n=5)*
Δweight	0.670 [‡]	-0.990 [‡]
Δwaist circumference	0.660 [‡]	0.974 [‡]
Δhip circumference	0.109	0.532
Δbody fat mass	0.658 [‡]	-0.427
Δnon-fat mass	0.658 [‡]	-0.427
ΔBMI	0.753 [§]	-0.965 [‡]

*number of twin pairs; [‡]p<0.05; [§]p<0.01
BMI, body mass index

produce information on the relative contribution of genes and environment. Genetic studies using the twin design are based upon the assumption that twins are representative of the general population for the outcomes being studied. To our knowledge, this is the first longitudinal twin study investigating the relative contribution of genetic factors to changes in body composition variables, assuming that not only the measured traits, but also their pattern of longitudinal change, could be genetically determined. We chose these dynamic phenotypes because of their known tendency to increase during adult lifespan and they can be comprehensively evaluated by repeated measurements over the span of the study. Our analysis is based on data obtained from a cohort of adult twin pairs during a short time interval. We report that there was a suspected genetic influence during the two-year change in weight, body fat mass, non-fat mass, and BMI, but no significant influence on waist and hip circumferences.

During the two-year follow-up period, weight, BMI, and body fat slightly increased overall, while non-fat mass, waist circumference, and hip circumference decreased (Table 1). Since two previous longitudinal studies indicated stable heritability of BMI (10, 11) and there is strong evidence for the high heritability of body weight, waist and hip circumference, and BMI variances (4-7), we hypothesized that the heritability of these parameters would remain stable. Our short-term results seem to confirm stable heritability, not only for BMI, but also for other body composition variables. Although we

could not calculate exact heritability using the classical A-C-E model due to our sample size, the significantly higher concordance rate for the direction of change in body composition as well as the higher, significant intraclass correlation of MZ twins compared to DZ twins suggests the role of genetic factors. The short-term change in waist and hip circumferences showed no genetic influence, which may be associated with the increasing role of epigenetic factors. To date, no other longitudinal study has investigated the role of changes in the genetic determination of waist and hip circumferences by age. According to our short-term findings, genetic determination tends to decrease by aging for these phenotypes. However, we could not confirm the exact extent of change in heritability (whether it increases or decreases) because of the lack of A-C-E analysis due to sample size.

Adverse body composition is associated with co-morbidities such as type 2 diabetes mellitus and other metabolic abnormalities, respiratory disturbances, cardiovascular conditions, and increased risk for cancer, particularly malignancies of the digestive system (21-24). Based on the longitudinal genetic determination of most of the body composition variables as corroborated by our findings, screening for these co-morbidities must be considered. Due to the possible increasing longitudinal role of environmental factors in waist and hip circumferences, the incidence of co-morbidities may occur with increasing waist and hip circumferences. It has to be taken into account that obesity-associated adverse health effects may not develop

in 'metabolically benign' obesity (i.e., obesity unaccompanied by hypertension, dyslipidemia, and diabetes) when body fat exerts a beneficial effect by soaking up toxic fatty acids and protecting from deleterious effects primarily associated with visceral obesity (25). However, women with metabolically benign overweight or obesity were reported to have a greater subclinical cardiovascular disease burden compared to normal weight women (26).

Our study has several limitations. Obvious limitations are the relatively small sample size (especially of the dizygotic pairs) and short observation period which limited a concrete conclusion of gene environment interactions. A major strength of the present study is that the tests were performed by the same trained researchers using the same instrument and novel findings are presented.

In conclusion, our study suggests a genetic influence during the two-year change in weight, body fat mass, non-fat mass, and BMI. These results indicate the possibility of short-term longitudinal genetic determinants in these phenotypes. In contrast, short-term longitudinal roles of environmental factors can be suspected for changes in waist and hip circumferences. Accordingly, our findings underscore the importance of lifestyle in the long-term, primary prevention of increased waist and hip circumferences. Large sample sizes and longer longitudinal observation periods are warranted for confirmation of our findings. The phenotypes derived from longitudinal data could then be used to find the locations of genes involved in

long-term regulation of the investigated anthropometric variables, since those long-term regulatory genes may play key roles in common diseases with late onset.

Conflict of interest

We declare that there is no conflict of interest.

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