

# Independent Genetic Factors Determine the Amount and Distribution of Fat in Women after the Menopause\*

K. SAMARAS, T. D. SPECTOR, T. V. NGUYEN, K. BAAN, L. V. CAMPBELL,  
AND P. J. KELLY

*Twin Research Unit (T.D.S., K.B., P.J.K.), St. Thomas' Hospital, London SE1 7EH, United Kingdom; Garvan Institute of Medical Research (K.S., T.V.N., L.V.C.), Sydney, Australia; and Department of Endocrinology (L.V.C., P.J.K.), St. Vincent's Hospital, Sydney NSW 2010, Australia*

## ABSTRACT

Central adiposity is a strong predictor of cardiovascular disease in women. We studied postmenopausal twins to explore the strength and the relationship between genetic influences on body fat and its distribution in a group where cardiovascular disease is the major cause of mortality. Healthy twin women were recruited from a national media campaign. One hundred nineteen monozygotic (MZ) and 97 dizygotic twin pairs were studied (mean  $\pm$  SE age  $60 \pm 0.3$  yr,  $10 \pm 0.4$  yr post menopausal). Total and central body fat were measured by dual-energy x-ray absorptiometry. Intrapair resemblance was significantly greater in MZ pairs for total fat (MZ vs. dizygotic,  $r = 0.70 \pm 0.05$  vs.  $r = 0.46 \pm 0.08$ ,  $P = 0.005$ ) and central fat ( $r = 0.62 \pm 0.06$  vs.  $r = 0.35 \pm 0.09$ ,  $P = 0.005$ ), suggesting a strong genetic influence

on these traits. Model-fitting analysis indicated that genetic factors contribute up to 60% of total population variance in both total and central body fat. The heritability of central fat remained, after adjustment for the heritability of total fat, suggesting an independent genetic influence on fat distribution. These results were unchanged after adjusting for the effects of estrogen replacement and smoking.

In conclusion, total adiposity and central abdominal fat mass in normal postmenopausal women are under strong genetic influence. The data suggest that some of the genes responsible for central adiposity and its metabolic sequelae will be different from those responsible for total adiposity. (*J Clin Endocrinol Metab* 82: 781–785, 1997)

**O**BESITY is increasingly prevalent and is associated with increased risk for cardiovascular disease and noninsulin dependent diabetes mellitus (NIDDM). This risk relates more to the distribution than to the amount of fat. Central adiposity predicts insulin resistance in the obese and non-obese (1–3), as well as development of NIDDM (4, 5) and cardiovascular disease (6–9). The Nurses Health Study found the relative risk of death from coronary disease was increased eight times in women with a greater waist-hip ratio (10), an indirect estimate of central adiposity. Waist-hip ratio at baseline predicted cardiac morbidity and mortality at the 20-yr follow-up of the Gothenburg Study, independent of body mass index (BMI) and cholesterol level (11).

Despite consensus that obesity is strongly genetically determined, estimates of the proportion of variance of total body fat attributable to genetic factors vary between 5 and 80% (12–14). Possible explanations for this include use of different estimates of body fat (BMI, skinfolds, and weight), variations of study design (twin, family, and adoption), and different statistical approaches. Some studies have relied solely on self-reported weight and height for derived estimates of body fatness (14, 15). When body fat was assessed more directly, strong genetic effects were found (12, 13).

The determinants of fat distribution distinct from total fat mass are only partially understood. Genetic influences on fat

distribution have been suggested in a large family study, albeit using truncal and abdominal skinfolds as a marker of central adiposity (12). A genetic effect is inferred from over- and underfeeding experiments in monozygotic (MZ) twins, where changes in intraabdominal fat were measured by computed tomography (16, 17). The response to over- or underfeeding was more similar within pairs than between pairs (16, 17), implying that genetic factors direct the deposition of fat to different body stores. By studying only MZ twins, a contribution from intrapair environmental similarity can not be excluded. Strong genetic influences on total and abdominal fat have been demonstrated recently in a premenopausal Australian twin sample (18).

An accurate estimate of the genetic and environmental influences on adiposity and fat distribution in postmenopausal women is lacking, although it is this group for which coronary disease is the major cause of mortality. We present results from 216 pairs of postmenopausal twins.

## Materials and Methods

### Study sample

Data from women who participated in a twin study of the genetic regulation of bone density (19) were analyzed. Volunteers were recruited by a media campaign and were unaware of any hypotheses regarding adiposity. Studies were carried out at St. Thomas' Hospital, London, between September of 1992 and February of 1995. Twin pairs were assessed on the same day. Weight and height were measured. Zygosity was determined with a standardized questionnaire and confirmed with multiplex DNA fingerprinting.

Menopausal status and duration were determined by interview. The menopause was defined as amenorrhea greater than 12 months duration. The mean age ( $\pm$ SD) of menopause was  $48.7 \pm 4.5$  yr. An age of 57 (two SD above the mean menopausal age) was used to define meno-

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Address all correspondence and requests for reprints to: Dr. Paul Kelly, St. Vincent's Clinic, Victoria Street, Sydney NSW 2010, Australia. E-mail: paulk@infinet.net.au.

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pausal status in hysterectomized women ( $n = 134$ ) or where status was unknown ( $n = 11$ ). Data from 432 postmenopausal women were analyzed. Smoking habit and use of estrogen replacement were assessed by standardized questionnaire. Twenty percent of the subjects were using estrogen replacement, and 15% were current smokers.

### Measurements of fat distribution

Body composition was measured using dual-energy x-ray absorptiometry (DEXA) (Hologic QDR-2000, Vertec, Waltham, MA). Total fat was determined directly from the standard software calculation (software version 710). The accuracy of this method has been validated against other measures of body fatness: skinfold thickness  $r = 0.82$ ; bioelectrical impedance analysis  $r = 0.97$ ; deuterium oxide dilution  $r = 0.79$  (20). Scans were reanalyzed to quantify abdominal fat by a single-blinded investigator (K.S.). The central fat region was expressed both in kilograms and as percentage. The test-retest variability for the central fat measurement was 8%. DEXA measurement of central fat correlates strongly with insulin resistance (3), with fasting insulin levels (23), and with CT measures of intraabdominal fat ( $r = 0.99$ ) (24, 25), despite the inclusion of some sc fat (26).

### Statistical analyses

Using the twin model, the observed variance in body fat is partitioned into genetic and environmental components. Because MZ twins share 100% of their genes, any differences between them are theoretically caused by differences in environment and measurement error. Dizygotic (DZ) twins are no more genetically alike than siblings, sharing 50% of their genes; any differences between them are caused by both environmental and genotypic differences. The extent to which MZ pairs are more similar than DZ pairs in body fat reflects the genetic contribution to the population variance in body fat. The twin model assumes: there is no gene-gene interaction (epistasis); there are no dominant genetic effects; and there is no interaction between genetic and environmental factors (27). Further assumptions are: that environmental covariance is equal in both MZ and DZ twin pairs; and that there is equality of total variances between the two zygositys.

### Estimates of heritability

*Within twin-pair correlations.* Intraclass correlation coefficients were used as a measure of within-pair similarity and derived as follows. Let  $y_{ij}$  be a fat measurement for a  $j$ th individual ( $j = 1, 2$ ) within an  $i$ th pair ( $i = 1, 2, 3, \dots, N$ ); then  $y_{ij}$  was modeled as being equal to an overall mean  $\mu$  plus or minus the additive effects of the  $i$ th pair (denoted by  $a_i$ ) and residual errors (denoted by  $e_{ij}$ ), i.e.  $y_{ij} = \mu + a_i + e_{ij}$ . Under the assumptions that  $a_i$  and  $e_i$  are both normally independently distributed with zero mean and variance  $\sigma_a^2$  and  $\sigma_e^2$ , respectively, then the intraclass correlation ( $r$ ) is defined as:  $r = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$ . To estimate  $r$ , an ANOVA was performed to partition the total variation of abdominal fat into two parts, namely between- and within-pair variations. The estimate of  $r$  is given by the difference between between-pair and within-pair over their sum. If rMZ is significantly greater than rDZ, a genetic influence is suggested. If rDZ is greater than zero, a simple index of heritability can be calculated from the formula: heritability =  $2(rMZ - rDZ)$ .

*Model-fitting analysis.* Model-fitting analyses were used to estimate the genetic contribution to the population variance in each trait more robustly, to adjust for potential confounders, and to estimate the genetic influence on central fat independent of that upon total body fat.

Variances-covariances among twins were expressed as a function of three effects: genetic (G) (additive and nonadditive); common environmental (C); and influences specific to an individual twin, including measurement error (E). Assuming the nonadditive genetic effect to be zero, three models were tested: model 1 incorporates the effects of G, C, and E; model 2 incorporates G and E; and model 3 incorporates C and E. Comparison of models 1 and 2 is a test for the effect of common (shared) environment, whereas comparison of models 1 and 3 is a test

for a genetic effect. The statistical criterion of comparisons between models was based on the likelihood ratio statistic. Index of heritability (proportion of phenotypic variance accounted for by additive genetic factors) was calculated using estimates of model 1 as the ratio of variance due to G divided by total phenotypic variance.

## Results

The characteristics of 119 MZ and 97 DZ twin pairs are presented in Table 1. Age, weight, and BMI distributed similarly in both groups. Weight and total fat (kg and %) were slightly lower in MZ twins.

Both total and central fat were significantly related to weight ( $r = 0.78, P < 0.001$ ; and  $r = 0.71, P < 0.001$ , respectively). Total fat correlated to central fat ( $r = 0.83, P = 0.0001$ ). No relationship was found between fat measures and age and duration of menopause (data not shown). BMI was related to both total fat and central fat ( $r = 0.68, P = 0.0001$ ; and  $r = 0.67, P = 0.0001$ , respectively).

Intraclass correlation coefficients for all fat measures were significantly greater in MZ twins (Table 2, Fig. 1), supporting strong genetic influences on these traits. For BMI, however, there was no difference between rMZ and rDZ, suggesting no genetic influence in this group.

### Model fitting analysis

Further analysis was performed to quantify the genetic contribution to the variance in fat mass and ascertain whether total and central fat were under independent genetic influences. Possible confounding factors were also addressed. Twenty MZ and 21 DZ pairs were discordant for estrogen replacement, and 33 MZ and 46 DZ pairs were discordant for current smoking. Current smokers had 4% lower total fat ( $P = 0.02$ ) and 4% lower central fat ( $P < 0.005$ ) compared with nonsmokers. Estrogen replacement was associated with 3% lower total fat ( $P = 0.02$ ) and 4% lower central fat ( $P < 0.005$ ) compared with nonusers. The intrapair correlations were unchanged after adjusting for weight, smoking, estrogen replacement, and (in the case of central fat) total fat mass (Table 3). Based on model 1, the heritabilities (proportion of population variance attributable to genetic factors) of total and central fat were  $56 \pm 17\%$  and  $64 \pm 20\%$ , respectively. The heritability of central fat was not reduced after adjustment for total fat, suggesting independent genetic regulation of central fat. Removal of the common environmental source of variance (model 2) did not improve the fit of the model to the observed data, suggesting

**TABLE 1.** Characteristics of the postmenopausal cohort (mean  $\pm$  SE)

Variable	MZ	DZ	P value
Number of pairs	119	97	
Age (yr)	59.8 $\pm$ 0.3	59.7 $\pm$ 0.4	0.84
Wt (kg)	61.7 $\pm$ 0.6	64.0 $\pm$ 0.8	0.02
BMI† (kg/m <sup>2</sup> )	24.1 $\pm$ 0.2	24.5 $\pm$ 0.3	0.24
Age at menopause (yr)	48.8 $\pm$ 0.3	48.6 $\pm$ 0.4	0.64
Duration menopause	10.6 $\pm$ 0.4	10.4 $\pm$ 0.5	0.79
Total fat (kg)	23.6 $\pm$ 0.5	25.8 $\pm$ 0.6	0.004
% Total fat	37.2 $\pm$ 0.4	39.0 $\pm$ 0.5	0.01
Central fat (kg)	1.5 $\pm$ 0.04	1.6 $\pm$ 0.05	0.07
% Central fat	36.6 $\pm$ 0.6	38.1 $\pm$ 0.7	0.11
Fat-free mass (kg)	36.9 $\pm$ 0.3	36.9 $\pm$ 0.3	0.84

that if common environmental factors regulate fat mass, their effects are weak in postmenopausal Caucasian women.

**Discussion**

Although the prevalence of obesity is increasing worldwide, the adverse health impact of adiposity may lie in its site rather than its total mass. The centrally obese individual suffers an increased risk of heart disease, stroke, hypertension, and diabetes. After the menopause, coronary disease rises in women to approximately that in men (28), and a role for central adiposity in this increment is implied in a number of studies (6–9). The relative risk of death caused by coronary disease is increased 8 times in women with abdominal fat deposition, independent of weight or BMI (10). Central adiposity predicts insulin sensitivity (1–3), and insulin resis-

tance is postulated to be a pivotal factor for the development of atheroma in the metabolic syndrome (29).

Little has been published on the genetic regulation of regional fat deposition in women. By using a large sample of twins, this study demonstrates that in postmenopausal women, genetic factors explain the majority of the population tendency to deposit fat centrally. Importantly, multivariate analysis shows that after adjusting for the genetic influences on total fat mass, the strength of the genetic effect on central fat was unchanged. This indicates that in postmenopausal women, specific genetic factors determine central deposition of fat with its metabolic correlates, including the risk of cardiovascular disease.

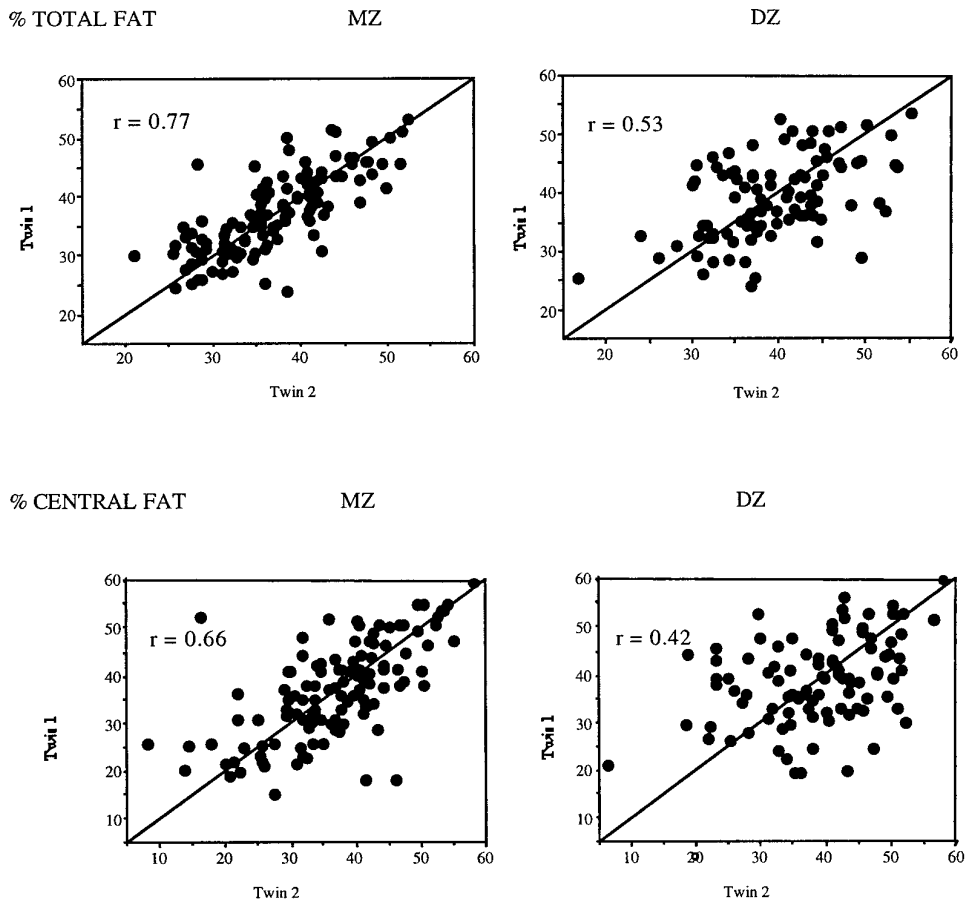
This study found that total fat is strongly heritable with up to 60% of the population variance caused by genetic factors, whereas previous estimates lie between 5 and 80%. The use of indirect estimates of body fat, variations of study design, and differing populations may explain this wide variation. In contrast to some of the studies, we studied women with a wide range of body weight and measured adiposity directly with DEXA. Several studies have used BMI (13–15), an indirect estimation of fatness, derived from self-reported weight and height (14, 15), with the risks of underreporting and unreliability. We found that although BMI correlated with measures of fat by DEXA, it was not accurate enough a measure of fat to reflect the differences in total fat between MZ and DZ pairs. Additionally, the rMZ and rDZ for BMI were similar, indicating that any genetic effect on BMI in this

**TABLE 2.** Unadjusted intraclass correlation coefficients ( $\pm$ SE) for MZ and DZ twin pairs

Variable	rMZ	rDZ	P value <sup>a</sup>	Heritability <sup>b</sup>
Number of pairs	117	97		
Wt	0.66 $\pm$ 0.05	0.46 $\pm$ 0.08	0.024	0.40
BMI	0.57 $\pm$ 0.02	0.51 $\pm$ 0.08	0.261	
Total fat	0.70 $\pm$ 0.05	0.46 $\pm$ 0.08	0.005	0.48
% Total fat	0.77 $\pm$ 0.04	0.53 $\pm$ 0.07	0.001	0.48
Central fat	0.62 $\pm$ 0.06	0.35 $\pm$ 0.09	0.005	0.54
% Central fat	0.66 $\pm$ 0.05	0.43 $\pm$ 0.08	0.008	0.47

<sup>a</sup> rMZ vs. rDZ.

<sup>b</sup> Heritability derived from intraclass correlation coefficients.



**FIG. 1.** Comparison of the intrapair correlations in MZ and DZ twin pairs for total and central body fat.

**TABLE 3.** The genetic influence on body fat distribution: analysis based on adjusted data

		Total fat <sup>a</sup>	Central fat <sup>b</sup>
Intraclass correlation ( $\pm$ SE)			
	rMZ	0.69 $\pm$ 0.05	0.63 $\pm$ 0.05
	rDZ	0.45 $\pm$ 0.08	0.31 $\pm$ 0.09
Model-fitting analyses: likelihood ratio statistics			
Model 1: G + C + E		3.61	8.52
Model 2: G + E		4.43	8.52
Model 3: C + E		16.82 <sup>c</sup>	20.18 <sup>c</sup>
P value model 1 vs. model 3		0.001	0.001
Estimated index of heritability ( $\pm$ SE) <sup>d</sup>		0.56 $\pm$ 0.17	0.64 $\pm$ 0.20

<sup>a</sup> Adjusted for current estrogen replacement therapy and current smoking.

<sup>b</sup> Adjusted for total fat, current estrogen replacement, and current smoking.

<sup>c</sup> Difference between models 1 and 3 at  $P < 0.001$ .

<sup>d</sup> Estimated heritability was based on model 1.

G, Additive and nonadditive genetic effects; C, common environmental effects; E, individual-specific environmental effects and measurement error.

group is weak and that within-pair similarity may be caused by similarities in environment. This is consistent with other studies (13, 30), where the heritability of BMI was 5%. There are several possible explanations. Genetic effects on BMI (or its nonfat components) may lessen with age or menopause. The accuracy of BMI as a marker of adiposity may decrease postmenopausally (e.g. caused by loss of height). Environmental effects on other components of BMI (such as muscle mass) may become more evident with age or after the menopause.

Environmental effects on adiposity also were evident. In this sample of twins, current smokers had lower total fat, consistent with larger cross-sectional studies (31–34). Central fat also was lower in smokers, although previous studies have reported an increase (33–36). These studies relied on waist-hip ratio as an index of abdominal adiposity, which relates weakly with central adiposity, particularly amongst postmenopausal women (37–39). Current estrogen replacement was associated also with lower total body fat and central fat, consistent with findings from cross-sectional (40) and prospective (41) work.

In conclusion, total adiposity and central abdominal fat mass in normal postmenopausal women are under strong genetic influence. Moreover, the data suggest that some of the genes responsible for central adiposity and its metabolic sequelae will be different from those responsible for total adiposity.

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