0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 12
Printed in U.S.A.

Familial Clustering of Insulin and Abdominal Visceral Fat: The HERITAGE Family Study*

YULING HONG, TREVA RICE, JACQUES GAGNON, JEAN-PIERRE DESPRÉS, ANDRÉ NADEAU, LOUIS PÉRUSSE, CLAUDE BOUCHARD, ARTHUR S. LEON, JAMES S. SKINNER, JACK H. WILMORE, D. C. RAO

Division of Biostatistics (Y.H., T.R., D.C.R.), Washington University School of Medicine, St. Louis, Missouri 63110-1093; Physical Activity Sciences Laboratory (J.G., J.-P.D., L.P., C.B.), Laval University, Québec GIK 7P4, Canada; Lipid Research Center (J.-P.D.), Laval University, Quebéc GIV 492, Canada; Diabetes Research Unit (A.N.), Laval University, Quebéc GIV 492, Canada; School of Kinesiology and Leisure Studies (A.S.L.) University of Minnesota, Minneapolis, Minnesota 55455; Department of Kinesiology (J.S.S.), Indiana University, Bloomington, Indiana 46202; Department of Health and Kinesiology (J.H.W.), Texas A&M University, College Station, Texas 77843; and Departments of Genetics and Psychiatry (D.C.R.), Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT

Abdominal visceral fat (AVF) is an obesity-related phenotype thought to be associated with insulin resistance, diabetes mellitus, and atherosclerosis. Significant genetic influences on both AVF and insulin levels have been reported. However, information is lacking as to whether common genetic influences on AVF and insulin levels exist.

AVF was assessed by computed tomography scan, and fasting insulin was measured by RIA in 512 members of 98 sedentary Caucasian families participating in the HERITAGE Family Study. Baseline data, collected before exercise training, were used in the present investigation. A bivariate familial correlation model was applied to evaluate whether there are familial influences that are common to insulin and AVF before and after adjustment for total fat mass (FM), and to assess the overall heritability of insulin and AVF. The maximal heritability for AVF, before and after adjustment for total FM, was 42% and 50%, respectively; and for insulin, it was 21%. Interestingly, 29% of the familial influences on insulin were also common to AVF, whereas 14% of the familial influences on AVF were shared by insulin. Furthermore, after AVF was adjusted for total FM, these common familial influences were increased to 48% and 20%.

Genes and/or familial nongenetic factors with pleiotropic effects seem to influence both AVF and plasma insulin levels to a certain degree. Genes involved in the regulation of lipid storage and mobilization in the abdominal fat depot are potential candidates for these genetic pleiotropic effects. (*J Clin Endocrinol Metab* **83:** 4239–4245, 1998)

INSULIN RESISTANCE, or low insulin sensitivity, and obesity are two important risk factors for atherosclerosis and noninsulin-dependent diabetes mellitus (NIDDM) (1–8) and represent two central features of the metabolic syndrome (9–12). Fasting insulin has been considered an acceptable measure of insulin resistance in population studies (13). A number of indices have been used to measure the degree and distribution of adiposity, such as body mass index (BMI); waist-to-hip ratio (WHR), a rough measure of the proportion of upper body fat (android obesity) relative to lower body fat (gynoid obesity); total fat mass (FM); skinfold thickness, such as the sum of several skinfold measures and trunk-to-extremity ratios, representing the distribution of sc fat; and abdominal visceral fat (AVF) level. These adiposity indices are correlated with each other and also with insulin resistance (14–16). The observed correlation between insulin resistance and obesity could be caused by causal associations, by common environmental or genetic backgrounds (pleiotropy), or both causation and common etiology. However, a causal association apparently cannot explain the entire correlation between obesity and insulin resistance. Alternatively, genetic or environmental factors common to both insulin resistance and adiposity indices, such as BMI, WHR, FM, skinfold measures, and trunk-to-extremity ratios, have been reported (15, 17–19). These results from quantitative genetic analyses have been supported by molecular findings that indicate that some genes influence both insulin resistance and BMI (20, 21).

More interestingly, AVF, a useful abdominal obesity phenotype, is considered by some to be one of the most important predictors of NIDDM and atherosclerosis (3, 10, 11, 22). Furthermore, the correlation between insulin resistance and AVF has been reported to be slightly stronger than correlations between insulin resistance and other adiposity indices in some studies (23–25), although not in all (26). Genetic influences on AVF have been reported (27–29), and these

Received June 4, 1998. Revision received August 19, 1998. Accepted August 24, 1998.

Address all correspondence and requests for reprints to: Dr. Yuling Hong, Division of Biostatistics, Campus Box 8067, Washington University School of Medicine, St. Louis, Missouri 63110. E-mail: yuling@wubios.wustl.edu.

* The HERITAGE Family Study is supported by the National Heart, Lung, and Blood Institute through the following grants: HL-45670 (to C.B., Principal Investigator), HL-47323 (to A.S.L., Principal Investigator), HL-47317 (to D.C.R., Principal Investigator), HL-47327 (to J.S.S., Principal Investigator), and HL-47321 (to J.H.W., Principal Investigator). A. S. Leon is also supported by NIH-funded University of Minnesota's Clinical Research Center (M01-RR-00400).

effects are slightly stronger than those on sc adipose tissue and are thought to be different from those involved in determining total body FM (28, 29). In addition, there are indications of major gene effects on AVF, although the issue has not been fully resolved yet (30, 31). However, whether insulin resistance and AVF share common genetic or environmental etiologies has not yet been explored.

In the present investigation, baseline data from the HERITAGE Family Study (32) on fasting insulin and AVF, as measured by computed tomography scan, were used. One unique feature of this data set is the control of a potentially confounding source (namely, physical activity levels), because these families were selected, among other criteria, to be sedentary. Cross-trait familial correlations between fasting insulin and AVF, before and after adjustment for total FM (AVF-FM), were evaluated to determine whether there are genetic and/or familial environmental influences common to both insulin resistance and central obesity.

Subjects and Methods

Study subjects

The HERITAGE Family Study is a multicenter exercise-training study. The main objective of the study is to assess the role of genetic factors in the cardiovascular, metabolic, and hormonal responses to aerobic exercise training in sedentary families. The HERITAGE sampling procedure, outlining the inclusion and exclusion criteria, has been described in detail elsewhere (32).

In brief, several criteria were used to screen subjects for participation. First, children were required to be 17–40 yr old, and parents were required to be 65 yr old or less, to reduce maturation (low end) and aging (high end) complications. Second, families were required to be sedentary, defined at baseline as having not engaged in regular vigorous physical activity over the previous 6 months (*i.e.* any activity lasting 30 min or more and involving a rate of energy expenditure of 7 METS or more (1 MET = 3.5 mL O_2 uptake per kg body weight per min and represents the rate of energy expenditure at rest) in individuals 50 yr old or more, or 8 METS or more for younger individuals, and occurring more than once a week. Families with some nonsedentary members were included, provided that the nonsedentary individual(s) remained inactive for at least 6 months. Third, individuals with a BMI greater than 40 kg/m^2 were usually excluded because of metabolic abnormalities and exercise difficulties associated with extreme obesity, unless shown to be capable of exercising on a cycle ergometer. Fourth, individuals with blood pressure levels greater than 159 mm Hg systolic and/or 99 mm Hg diastolic also were excluded. Fifth, individuals with any condition or disease that was life-threatening or that could be aggravated by cycle exercise were excluded (*e.g.* a malignancy; uncontrolled endocrine and metabolic disorders, including diabetes; definite or possible coronary heart disease; and chronic or recurrent respiratory problems). Finally, individuals requiring lipid-lowering or antihypertensive drugs were excluded.

In all, 98 nuclear families of Caucasian descent, each with both biological parents, and at least two biological children, were used for the present study. The sample size was 512 subjects. Families of African-American descent were also recruited, but their data are not reported here.

Measures

All participants underwent a battery of tests both before and after completing the 20-week standardized exercise training program. Results are limited to the baseline (preexercise training) evaluation in the present study.

Fasting plasma insulin. Blood samples were collected under EDTA, and the tubes were centrifuged at 1000 × *g*, at a temperature of 4 C, for 10 min. Plasma was kept frozen at −20 C until the time of assay. Plasma insulin levels were measured by RIA after polyethylene glycol separation, as described by Desbuquois and Aurbach (33). Polyclonal antibodies that cross-react more than 90% with proinsulin (and presumably, with its conversion intermediates) were used (34). Therefore, in this study [as in others (34, 35)], insulin refers to immunoreactive insulin (defined as the sum of insulin, proinsulin, and split-proinsulin). In the present cohort, with normal fasting glucose levels and no history of diabetes, it is estimated that about 10% of the immunoreactive insulin is in the form of proinsulin and its conversion intermediates (35). Insulin levels were treated as missing, for three individuals with insulin antibodies, four individuals with extremely low glucose disappearance rate, and one individual with both conditions. All the assays were performed at a central laboratory in Quebec. The intra- and interassay coefficients of variation were 7.7% and 10.3%, respectively.

AVF, abdominal sc fat (ASF), and total FM. AVF and ASF levels were measured by computed tomography scan (36). Subjects were examined in a supine position with their arms stretched above the head. The abdominal scan was obtained between the fourth and fifth lumbar vertebrae. The attenuation interval used in the quantification of the areas of adipose tissue ranged from −190 to −30 Hounsfield units. The AVF area was defined by drawing a line within the muscle wall surrounding the abdominal cavity. The ASF area was calculated by subtracting the amount of visceral fat from the total abdominal fat area. Underwater weighing was performed to determine FM, which was converted to percent fat by using the equation of Siri (37). A correction was made for residual lung volume by the oxygen dilution method (38). At the Laval University Clinical Center, residual lung volume was assessed by the helium-dilution technique (39).

Statistical analysis

Because fasting insulin tends to have a skewed distribution, the data were transformed using a natural logarithm before any data analysis.

Age adjustment. Adjustments for the effects of age on fasting insulin and AVF were carried out separately in the four sex-by-generation groups (fathers, mothers, sons, and daughters) using a stepwise multiple-regression procedure. Age, age^2, and age^3 were included in the regression model. The significance level for retaining the terms in the stepwise regression analysis was 5%. Age was a significant predictor for AVF in fathers (age term accounting for 6.3% of the variance), mothers (age^3 term explaining 4.7% of the variance), sons (age term accounting for 30.1% of the variance), and daughters (age^3 term explaining 17.1% of the variance). When FM was added into the regression model, FM and age were significant predictors for AVF in fathers (FM and age terms accounting for 29.9% and 3.2% of the variance, respectively), mothers (FM explaining 55.9% of the variance), sons (FM, age, and age^3 terms accounting for 73.3%, 4.7%, and 0.9% of the variance, respectively), and daughters (FM and age^3 explaining 57.5% and 4.9% of the variance). Age was also a significant predictor for fasting insulin in daughters (age and age^3 terms accounting for 11.4% of the variance) but not in fathers, mothers, and sons. Standardized residuals from the best regression models were used in the following bivariate familial correlation analysis.

Bivariate familial correlation model. The bivariate correlation model is the multivariate extension of the univariate case. In the univariate case, there are four types of family members (f, fathers; m, mothers; s, sons; and d, daughters), leading to eight interindividual correlations (fm, fs, fd, ms, md, ss, sd, and dd). In addition to estimating these interindividual correlations for each trait, the bivariate analysis also evaluates the inter- and intraindividual cross-trait correlations. For example, the interindividual cross-trait correlation between father's trait 1 and son's trait 2 (f_1s_2) and the intraindividual cross-trait correlation in fathers (f_{12}) are estimated. Here, subscript 1 refers to fasting insulin, and subscript 2 refers to AVF or AVF-FM. These cross-trait correlations are the focus of the current study.

The computer program SEGPATH (40) was used to estimate the familial correlations based on maximum likelihood methods. SEGPATH is a general-purpose program that can be used to generate any linear model for analyzing pedigree data and is based on flexible, model-specification syntax. Here, the statistical method of analysis fits the model directly to the family data, under the assumption that the phe-

notypes in a family follow jointly a multivariate normal distribution. The application of SEGPATH for bivariate analysis has been published elsewhere (16). In summary, 34 correlations are estimated in the bivariate analysis (in contrast to 8 in the univariate analysis). There are 18 cross-trait correlations: 14 interindividual, for siblings (s_1s_2, s_1d_2, s_2d_1, and d_1d_2), parent-offspring (f_1s_2, f_2s_1, f_1d_2, f_2d_1, m_1s_2, m_2s_1, m_1d_2, and m_2d_1), and spouse (f_1m_2 and f_2m_1); and 4 intraindividual (s_{12}, d_{12}, f_{12}, and m_{12}). The remaining 16 are interindividual correlations within a trait, 8 for each of the 2 traits (*e.g.* s_1d_1, s_1s_1, d_1d_1, f_1s_1, f_1d_1, m_1s_1, m_1d_1, and f_1m_1 for trait 1) (see Table 1 for details).

Hypothesis tests (Table 2). The significance of each set of familial correlations is tested by comparing the log likelihood of a reduced model, where some of the correlations are fixed to zero, against the log likelihood obtained from the general model, where all familial correlations are estimated. The likelihood ratio test, which is the difference between twice the log likelihoods, is distributed as a χ^2. The degrees of freedoms are given by the difference in the number of parameters estimated in the two nested models. A χ^2 with a P value of less than 0.05 is taken to suggest that the set of familial correlations (set to zero under a null hypothesis) is significant. Moreover, a P value less than 0.10, but greater than 0.05, will be considered here as indicating a correlation of marginal significance. The most parsimonious model is derived from combining all nonrejected models.

The familial patterns of the cross-trait correlations are the main focus of the present study. Under the assumptions that sibling and parent-offspring pairs share half of their genes, besides some familial environmental effects, and that spouse pairs share only familial environmental effects (provided mating is random, with regard to the two traits), significant cross-trait correlations among siblings and/or between parents and offspring, but not between spouses, would suggest that there are common genetic influences on the two traits. Significant spouse cross-trait correlations, in addition to sibling and/or parent-offspring cross-trait correlations, indicate that at least some of the familial effects may be caused by familial environments shared by the two traits. The intraindividual cross-trait correlations simply reflect the phenotypic correlation between the two traits (see Table 1 for details about model tests). The maximal cross-trait heritability can be computed using the following equation: $h^2 = (r_{sibling} + r_{parent\text{-}offspring})(1 + r_{spouse}) / (1 + r_{spouse} + 2\, r_{spouse} r_{parent\text{-}offspring})$, where, r represents the interindividual cross-trait correlations, and the heritability is adjusted for the degree of spouse resemblance, if present. The heritability for each trait also can be estimated, based on the above mentioned equation, where r represents interindividual correlations within a trait. It should be noted that this is a generalized heritability: both genetic and familial environmental (if significant) effects are included.

Results

Table 3 presents the means and SDs of the unadjusted fasting insulin and AVF levels by four sex and generation groups. Based on a comparison of SEs, there are sex and generation differences in fasting insulin and AVF. In general, parents have higher levels of fasting insulin and AVF than offspring, and males have higher levels of these two variables than females, in both generations. Means and SDs of BMI, FM, and ASF, across four sex and generation groups, are also given in Table 3, for comparison purposes.

The results of the hypothesis tests are summarized in Table 4. There are marginal cross-trait correlations in siblings and parent-offspring (models 5 and 6, with $P = 0.061$ and 0.092, respectively). The cross-trait spouse correlations are not significant (model 7, with $P = 0.325$), suggesting that any cross-trait family resemblance is likely to be caused by genetic influences in common to fasting insulin and AVF. Model 8 shows that there are significant intraindividual cross-trait correlations ($P < 0.001$), confirming the significant phenotypic correlations between fasting insulin and AVF. The hypothesis of no cross-trait correlations, at all (model 10, with $P < 0.001$), was rejected, and that of no interindividual cross-trait correlations (model 9, with $P = 0.069$) was borderline, as expected (given the results from the individual tests). Together, this pattern endorses familial etiologies that are shared by fasting insulin and AVF. In addition, no sex or generation differences in the familial correlations were found (models 2, 3, and 4).

The cross-trait familial correlations and SEs from both the general model and the most parsimonious model are given in Table 5. The intraindividual cross-trait correlation (0.484) was significant, on the basis of SE comparisons, in accordance with the results from likelihood ratio tests. Although not all cross-trait correlations in siblings and parent-offspring are significant, on the basis of SE comparisons, they were marginally significant as a group, based on the likelihood ratio test. The maximum cross-trait heritability between fasting insulin and AVF is 6% (calculated from the equation mentioned previously) (Table 6). Because the cross-trait spouse correlations were not significant, this heritability may be predominantly genetic in etiology. Based on the interindividual correlations within trait, heritabilities for fasting insulin and AVF are calculated as 21% and 42%, respectively (Table 6). Thus, of the heritability of 21% for fasting insulin, 6% is caused by genetic influences in common with AVF, suggesting that 29% (6% of 21%) of genetic influences on insulin are shared by AVF, whereas only 14% (6% of 42%) of the genetic influences on AVF are shared by fasting insulin. Therefore, although a modest part of the genetic etiology of fasting insulin is related to that of AVF, only a small part of the genetic etiology of AVF seems to be related to that of fasting insulin.

After AVF was adjusted for FM, the results of hypothesis tests have changed slightly (Table 4). The cross-trait correlations in siblings, between insulin and AVF-FM, were significant (model, 5 with $P = 0.01$), whereas the cross-trait parent-offspring correlations were not significant (model 6, with $P = 0.29$). The cross-trait spouse correlation remained nonsignificant (model 7, with $P = 0.33$). Parameter estimates from the most parsimonious model are given in Table 5. From these estimates, we calculated the heritability estimate for AVF-FM as 50% and the cross-trait heritability as 10%. Thus, 48% (10% of 21%) of the genetic influences on insulin are in common to AVF-FM, whereas 20% (10% of 50%) of the genetic influences on AVF-FM are shared by insulin.

TABLE 1. Correlations in the bivariate familial correlation model

	Cross-trait		Within trait (all interindividual)	
	Interindividual	Intraindividual	Trait 1	Trait 2
Siblings	s_1s_2, s_1d_2	s_{12}	s_1s_1, s_1d_1	s_2s_2, s_2d_2
	s_2d_1, d_1d_2	d_{12}	d_1d_1	d_2d_2
Parent-offspring	f_1s_2, f_2s_1		f_1s_1	f_2s_2
	f_1d_2, f_2d_1		f_1d_1	f_2d_2
	m_1s_2, m_2s_1		m_1s_1	m_2s_2
	m_1d_2, m_2d_1		m_1d_1	m_2d_2
Spouse	f_1m_2, f_2m_1	f_{12}, m_{12}	f_1m_1	f_2m_2

s, Sons; d, daughters; f, fathers; m, mothers; 1, trait 1 (insulin); 2, trait 2 (adiposity).

TABLE 2. Summary of hypothesis tests

Hypothesis	df	Parameter reductions
1. General		All 34 correlations estimated
2. No sex difference in offspring	16	$s_1s_1 = d_1d_1 = s_1d_1$, $s_1s_2 = d_1d_2 = s_1d_2 = s_2d_1$, $s_2s_2 = d_2d_2 = s_2d_2$, $f_1s_1 = f_1d_1$, $f_1s_2 = f_1d_2$, $f_2s_1 = f_2d_1$, $f_2s_2 = f_2d_2$, $m_1s_1 = m_1d_1$, $m_1s_2 = m_1d_2$, $m_2s_1 = m_2d_1$, $m_2s_2 = m_2d_2$, $s_{12} = d_{12}$
3. No sex differences in offspring or parents	22	$s_1s_1 = d_1d_1 = s_1d_1$, $s_1s_2 = d_1d_2 = s_1d_2 = s_2d_1$, $s_2s_2 = d_2d_2 = s_2d_2$, $f_1s_1 = f_1d_1 = m_1s_1 = m_1d_1$, $f_1s_2 = f_1d_2 = m_1s_2 = m_1d_2$, $f_2s_1 = f_2d_1 = m_2s_1 = m_2d_1$, $f_2s_2 = f_2d_2 = m_2s_2 = m_2d_2$, $f_1m_2 = f_2m_1$, $f_{12} = m_{12}$, $s_{12} = d_{12}$
4. No sex or generation differences	27	$f_1s_1 = f_1d_1 = m_1s_1 = m_1d_1 = s_1s_1 = d_1d_1 = s_1d_1$, $f_1s_2 = f_1d_2 = m_1s_2 = m_1d_2 = f_2s_1 = f_2d_1 = m_2s_1 = m_2d_1 = s_1s_2 = d_1d_2 = s_1d_2 = s_2d_1$, $f_2s_2 = f_2d_2 = m_2s_2 = m_2d_2 = s_2s_2 = d_2d_2 = s_2d_2$, $f_1m_2 = f_2m_1$, $f_{12} = m_{12} = s_{12} = d_{12}$
5. No cross-trait correlations in siblings	4	$s_1s_2 = d_1d_2 = s_1d_2 = s_2d_1 = 0$
6. No cross-trait correlations in parent-offspring	8	$f_1s_2 = f_1d_2 = m_1s_2 = m_1d_2 = f_2s_1 = f_2d_1 = m_2s_1 = m_2d_1 = 0$
7. No cross-trait correlations between spouses	2	$f_1m_2 = f_2m_1 = 0$
8. No cross-trait correlations in intraindividual	4	$f_{12} = m_{12} = s_{12} = d_{12} = 0$
9. No cross-trait correlations in interindividual	14	$s_1s_2 = d_1d_2 = s_1d_2 = s_2d_1 = 0$, $f_1m_2 = f_2m_1 = 0$, $f_1s_2 = f_1d_2 = m_1s_2 = m_1d_2 = f_2s_1 = f_2d_1 = m_2s_1 = m_2d_1 = 0$
10. No cross-trait correlations at all	18	$s_1s_2 = d_1d_2 = s_1d_2 = s_2d_1 = 0$, $f_{12} = m_{12} = s_{12} = d_{12} = 0$, $f_1s_2 = f_1d_2 = m_1s_2 = m_1d_2 = f_2s_1 = f_2d_1 = m_2s_1 = m_2d_1 = 0$, $f_1m_2 = f_2m_1 = 0$
11. Most parsimonious model		Combination of all nonrejected hypotheses above

TABLE 3. Characteristics of study variables by sex and generation groups

Variable	Group	n	mean	SD
Fasting insulin (pmol/L)	Fathers	90	78.28	59.04
	Mothers	90	61.81	29.25
	Sons	151	67.23	40.77
	Daughters	166	58.73	26.36
AVF (cm^2)	Fathers	95	156.68	61.17
	Mothers	92	120.18	59.35
	Sons	155	77.11	43.52
	Daughters	166	52.20	28.77
BMI (kg/m^2)	Fathers	95	28.28	4.48
	Mothers	93	27.65	4.98
	Sons	157	25.65	4.92
	Daughters	167	23.68	4.44
FM (kg)	Fathers	89	24.47	9.08
	Mothers	85	26.98	10.38
	Sons	146	16.90	11.10
	Daughters	167	18.01	9.77
ASF (cm^2)	Fathers	95	267.41	109.26
	Mothers	92	363.34	122.12
	Sons	155	202.34	146.72
	Daughters	166	251.46	145.86

Discussion

The present study is the first to explore common genetic and/or familial environmental influences on insulin resistance and AVF. We found that AVF and fasting insulin share a certain degree of genetic influences but, generally, of small magnitude. Although fasting insulin is less heritable than AVF, approximately one third and one half of the genetic influences on insulin were in common with AVF and AVF-FM, whereas only one sixth and one fifth of the genetic influences on AVF and AVF-FM were shared by insulin. The overall heritability for AVF, AVF-FM, and insulin were 42%, 50%, and 21%, respectively.

The present estimates of common genetic and/or familial environmental influences on AVF and insulin are based on cross-trait familial resemblance among relatives. Although it is difficult to distinguish between genetic and familial environmental factors in intact nuclear families, the pattern of significant sibling and parent-offspring cross-trait correlations (but no spouse cross-trait correlations) found in the present study suggests that common factors for AVF and insulin are likely to be predominantly genetic. Interestingly, the magnitudes of common genetic influences shared by insulin and AVF increased after AVF was adjusted for FM, suggesting that genetic influences on AVF are partly independent from those of FM.

There has been some discussion about which adiposity phenotype is the best correlate of insulin resistance (26, 41). AVF seems to be a better predictor of insulin resistance and NIDDM (3, 10, 11, 22), as compared to other adiposity indices, although several studies have not controlled the AVF data for the concomitant effects of FM (41), and there are contradictory results (26). The present finding, that the magnitudes of common genetic influences shared by insulin and AVF increased after AVF was adjusted for FM, also indirectly supports this notion that AVF is an important independent correlate of insulin resistance.

Several other studies have investigated the common genetic and environmental influences on diabetes or insulin resistance (fasting insulin, 2-h insulin, insulin-to-glucose ratio, and homeostasis (HOMA) insulin resistance index) and adiposity phenotypes (BMI, WHR, sum of skinfold thicknesses, or various ratios of skinfold thicknesses). Moderate-to-substantial shared-genetic influences were observed for BMI, with NIDDM or HOMA insulin resistance index, in studies of middle-aged and elderly Caucasian twins from Sweden and the USA (17, 19). The pleiotropic effects of genes influencing fasting insulin levels and adiposity measures (BMI, WHR, and subscapular/triceps skinfold thickness ratio) were also found to be moderate in Mexican Americans, in a three-generation family heart study (18). The magnitude of the common genetic influences on insulin levels and adiposity seems to be lower in the present study than in the previous reports (17–19); however, the present study is the first to consider AVF. Because the genetic influences on AVF are reported to be different from those on sc adipose tissue and, in part, independent of total body FM, the pattern of common genetic influences may also be different.

Differences among the study results may also relate to different study designs and sample sources. Age-related dif-

TABLE 4. Results of hypothesis tests across sex, generation, and traits for fasting insulin and abdominal visceral fat (AVF)

Hypothesis[a]	Between insulin and AVF			Between insulin and AVF-FM		
	df	χ^2	P	df	χ^2	P
1. General model						
2. No sex differences in offspring	16	19.62	0.238	16	24.02	0.09
3. No sex differences in offspring or parents	22	26.14	0.246	22	33.38	0.06
4. No sex or generation differences	27	27.97	0.412	27	44.53	0.02
5. No cross-trait correlations in siblings	4	8.99	0.061	4	12.45	0.01
6. No cross-trait correlations in parent-offspring	8	13.63	0.092	8	9.70	0.29
7. No cross-trait correlations in spouse	2	2.25	0.325	2	2.19	0.33
8. No cross-trait correlations in intraindividual	4	93.08	<0.01	4	34.67	<0.01
9. No cross-trait correlations in interindividual	14	22.50	0.069	14	23.30	0.05
10. No cross-trait correlations at all	18	180.31	<0.01	18	59.14	<0.01
11. Most parsimonious model	28	30.38	0.345	28	38.01	0.05

[a] See Table 2 for details about parameter reductions in each model.

TABLE 5. Parameter estimates ± standard errors from general and most parsimonious models

Correlations	Between insulin and AVF		Between insulin and AVF-FM	
	General	Parsimonious[a]	General model	Parsimonious[a]
Sibs				
s_1s_2	0.188 ± 0.079	0.032 ± 0.033	0.211 ± 0.074	0.097 ± 0.044
d_1d_2	0.060 ± 0.098	[0.032]	0.172 ± 0.087	[0.097]
s_1d_2	−0.086 ± 0.087	[0.032]	0.071 ± 0.093	[0.097]
s_2d_1	−0.028 ± 0.096	[0.032]	−0.053 ± 0.088	[0.097]
Parent-offspring				
f_1s_2	0.126 ± 0.085	[0.032]	0.084 ± 0.088	[0]
f_2s_1	0.081 ± 0.089	[0.032]	0.024 ± 0.090	[0]
f_1d_2	0.110 ± 0.095	[0.032]	0.176 ± 0.094	[0]
f_2d_1	−0.200 ± 0.079	[0.032]	−0.139 ± 0.083	[0]
m_1s_2	0.109 ± 0.090	[0.032]	0.080 ± 0.092	[0]
m_2s_1	0.121 ± 0.086	[0.032]	0.127 ± 0.090	[0]
m_1d_2	−0.016 ± 0.090	[0.032]	0.578 ± 0.095	[0]
m_2d_1	−0.020 ± 0.086	[0.032]	0.013 ± 0.082	[0]
Spouse				
f_1m_2	0.143 ± 0.105	[0]	0.108 ± 0.103	[0]
f_2m_1	0.111 ± 0.104	[0]	0.124 ± 0.104	[0]
Intraindividual				
f_{12}	0.553 ± 0.062	0.496 ± 0.035	0.466 ± 0.073	0.326 ± 0.060
m_{12}	0.495 ± 0.070	[0.496]	0.238 ± 0.095	0.326 ± 0.060
s_{12}	0.531 ± 0.059	[0.496]	0.160 ± 0.082	0.165 ± 0.053
d_{12}	0.430 ± 0.064	[0.496]	0.188 ± 0.079	0.165 ± 0.053

[a] Square brackets, Correlation was equated with a preceding parameter or fixed at zero.

TABLE 6. Heritability and cross-trait heritability for fasting insulin and abdominal visceral fat (AVF)

Variables	Heritability (%)
Insulin	21
AVF	42
AVF-FM	50
Insulin and AVF (bivariate)	6
Insulin and AVF-FM (bivariate)	10

ferences in insulin resistance and obesity have been reported (42). These differences could indicate that genetic effects are also age-dependent, so that genes related to both adiposity and insulin resistance may turn on later in life. For example, in a follow-up twin study, a major gene effect was reported to be responsible for the age-related change of diastolic blood pressure (43). Alternatively, genes associated with adipose tissue accretion, over time, may be detectable well before those specifically involved in tissue-specific insulin resistance become manifest, *i.e.* the expression of the obesity-related genes would precede the expression of those associated with the insulin resistance phenotypes. However, longitudinal data are needed to identify properly the age-related changes in the genetic influences that are in common between adiposity and insulin, and such data are lacking. Another reason that may explain differences across studies is exclusion criteria (health condition, activity levels, etc.). Strict exclusion criteria were applied in the present study. Subjects with NIDDM, extreme obesity, hypertension, and definite or possible coronary heart disease were not included in the HERITAGE study. Because of the fact that these diseases or disease conditions may entail high genetic susceptibility, low estimates of heritability may have been obtained in this study, in comparison with other studies.

The overall heritabilities of AVF and AVF-FM, from the univariate analysis (42% and 50%), are comparable with those found in previous studies (28, 29, 31), where the heritability ranged from 47–56%. The slight differences of heritability estimates for AVF and AVF-FM may reflect the fact that subjects with newly diagnosed diabetes or with insulin antibodies were excluded in the present study. However, the heritability estimate of 21% for insulin in the present study

is lower than reported in several other studies, in which the heritability estimates ranged from 40–53% (18, 44–47); but it is comparable with several others, including a Dutch study with a heritability of 21% in relatively younger subjects (48). Again, the age-related nature of genetic influences could be part of the reason for different heritability estimates across studies. Longitudinal studies could provide clearer answers. Another factor leading to differences across studies is gene-environment interaction. Heritability estimates could be biased if gene-environment interactions are age-dependent.

Genes or genetic markers with effects on both insulin and obesity have been reported (20, 21), an observation that provides some support for the findings from these multifactorial analyses. Neuroendocrine mechanisms are considered to be among the primary reasons for a high AVF level and insulin resistance or hyperinsulinemia (49). Several genes, related to the hypothalamic-pituitary-adrenal axis, could be perceived as useful candidates to be investigated. For instance, a polymorphism at the glucorticoid receptor gene has been reported to contribute to the accumulation of AVF, particularly in normal-weight people (50). Whether this gene also affects the insulin levels remains to be determined. Another reasonable candidate gene for the common genetic influences on AVF and fasting insulin could be the lipoprotein lipase (LPL) gene. Alterations in LPL activity levels have been found in insulin-resistant individuals (51, 52). In addition, LPL gene polymorphisms are known to be associated with both diabetes and severity of coronary artery disease (53). The LPL gene or other genes affecting LPL activity, particularly in visceral adipocytes, could be of interest as candidate genes for the common genetic influences on insulin and AVF. Moreover, as is well established, lipolytic rates vary among human fat depots (54), and these variations in lipid mobilization from the adipose tissue may play a role in the etiology of insulin resistance and the metabolic syndrome (55). Of particular interest in the search for candidate genes with pleiotropic effects on fasting insulin and AVF are the observations that lipolysis is higher in visceral adipocytes than in abdominal sc and gluteal-femoral sc tissues and that visceral adipocytes are more resistant to the antilipolytic effects of insulin (54, 55).

In conclusion, genes and/or familial nongenetic factors with pleiotropic effects seem to influence both AVF and plasma insulin levels. However, these genes and/or familial nongenetic factors have only low effects on the covariation between AVF and fasting insulin levels. Among potential candidate genes to consider, those involved in the regulation of lipid storage and mobilization in the abdominal fat depot should be investigated, with the aim of defining these genetic pleiotropic effects.

Acknowledgments

Thanks are expressed to all the coprincipal investigators, investigators, coinvestigators, local project coordinators, research assistants, laboratory technicians, and secretaries who have contributed to the study.

References

1. **Vague J.** 1956 The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. Am J Clin Nutr. 4:22–34.
2. **Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibbin G.** 1984 Abdominal adipose tissue distribution, obesity and risk of cardiovascular disease and death: 13-year follow up of participants in the study of men born in 1913. Br Med J. 288:1401–1404.
3. **Bouchard C, Bray GA, Hubbard VS.** 1990 Basic and clinical aspects of regional fat distribution. Am J Clin Nutr. 52:946–950.
4. **Manson JE, Colditz GA, Stampfer MJ, et al.** 1990 A prospective study of obesity and risk of coronary heart disease in women. N Engl J Med. 322:882–889.
5. **Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP.** 1992 Prospective analysis of the insulin-resistance (syndrome X). Diabetes. 41:715–722.
6. **Mykkänen L, Kuusisto J, Pyörälä K, Laasko M.** 1993 Cardiovascular disease risk factors as predictors of type 2 (non-insulin-dependent) diabetes mellitus in elderly subjects. Diabetologia. 36:553–559.
7. **Despres J-P, Lamarche B, Mauriege P, et al.** 1996 Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med. 334:952–957.
8. **Howard G, O'Leary DH, Zaccaro D, et al.** 1996 Insulin sensitivity and atherosclerosis. Circulation. 93:1809–1817.
9. **Ferrannini E, Haffner SM, Mitchell BD, Stern MP.** 1991 Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia. 34:416–422.
10. **Björntorp P.** 1993 Visceral obesity: a "civilization syndrome". Obes Res. 1:206–222.
11. **Després J-P.** 1993 Abdominal obesity as important component of insulin-resistance syndrome. Nutrition. 9:452–459.
12. **Schmidt MI, Watson RL, Duncan BB, et al.** 1996 Clustering of dyslipaemia, hyperuricemia, diabetes, and hypertension and its association with fasting insulin and central and overall obesity in a general population. Metabolism. 45:699–706.
13. **Laakso M.** 1993 How good a marker is insulin level for insulin resistance? Am J Epidemiol. 137:959–965.
14. **Kissebah AH, Vydelingun N, Murray R, et al.** 1982 Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab. 54:254–260.
15. **Rice T, Nadeau A, Pérusse L, Bouchard C, Rao DC.** 1996 Familial correlations in the Québec family study: cross-trait familial resemblance for body fat with plasma glucose and insulin. Diabetologia. 39:1357–1364.
16. **Liese AD, Mayer-Davis EJ, Tyroler HA, et al.** 1997 Development of the multiple metabolic syndrome in the ARIC cohort: joint contribution of insulin, BMI, and WHR. Ann Epidemiol. 7:407–416.
17. **Carmelli D, Cardon LR, Fabsitz R.** 1994 Clustering of hypertension, diabetes, and obesity in adult male twins: same genes or same environments? Am J Hum Genet. 55:566–573.
18. **Mitchell BD, Kammerer CM, Mahaney MC, et al.** 1996 Genetic analysis of the IRS: pleiotropic effects of genes influencing insulin levels on lipoprotein and obesity measures. Arterioscler Thromb Vasc Biol. 16:281–288.
19. **Hong Y, Pedersen NL, Brismar K, de Faire U.** 1997 Genetic and environmental architecture of the features of the insulin-resistance syndrome. Am J Hum Genet. 60:143–152.
20. **Duggirala R, Stern MP, Mitchell BD, et al.** 1996 Quantitative variation in obesity-related trait and insulin precursors linked to the OB gene region on human chromosome 7. Am J Hum Genet. 59:694–703.
21. **Fleury C, Neverova M, Collins S, et al.** 1997 Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet. 15:269–272.
22. **Kissebah AH, Peiris AN.** 1989 Biology of regional body fat distribution: relationship to non-insulin-dependent diabetes mellitus. Diabetes Metab Rev. 5:83–109.
23. **Peiris AN, Sothmann MS, Hoffmann RG, et al.** 1989 Adiposity, fat distribution, and cardiovascular risk. Ann Intern Med. 110:867–872.
24. **Kissebah AH.** 1991 Insulin resistance in visceral obesity. Int J Obes. [Suppl 2] 15:109–115.
25. **Park KS, Kim SY, Lee HK, Koh CS, Min HK.** 1991 Intraabdominal fat is associated with decreased insulin sensitivity in healthy young men. Metabolism. 40:600–603.
26. **Abate N, Garg A, Peshock RM, Stray-Gundersen J, Adams-Huet B, Grundy SM.** 1996 Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. Diabetes. 45:1684–1693.
27. **Bouchard C, Tremblay A, Després JP, et al.** 1990 The response to long-term overfeeding in identical twins. N Engl J Med. 322:1477–1482.
28. **Pérusse L, Després J-P, Lemieux S, Rice T, Rao DC, Bouchard C.** 1996 Familial aggregation of abdominal viscerl fat level: results from the Quebec Family Study. Metabolism. 45:378–382.
29. **Rice T, Després J-P, Daw EW, et al.** 1997 Familial resemblance for abdominal visceral fat: the HERITAGE Family Study. Int J Obes. 21:1024–1031.
30. **Bouchard C, Rice T, Lemieux S, Després J-P, Perusse L, Rao DC.** 1996 Major gene for abdominal visceral fat area in the Québec Family Study. Int J Obes. 20:420–427.
31. **Rice T, Després J-P, Pérusse L, et al.** 1997 Segregation analysis of abdominal visceral fat: the HERITAGE Family Study. Obes Res. 5:417–424.
32. **Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, Gagnon J.** 1995 The

HERITAGE Family Study: aims, design, and measurement protocol. Med Sci Sports Exerc. 27:721–729.
33. **Desbuquois B, Aurbuch GD.** 1971 Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab. 33:732–738.
34. **Røder ME, Porte Jr D, Schwartz RS, Kahn SE.** 1998 Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 83:604–608.
35. **Kahn SE, Leonetti DL, Prigeon RL, Boyko EJ, Bergstrom RW, Fujimoto WY.** 1995 Relationhip of proinsulin and insulin with noninsulin-dependent diabetes mellitus and coronary heart disease in Japanese-American men: impact of obesity - Clinical Research Center Study. J Clin Endocrinol Metab. 80: 1399–1406.
36. **Sjöström L, Kvist H, Cederblad A, Tylen U.** 1986 Determination of the total adipose tissue and body fat in women by computed tomography: comparison with 40K and tritium techniques. Am J Physiol. 250:E736–E745.
37. **Behnke AR, Wilmore JH.** 1974 Evaluation and regulation of body build and composition. Englewood Cliffs, NJ: Prentice-Hall; 21–27.
38. **Wilmore JH, Vodak PA, Parr RB, Girandola RM, Billing JE.** 1980 Further simplification of a method for determining residual lung volume. Med Sci Sports Exerc. 12:216–218.
39. **Motley HL.** 1957 Comparison of simple helium closed with the oxygen open-circuit method for measuring residual air. Am Rev Tuberc Pulm Dis. 76:601–615.
40. **Province MA, Rao DC.** 1995 A general purpose model and a computer program for combined segregation and path analysis (SEGPATH): automatically creating computer programs from symbolic language model specification. Genet Epidemiol. 12:203–219.
41. **Seidell JC, Bouchard C.** 1997 Visceral fat in relation to health: is it a major culprit or simply an innocent bystander? Int J Obes. 21:626–631.
42. **Davidson MB.** 1979 The effect of aging on carbohydrate metabolism: a review of the English literature and practical approach to the diagnosis of diabetes mellitus in the elderly. Metab Clin Exp. 28:688–705.
43. **Cheng LS-C, Carmelli D, Hunt SC, Williams RR.** 1995 Evidence for a major gene influencing 7-year increases in diastolic blood pressure with age. Am J Hum Genet. 57:1169–1177.
44. **Iselius L, Lindsten J, Morton NE, et al.** 1982 Evidence for an autosomal recessive gene regulating the persistence of the insulin response to glucose in man. Clin Genet. 22:180–194.
45. **Schumacher MC, Hasstedt SJ, Hunt SC, Williams RR, Elbein SC.** 1992 Major gene effects for insulin levels in familial NIDDM pedigree. Diabetes. 41:416–423.
46. **Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U.** 1996 Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. J Clin Endocrinol Metab. 81:1791–1797.
47. **Mayer EJ, Newman B, Austin MA, et al.** 1996 Genetic and environmental influences on insulin levels and the insulin resistance syndrome: an analysis of women twins. Am J Epidemiol. 143:323–332.
48. **Snieder H, Boomsma DI, van Doornen LJP, Neale MC.** 1996 A bivariate genetic analysis of fasting insulin and glucose. Ph.D. dissertation. Vrije Universiteit, The Netherlands.
49. **Björntorp P.** 1997 Obesity and the adipocyte: neuroendocrine factors in obesity. J Endocrinol. 155:193–195.
50. **Buemann B, Vohl M-C, Chagnon M, et al.** 1997 Abdominal visceral fat is associated with a BclI restriction fragment length polymorphism at the glucocordicoid receptor gene locus. Obes Res. 5:186–192.
51. **Eckel RH, Yost TJ, Jensen DR.** 1995 Alterations in lipoprotein lipase in insulin resistance. Int J Obes. [Suppl 1] 19:S16–S21.
52. **Potts JL, Coppack SW, Fisher RM, Humphreys SM, Gibbons GF, Frayn KN.** 1995 Impaired postprandial clearance of triacylglycerol-rich lipoproteins in adipose tissue in obese subjects. Am J Physiol. 268:E588–E594.
53. **Wang XL, McCredie RM, Wilcken DEL.** 1996 Common DNA polymorphisms at the lipoprotein lipase gene: association with severity of coronary artery disease and diabetes. Circulation. 93:1339–1345.
54. **Bouchard C, Després JP, Mauriège P.** 1993 Genetic and nongenetic determinants of regional fat distribution. Endocr Rev. 14:72–93.
55. **Arner P.** 1993 Regulation of adipose tissue lipolysis, importance for the metabolic syndrome. Adv Exp Med Biol. 334:259–267.