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Monozygotic Twin Resemblance in Fatness and Fat Cell Lipolysis

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Abstract. Six skinfold measurements, and percent body fat and fat-free weight derived from the underwater weighing technique were obtained in 43 pairs of male and 44 pairs of female monozygotic (MZ) twins. A fat tissue biopsy was performed in the suprailiac region in 20 male and 16 female pairs in order to determine mean adipocyte diameter and basal lipolysis as well as epinephrine maximally stimulated lipolysis (10^{-4} M). Twin resemblance in body fatness is clearly demonstrated by the analysis of the between MZ sibships over the within MZ shibship means of squares for all skinfold measurements, percent body fat and fat free weight ($P < 0.01$). Within MZ pair similarity is as high in female as in male pairs for body fatness. Moreover, members of the same twin pair resemble one another significantly for fat cell size and fat cell lipolytic activities, particularly when epinephrine stimulated. In female MZ pairs, additional studies with control over the menstrual cycle are needed to clarify the case of isolated fat cell basal lipolysis.

Key words: Body fat, Sex differences, Twins, Adipocyte diameter, Fat cell lipolysis

INTRODUCTION

Body fatness is an important health-related physical attribute whose variations are determined by both genetic and environmental factors. In experiments designed to investigate the contribution of heredity to body fatness variation, different approaches have been used. Several investigators have studied skinfold measurements in biologically related brothers and sisters [1,6,11,14,15]. Savard et al [19] have studied familial similarity in fatness in 481 relatives from 114 families and found significant covariation in adiposity between biologically related individuals after controls over energy intake and energy expenditure, thus suggesting a genetic effect in body fatness.

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Other investigators have considered skinfold data in pairs of twins living together [10,16]. Brook et al [3] measured triceps and subscapular skinfolds in 222 pairs of twins (78 MZ and 144 DZ), aged 3 to 15 years, and obtained high heritability estimates in both sites after the age of 10.

These studies generally suggest that genetic variation could be of considerable importance in the determination of body fatness. However, these results have relied only on skinfold data as indicator of total body fatness. In the present experiment, several indicators of body fatness (six subcutaneous skinfolds, percent body fat, fat free weight) have been obtained in 43 male and 44 female MZ twin pairs. Moreover, measurements of isolated fat cell morphology and metabolism have been obtained from a subsample of MZ pairs of both sexes.

METHODS

Subjects

Subjects of this study were recruited from the greater Quebec city area. Forty-four female (mean age, 16.9 ± 4.4 years) and 43 male (16.8 ± 4.7 years) MZ twin pairs agreed to participate. Among them, 20 pairs of males and 16 pairs of females (all above 18 years of age) gave their written consent and were biopsied for a sample of adipose tissue in the suprailiac region.

Twin zygosity was established from questionnaire and from several red blood cell antigen and enzyme markers as well as from the A, B and C loci of the HLA system.

Body weight of male twins reached a mean of 55.3 kg (SD = 16.1), while mean percent body fat was 11.6% (SD = 5.7). In the case of female twins, mean body weight and body fat were 46.3 kg (SD = 10.0) and 20.0% (SD = 5.8), respectively.

Measurements of Body Composition and Skinfolds

Percent body fat was estimated from body density obtained by underwater weighing, using the Siri [20] equation. Underwater weighing was performed in the fasting state after a moderate inspiration. The mean of six valid measurements was used in the calculation of body density. Water temperature was recorded after each trial.

Residual volume was assessed by the method of Wilmore et al [24]. Fat free weight was computed from percent body fat and body weight in kg.

Subcutaneous skinfolds were measured on the left side of the body with a Harpended skinfold caliper as recommended by the International Biological Program [23]. Six skinfold were used in the study: biceps, triceps, subscapular, suprailiac, abdominal, and calf. The sum of 6 skinfolds was also chosen as another fatness indicator.

Adipose Tissue Biopsy and Adipocyte Isolation

The biopsy of subcutaneous fat was performed as previously described [5]. Briefly, after an overnight fast, subjects were locally anesthesized in the suprailiac region with xylocaine 1% and 200 mg of adipose tissue were removed using the Ritthaler et al [17] technique. Adipocytes were collagenase isolated for 25 min by a modification [5] of Rodbell method [18].

The digested tissue was then filtered and washed 4 times to eliminate collagenase. An aliquot of the final cell suspension was taken. Fat cell size and concentration were measured using a Leitz microscope equipped with an hemacytometer and a graduater ocular. Five hundred cells were counted per subject.

Measurement of Fat Cell Lypolysis

Incubation conditions were a modification [5] of the procedure of Bukowiecki et al [4]. Briefly, a 350 μl of the final adipocyte suspension is incubated for 30 min in polyethylene vials containing a final volume of 1.5 ml of Krebs Ringer bicarbonate buffer (glucose 50 mg/100 ml, albumin 4%) maintained at 37°C under a 95% 0_2/5% CO_2 atmosphere. Basal and maximal epinephrine stimulated

Table 1 - Body Fatness Indicators in MZ twins

Variable	MZ males					MZ females				
	N pairs	MS_b	MS_w	F	r_i	N pairs	MS_b	MS_w	F	r_i
Triceps skinfold[1]	43	0.04	0.00	12.12	0.85	44	0.03	0.01	5.29	0.68
Biceps skinfold[1]	43	0.03	0.00	9.80	0.81	44	0.04	0.01	7.60	0.77
Subscapular skinfold[1]	43	0.03	0.00	17.17	0.89	44	0.04	0.00	10.31	0.82
Suprailiac skinfold[1]	43	0.11	0.00	23.50	0.92	44	0.08	0.00	18.41	0.90
Abdominal skinfold[1]	43	0.09	0.00	19.00	0.90	44	0.09	0.01	12.64	0.85
Calf skinfold[1]	43	0.05	0.01	8.45	0.79	44	0.05	0.01	7.11	0.75
Σ 6 skinfolds[1]	43	0.04	0.00	16.40	0.88	44	0.04	0.00	9.41	0.81
% body fat	37	58.93	6.02	9.79	0.81	39	53.26	10.10	5.27	0.69
Fat free weight	37	383.21	3.00	127.77	0.98	39	122.95	2.3	53.38	0.96

MS_b = Mean squares between, MS_w = mean squares within pairs; r_i = intraclass correlation coefficient. All F ratios and coefficients significant: $P < 0.01$.

[1] Scores transformed in $\log_{10}$.

Table 2 - Adipocyte Diameter and Lipolytic Activity Measurements in MZ twins

Variable	MZ males					MZ females				
	N pairs	MS_b	MS_w	F	r_i	N pairs	MS_b	MS_w	F	r_i
Mean adipocyte diameter	20	286.85	25.78	11.1*	0.84*	16	187.6	18.22	10.3*	0.82*
Basal lipolysis	20	0.02	0.00	12.7*	0.85*	16	0.01	0.00	2.3	0.39
Maximal epinephrine stimulated lipolysis	20	0.72	0.19	3.8*	0.58*	16	0.50	0.09	5.7*	0.70*

MS_b = mean squares between, MS_w = mean squares within pairs; r_i = intraclass correlation coefficient.

* $P < 0.01$.

lipolysis are measured by assessing fluorometrically [13] the release of glycerol from the isolated fat cell. A concentration of 100 μM of epinephrine has been shown to yield a maximal stimulation of suprailiac fat cell lipolysis [5]. Incubation is stopped on ice and adipocytes are removed by aspiration. Glycerol assays were performed in triplicate. Results were expressed in μmol of glycerol released in 30 min per 10^6 cells.

Statistical Analysis

The analysis of variance and the F ratio computed from the between sibship over the within sibship means of squares were obtained as outlined by Haggard [7]. Intraclass correlation coefficients were computed according to procedures described by Winer [25]. Tests for homogeneity of a subset of correlations were performed following the procedures described in Snedecor and Cochran [21].

RESULTS

Table 1 describes the results of the analysis of variance along with the intraclass correlation coefficients (r_i) for body fatness indicators of the study. In both sexes, ANOVA revealed a significant within-pair resemblance (all F ratios < 0.01) in all skinfolds, in percent body fat and in fat free weight. Intraclass coefficients ranged form 0.79 to 0.98 in males and from 0.68 to 0.96 in females. Interclass correlations were also computed for each fatness indicator within each sex separately and chi-square tests for the homogeneity of correlations revealed no significant differences between males and females.

Sample of adipose tissue were obtained in a subset of these MZ twins for the purpose of fat morphology and metabolism analysis. Results of these studies are presented in Table 2. Significant within-pair resemblance is observed for mean adipocyte diameter and maximal epinephrine stimulated lipolysis. However, only male MZ pairs have a significant level of similarity in basal lipolysis.

DISCUSSION

The present study suggests that sibs of the same sex, genetically identical by descent, are very similar in body fatness. Indeed, intraclass coefficients computed separately in each sex for body fatness indicators range from 0.79 to 0.98 in males and from 0.68 to 0.96 in females. No sex difference in the within MZ pair resemblance could be found in body fatness.

A significant within-pair resemblance for mean adipocyte diameter was observed in both sexes. In this case, the twin resemblance was identical to that found for the non-invasive fatness indicators. To our knowledge, isolated fat cell diameter has never been assessed before in MZ twins.

Several studies have provided heritability estimates for body fatness using non-invasive indicators of fat morphology. Bouchard et al [2] studied somatotype components in 239 families and obtained significant heritability estimates for the level of endomorphy, a component of somatotype associated with body fat. Savard et al [19] reported a significant covariation in biological relatives for body fatness measurements, even after statistical control over energy balance and socioeconomic indicators. Brook et al [3] measured triceps and subscapular skinfold thicknesses in MZ and DZ twins and obtained high heritability estimates above the age of 10. These studies, along with the data reported herein, generally support the hypothesis that human variation in body fat is not independent of the genotype.

The present study also demonstrates that a significant contribution of heredity to fat cell lipolysis is entirely possible. In both sexes, the lipolytic response of isolated fat cell to a maximal catecholamine challenge tends to be similar for members of the same twin pair. However, only male twins exhibited significant covariation in basal lipolysis. In the present study, the phase of the menstrual cycle at the time of the fat biopsy was not controlled in women. Sex hormones have been shown to alter fat cell metabolism. Thus, recent studies have suggested that gonadal steroids were of great importance in the control of food intake and fat deposition [22]. These hormones could induce changes in fat cell lipolysis, lipogenesis and lipoprotein lipase activity [8,9,12]. One can consider the possibility that members of the same female pair were not biopsied at the same phase of their menstrual cycle, thereby masking the presumed similarity in fat cell basal lipolysis for females genetically identical by descent. However, one must remain cautious in this interpretation of the data as a significant within MZ female pair resemblance was observed for maximal epinephrine stimulated lipolysis, despite this lack of control over the phase of the menstrual cycle. Further investigation will have to be undertaken in order to establish whether isolated fat cell lipolysis in females is associated with sex hormone variation and/or the phases of the menstrual cycle.

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