Genetic Epidemiology 16:426–446 (1999)

Bivariate Genetic Analysis of Fasting Insulin and Glucose Levels

Harold Snieder,[1]* Dorret I. Boomsma,[1] Lorenz J.P. van Doornen,[1] and Michael C. Neale[2]

[1]*Department of Psychophysiology, Vrije Universiteit, Amsterdam, The Netherlands*
[2]*Department of Psychiatry, Medical College of Virginia, Richmond*

The main aim of this study was to estimate the relative influence of genes and environment on fasting insulin levels, which were considered a proxy of insulin resistance. Possible sex differences in genetic and environmental influences, and the origin of the covariance between fasting insulin and glucose were investigated. Subjects were 209 pairs of middle-aged twins, divided into 5 sex-by-zygosity groups. A general bivariate model and a reciprocal causation model including fasting insulin and glucose were used in the analyses. For both quantitative genetic models, a model specifying additive genetic and unique environmental factors, which were the same in males and females, showed the best fit to the data. Heritability estimates were modest and highly similar in both models: 20–25% of the variance in fasting insulin, and around 50% of the variance in fasting glucose levels could be attributed to genetic factors. The two models could not be discriminated on the basis of their fit to the data. A submodel of the general bivariate model suggested that the covariance between glucose and insulin has a unique environmental basis, whereas for the reciprocal causation model both causal paths were needed to explain the phenotypic correlation between insulin and glucose and estimates of the reciprocal paths were of opposite sign, an indication for the expected negative feedback loop. Genet. Epidemiol. 16:426–446, 1999. © 1999 Wiley-Liss, Inc.

Key words: heritability; twins; insulin resistance; truncated data; assay batch effects; reciprocal causation model

*Correspondence to: Dr. Harold Snieder, Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, Lambeth Palace Road, London, UK SE1 7EH. E-mail: h.snieder@umds.ac.uk

Received 5 December 1997; Revised 29 July 1998; Accepted 2 October 1998

Contract grant sponsor: Netherlands Heart Foundation; Contract grant number: 90.313.

INTRODUCTION

Resistance to insulin-stimulated glucose uptake, also called insulin resistance or impaired insulin sensitivity, is considered a major risk factor for progression to both coronary heart disease (CHD) and non-insulin-dependent diabetes mellitus (NIDDM) [Reaven, 1988; Groop and Eriksson, 1992; Beck-Nielsen and Groop, 1994; Pierce et al., 1995]. At first, insulin-resistant individuals are able to keep a normal glucose homeostasis by developing hyperinsulinemia [Reaven, 1988]. This compensatory response of the endocrine pancreas is not without its price, however. Insulin resistance and its resulting hyperinsulinemia may lead to a cluster of metabolic abnormalities, which comprise a syndrome of interrelated risk factors for cardiovascular disease. This syndrome is known as "syndrome X" [Reaven, 1988, 1993, 1994] or "insulin resistance syndrome" [Haffner et al., 1992; Wajchenberg et al., 1994; Grootenhuis, 1994]. Insulin resistance is most accurately measured by the euglycemic hyperinsulinaemic clamp technique. This method is, however, laborious and therefore not easily applicable in epidemiological studies. The best estimate of insulin resistance in population studies is fasting insulin level [Laakso, 1993]. Elevated fasting insulin precedes other features of the insulin resistance syndrome like hypertension, low HDL cholesterol, and high triglyceride concentrations [Reaven, 1990, 1991; Haffner et al., 1992; Reaven et al., 1996]. Moreover, evidence from large prospective studies shows that hyperinsulinemia is an independent predictor of ischemic heart disease in men [Fontbonne and Eschwège, 1991; Fontbonne, 1993; Deprés et al., 1996].

A number of studies have shown that insulin resistance and hyperinsulinemia are familial characteristics, which may point to a genetic basis of insulin resistance [Lillioja et al., 1987; Haffner et al., 1988; Bogardus et al., 1989; Erikkson et al., 1989; Gulli et al., 1992; Martin et al., 1992; Schumacher et al., 1992; Vaag et al., 1992; Mitchell et al., 1996; Sakul et al., 1997]. However, the search for the genetic basis of insulin action via candidate genes and linkage analyses has, to date, been only minimally positive, leaving most insulin resistance unexplained [Flier, 1992; Hansen, 1993; Raffel et al., 1994]. A number of factors are held responsible for this result. First, insulin resistance may have a polygenic basis: multiple loci with small effect may be involved [Rich, 1990; Groop and Eriksson, 1992; Beck-Nielsen and Groop, 1994]. Furthermore the penetrance is dependent on age and on lifestyle factors like smoking [Facchini et al., 1992; Attvall et al., 1993], diet [Lovejoy and DiGirolamo, 1992; Sharma, 1992; Mayer et al., 1993], and physical exercise [Laws and Reaven, 1991; Lampman and Schteingart, 1991; Mikines, 1992; Perseghin et al., 1996].

Quantitative genetic approaches enable estimation of the relative importance of genetic and environmental influences on a phenotype, provided that the data are collected in genetically informative subjects like, for example, nuclear families or twins. The relative influence of genes can be expressed as heritability, which is defined as the proportion of population variance attributable to genetic factors. A number of studies have estimated the heritability of fasting insulin. Iselius et al. [1982] applied a path analytic model to data from 155 nuclear families and reported a heritability of fasting insulin of .40 in both children and adults. Evidence was found for the effect of a single major locus in a segregation analysis of fasting insulin on data from 16 pedigrees ascertained through siblings with NIDDM [Schumacher et al., 1992]. A third of the variance in fasting insulin levels was attributed to this major locus and 11.4% to polygenic inheritance. In total,

44.5% of the variance was due to genetic factors. More recently, heritability estimates of fasting insulin were reported in Mexican Americans (44%) [Mitchell et al., 1996] and Pima Indians (65%) [Sakul et al. 1997]. Two twin studies, one in 178 adult women twin pairs [Mayer et al., 1996] and one in 248 middle-aged and elderly twin pairs of both sexes [Hong et al., 1996], rendered similar heritability estimates for fasting insulin of 0.53 and 0.48, respectively.

In normal circumstances, the concentration of blood glucose is regulated within close boundaries [Brück, 1983]. Insulin plays an important role in blood glucose homeostasis by stimulating the uptake of glucose in the cell. This homeostatic regulation can be represented by a feedback loop between glucose and insulin. Furthermore, fasting glucose and insulin levels tend to show a positive correlation in population samples of older age, which is probably a reflection of a weakening homeostatic control of the blood glucose concentration with aging [Grootenhuis, 1994; Beck-Nielsen and Groop, 1994].

The principal aim of this study was to estimate the relative influence of genes and environment on fasting insulin levels, considered as a proxy for insulin resistance. Sex differences in genetic and environmental estimates were investigated and the covariance between fasting insulin and glucose was modeled. Specifically, it was tested whether the origin of the covariance between fasting glucose and insulin levels could best be explained by correlated latent factors (genetic or environmental) or a reciprocal causation of insulin and glucose (i.e., a feedback loop on a phenotypic level). To resolve these questions, we measured levels of fasting insulin and glucose in middle-aged twins and used a general bivariate model and a reciprocal causation model to quantify the contributions of genes and environment to the variance and the covariance of these variables.

METHODS

Subjects

This study is part of a project in which cardiovascular risk factors were studied in 213 unselected middle-aged Caucasian twin pairs aged between 34 and 63 [Snieder et al., 1995, 1997a, 1997b; van Doornen et al., 1998]. Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Netherlands Twin Registry, and solicitation through the Dutch Twin Club. In addition, a small number of twins who heard from the study in another way volunteered to participate. Participating twins were unaware of the specific hypotheses tested and informed consent was obtained from all subjects. Data from 4 subjects were excluded from the sample. In one subject no blood could be obtained. Two subjects were dropped because they were insulin-dependent diabetics, and one subject was discarded because of a glucose value higher than 7.8 mmol/l, which is an indication of NIDDM according to WHO criteria [WHO report, 1985]. One (monozygotic) triplet was included in the sample by discarding the data from the second born subject. In total, 204 males (age: 43.7 ± 6.5) and 218 females (age: 44.7 ± 6.8) were included in the study. In all twin pairs, zygosity was determined by DNA fingerprinting [Jeffreys et al., 1985]. Grouped according to their zygosity and sex, the sample consisted of the following number of twin pairs: 43 pairs of monozygotic males (MZM), 39 pairs of dizygotic males (DZM), 49 pairs of monozygotic

females (MZF), 39 pairs of dizygotic females (DZF), and 39 dizygotic pairs of opposite sex (DOS).

Blood Sampling and Biochemical Assays

Twins arrived at the laboratory at about 10:00 a.m. They were requested to fast, refrain from smoking and the use of alcohol, coffee, and tea after 11:00 p.m. the preceding night. Blood was collected by venipuncture and sampled in EDTA tubes. The tubes were placed on ice and centrifuged promptly (30 minutes, 2,000*g*) at 4°C to separate plasma from cells. Aliquots of plasma were snap-frozen using liquid nitrogen and stored at –20°C until processing. Fasting insulin levels were measured by a commercial radioimunoassay kit (INS-RIA-100, Medgenix diagnostics, Brussels, Belgium). Intra- and interassay coefficients of variation were 4.7 and 6%, respectively, with a lower limit of sensitivity of 3.0 mU/L. Fasting glucose levels were determined with a Dimension clinical chemistry system (DuPont, Wilmington, DE). Intra- and interassay coefficients of variation were 2.6 and 2.8%, respectively. Each time a substantial number of samples had been collected, plasma samples were sent to the laboratory and analyzed subsequently. Analyses took place on 8 different occasions in 8 different assay batches. Assays were done at the laboratory of the University Hospital, Leiden, The Netherlands [Nijs et al., 1990], which participates on a regular basis in the proficiency schemes of the Dutch Foundation of Quality Control in Clinical Chemical Hospital Laboratories. Assays are standardized with in-house, external, and international controls and drift is tracked.

Twin Methodology

Quantitative genetic model fitting of twin data allows the separation of the observed phenotypic variance into its genetic and environmental components [Neale and Cardon, 1992]. This variance can be decomposed into several contributing factors. Additive genetic variance (G), dominance genetic variance (D), shared (common) environmental variance (C), and specific (unique) environmental variance (E). A general univariate genetic model can be represented by the following linear structural equations:

$$1)\ P_i = hG_i + dD_i + cC_i + eE_i$$
$$2)\ V_P = h^2 + d^2 + c^2 + e^2$$

where P is the phenotype of the *i*th individual, scaled as a deviation from zero. G, D, C, and E can be conceived of as uncorrelated latent factors with zero mean and unit variance. h, d, c, and e are factor loadings of the observed variable on the latent factors and V_P is the phenotypic variance. Squaring of the factor loadings yields the different components of variance. Extension of this univariate to a bivariate model allows exploration of the origin of the covariance between the two phenotypes [Neale and Cardon, 1992; Falconer, 1989].

Genetic Analysis of Fasting Insulin and Glucose Levels

Thirty of the 204 males (14.7%) and 53 of the 218 females (24.3%) had fasting insulin values below the detection limit of the assay (3 mU/L). Though it was certain that these subjects had a measured value below 3 mU/L, which is informative from a

clinical-diagnostic point of view, the exact value of their fasting insulin thus remained unknown. A simple solution to this problem would be to assign a fixed value (e.g., 2 mU/L) to those subjects. This, however, leads to a truncated, and thus non-normal, distribution of fasting insulin for both males and females, which may adversely affect the quantitative genetic modeling. We, therefore, decided to account for the truncation problem more accurately, within the genetic model fitting (see Appendix).

Analysis of the data of one randomly chosen member of each twin with ANOVA, showed a significant influence of the assay batch on both fasting insulin and glucose levels. As two members of a twin pair were always measured within the same batch, this effect could spuriously induce an increase in twin correlation. This assay batch effect was accounted for in the model fitting by estimating separate means for each assay batch.

Prior to all data analysis and model fitting, fasting insulin and glucose levels were transformed by natural logarithm to obtain a normal distribution.

Model fitting was done with Mx [Neale, 1995], a computer program specifically designed for the analysis of genetically informative data. Parameters were estimated by normal-theory maximum-likelihood, where the models were fitted to the raw data [Lange et al., 1976]. Initially, a saturated model was defined, which accounted for both truncation and assay batch, and in which the initial 4×4 variance-covariance matrices of glucose and insulin were estimated freely for each of the 5 sex-by-zygosity groups. Also, the initial means of glucose and insulin were estimated for each sex separately. The 4×4 variance-covariance matrices in each of the 5 zygosity groups were expressed in terms of a bivariate genetic model. Model fitting provided parameter estimates (h, d, c, e) and was done by a user defined fit function. Submodels were compared by hierarchic χ^2 tests, as the difference between the function value for a reduced model and that of the full model (Δ 2lnL) is χ^2 distributed. The degrees of freedom (df) for this test are equal to the difference between the df for the reduced and the full model.

Bivariate Genetic Models

A general bivariate model with glucose entered as the first and insulin as the second variable and a reciprocal causation model, which is a submodel of the general bivariate model [Heath et al., 1993], were fitted to the data. The general bivariate model is presented in Figure 1, the reciprocal causation model in Figure 2. In both models, the observed phenotypes for twin 1 and twin 2 are shown in squares, and latent factors are shown in circles. Of all possible latent factors, only G and E are shown for reasons of clarity. Factor loadings of observed variables on the different latent factors are depicted beside the arrows. Correlations between the latent genetic factors in both models are 1 in MZ twins and 0.5 in DZ twins.

In the general bivariate model, latent factors are subdivided into genetic and environmental factors common to glucose and insulin (G_c and E_c), and genetic and environmental factors specific to insulin (G_s and E_s). The total additive genetic variance components for insulin and glucose are $V_G\,ins = h'^2_c + h^2_s$ and $V_G glu = h^2_c$ respectively. Similar formulae apply to the total unique environmental variance components ($V_E\,ins = e'^2_c + e^2_s$ and $V_E\,glu = e^2_c$). The covariance between glucose and insulin consists of a genetic part ($h_c * h'_c$) and an environmental part ($e_c * e'_c$).

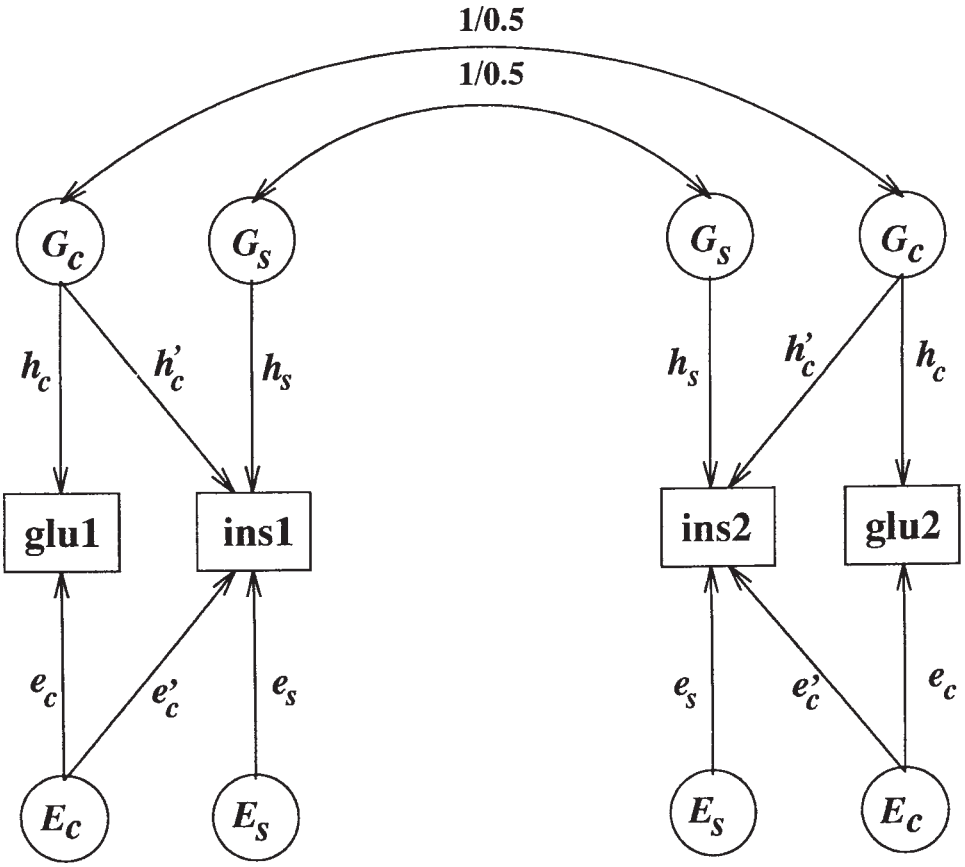

Fig. 1. General bivariate model with glucose entered as the first and insulin as the second variable. Observed phenotypes for twin 1 and twin 2 are shown in squares. Latent factors are shown in circles. G_c and E_c reflect genetic and environmental influences common to glucose and insulin. G_s and E_s reflect genetic and environmental influences specific to insulin. Factor loadings of observed variables on the different latent factors are depicted beside the arrows: h'_c = additive genetic influence common to glucose and insulin; h_s = additive genetic influence specific for insulin; e'_c = unique environmental influence common to glucose and insulin; e_s = unique environmental influence specific for insulin; h_c = additive genetic influence on glucose; e_c = unique environmental influence on glucose. Correlations between the latent genetic factors are 1 in MZ twins, 0.5 in DZ twins. Although models including C (ACE) and D (ADE) were also tested, C and D are not shown in the figure for reasons of clarity.

$h_c * h'_c$ divided by the square root of the product of V_G*glu* and V_G*ins* yields the genetic correlation between glucose and insulin. Accordingly, $e_c * e'_c$ divided by the square root of the product of V_E*glu* and V_E*ins* yields the environmental correlation between glucose and insulin. Within the general bivariate model, the genetic and/or environmental origin of the covariation between insulin and glucose can be tested by successively setting the respective connecting paths (h'_c and e'_c) to zero. Total (standardized) heritability of insulin is calculated as:

$$h^2 = (h'^2_c + h_s^2)/V_P\,ins.$$

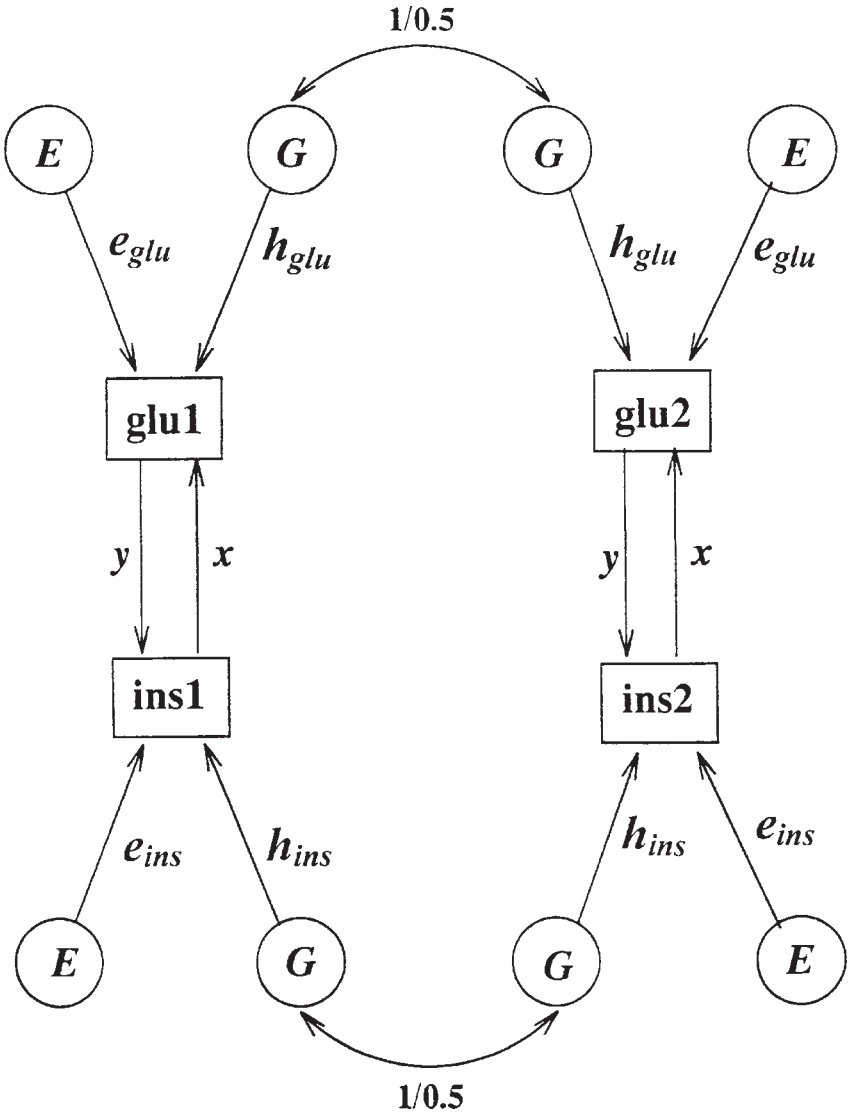

Fig. 2. Reciprocal causation model for glucose and insulin. Observed phenotypes for twin 1 and twin 2 are shown in squares. Latent factors are shown in circles. G and E reflect genetic and environmental influences on glucose and insulin. Factor loadings of observed variables on the different latent factors are depicted beside the arrows: h_{ins} = additive genetic influence on insulin; e_{ins} = unique environmental influence on insulin; h_{glu} = additive genetic influence on glucose; e_{glu} = unique environmental influence on glucose; × = causal path from insulin to glucose; y = causal path from glucose to insulin. Correlations between the latent genetic factors are 1 in MZ twins and 0.5 in DZ twins. Although models including C (ACE) and D (ADE) were also tested, C and D are not shown in the figure for reasons of clarity.

Homeostatic mechanisms can be represented by a reciprocal causation model [Turner and Stevens, 1959]. In this case, the physiological feedback loop between glucose and insulin, responsible for the maintainance of a constant glucose concentration in the blood plasma [Brück, 1983], was modeled. For the reciprocal causation model, it is assumed that the correlations between the genetic and environmental determinants of glucose with those of insulin are all zero, which implies that the association between glucose and insulin arises solely because of the reciprocal causal influences of insulin on glucose (x) and glucose on insulin (y). By setting x (or y) to zero within the reciprocal model it can be tested whether a unidirectional causation from insulin to glucose (or vice versa) is sufficient to explain the covariation between the two variables.

As the reciprocal causation model is a submodel of the general bivariate model [Heath et al., 1993], the fit of the two models can be compared using hierarchic χ^2 tests. However, at least three "sources of variation" (G, D, C, or E) are needed to test the reciprocal model against the general bivariate model [Neale and Cardon, 1992; Heath et al., 1993]. If there are only two "sources of variation," the reciprocal causation model will have the same degrees of freedom and, for models without sex differences, will give the same fit as the general bivariate model, which means that in that case a test of the model is not available.

Under the model as presented in Figure 2, total phenotypic variances (V_P) of insulin and glucose are equal to:

$$V_P ins = ((h^2 ins + e^2 ins) + y^2(h^2 glu + e^2 glu)) / (1 - xy)^2$$
$$V_P glu = ((h^2 glu + e^2 glu) + x^2(h^2 ins + e^2 ins)) / (1 - xy)^2$$

These formulae show that $V_P ins$ consists of a part due to variation in insulin and a part due to variation in glucose mediated through y, the path from glucose to insulin. The same reasoning applies to $V_P glu$. From these formulae it follows that the additive genetic component of variance (V_G) for insulin and glucose is equal to:

$$V_G ins = (h^2 ins + y^2 h^2 glu) / (1 - xy)^2$$
$$V_G glu = (h^2 glu + x^2 h^2 ins) / (1 - xy)^2$$

Standardized heritabilities (h^2) for insulin and glucose can be calculated from V_G and V_P: $h^2 = V_G/V_P$.

Sex differences in components of variance were examined by comparing general bivariate or reciprocal models in which parameter estimates are allowed to differ in magnitude between males and females, with a reduced model in which parameter estimates are constrained to be equal across the sexes. In a similar way, heterogeneity of the means of glucose and insulin was tested across males and females. This was done within the full model in which variances and covariances were estimated freely for each sex-by-zygosity group.

RESULTS

In Table I, means before truncation correction and after truncation correction of fasting insulin and glucose are shown for males and females. By hierarchic χ^2 tests a significant sex difference was found for insulin ($\chi^2_{[8]} = 21.41$, $P < .01$) but not for glucose ($\chi^2_{[8]} = 9.07$, n.s.). Insulin levels being higher in males than in females (see Table I). In subsequent model fitting, estimates of mean insulin levels were allowed to be different in males and females.

TABLE I. Means Before Truncation Correction and After Truncation Correction of Fasting Insulin and Fasting Glucose for Males and Females*

		Insulin (mU/l)		Glucose (mmol/l)	
	n	Before corr.	After corr.	Before corr.	After corr.
Males	204	5.45	5.65	6.12	6.11
Females	218	4.49	4.46	5.98	6.03

*n = number of subjects. Means of fasting insulin before truncation correction were calculated after all values below the detection limit were assigned a value of 2 mU/l. For insulin and glucose, antilogs are reported.

The effect of the correction for truncation only, and the correction for both truncation and assay batch on the twin correlations of fasting insulin and glucose levels in the 5 different zygosity groups can be seen in Table II. The corrections hardly affected the twin correlations of glucose. Only after correction for both effects, correlations showed the expected slight decrease. For fasting insulin, corrections had a substantial influence on the twin correlations. Especially the correction for assay batch induced a decrease in twin correlations. In general, after correction for truncation and assay batch, MZ correlations were larger than DZ correlations for both insulin and glucose, indicating a genetic influence. For insulin, however, MZ correlations were only slightly higher than DZ correlations, which implies that the proportion of variance due to genetic influences on fasting insulin levels is small.

Table III shows function values (-2lnL), and hierarchic χ^2 tests of submodels of the general bivariate model. In the upper part of Table III, models are shown, in which parameter estimates are allowed to be different between the sexes. The model estimating only additive genetic (G) and unique environmental (E) factors fitted best. In the lower part of Table III, estimates of variance components are set equal for males and females. Comparing the GE model without sex differences with the GE model that included sex differences showed that parameter estimates for males and females could be set equal without a significant loss in fit [$\Delta(2\text{lnL}) = 9.337$, df = 6]. This means that the GE model without sex differences in these variance components offers the most parsimonious solution. The lower part of Table III also shows various submodels of the GE model without sex differences: the origin of the covariance between glucose and insulin was tested by successively setting the connecting paths

TABLE II. Twin Correlations of Fasting Insulin and Glucose Levels Before Correction and After Correction for Truncation Only (Trunc corr.), and After Correction for Both Truncation and Assay Batch Effect (Both corr.)*

		Insulin			Glucose		
	N	Before corr.	Trunc. corr.	Both corr.	Before corr.	Trunc. corr.	Both corr.
MZM	43	.41	.28	.15	.56	.54	.48
DZM	39	.10	.19	.04	.02	.02	–.03
MZF	49	.38	.44	.45	.72	.73	.61
DZF	39	.42	.45	.29	.27	.27	.19
DOS	39	.25	.21	–.06	.19	.18	.17

*N = number of twin pairs; MZF = monozygotic females; MZM = monozygotic males; DZF = dizygotic females; DZM = dizygotic males; DOS = dizygotic opposite sex.

TABLE III. Function Values (–2lnL) and Hierarchic χ^2 Tests of Submodels of the General Bivariate Model, With and Without Sex Differences†

Model	–2lnL	df	Δdf	Δ(2lnL)	*P*
Sex differences					
GCE	2,661.425	961			
GDE	2,660.851	961			
GE	2,666.284	967	6	5.433	ns*
CE	2,677.533	967	6	16.682	<.025*
No sex differences					
GCE	2,675.514	970			
GDE	2,674.429	970			
GE	2,675.621	973	3	1.192	ns**
CE	2,688.867	973	3	14.438	<.001**
GE					
Full model	2,675.621	973			
$h'_c=0$	**2,676.874**	**974**	**1**	**1.253**	**ns*** **
$e'_c=0$	2,706.085	974	1	30.464	<.001***
$h'_c=0$ & $e'_c=0$	2,712.317	975	2	36.696	<.001***

†Submodels of the GE model without sex differences, testing for the origin of covariance between glucose and insulin, are also shown. –2lnL = minus twice the log-likelihood; df = degrees of freedom; Δdf = (df *submodel*) - (df *full model*); Δ(2lnL) = (2lnL *submodel*) – (2lnL *full model*), *P* = probability; ns = non-significant; G = additive genetic influence; D = dominance genetic influence; E = unique environmental variance; C = shared environmental variance; h'_c = additive genetic factor loading common to glucose and insulin; e'_c = unique environmental factor loading common to glucose and insulin. Most parsimonious solution is printed in **boldface** type.
*Compared to GDE (sex diff).
**Compared to GDE (no sex diff.).
***Compared to GE (no sex diff.).

between glucose and insulin (h'_c and e'_c) to zero. h'_c could be set to zero without a significant loss in fit. The best fitting general bivariate model suggests therefore, that the covariance between glucose and insulin has a unique environmental basis.

Table IV shows function values (-2lnL), and hierarchic χ^2 tests of submodels of the reciprocal causation model. Just as in Table III, the upper part of the table presents models in which parameter estimates are allowed to be different between the sexes, and the lower part shows models in which estimates of variance components are set equal between the sexes. Just like for the general bivariate model, a GE model without sex differences in variance components offered the most parsimonious solution. Submodels of the GE model without sex differences, testing whether the reciprocal paths (x or y) could be set to zero are also shown. None of these paths could be set to zero without a loss in fit, implicating that both causal paths are needed to explain the phenotypic correlation between insulin and glucose.

As expected [Neale and Cardon, 1992; Heath et al., 1993], GE models without sex differences in the general bivariate and the reciprocal causation case showed exactly the same fit and the same degrees of freedom (compare Tables III and IV). This means that there is no test available to compare the fit of the best fitting general bivariate and reciprocal causation model, because such a test requires at least three "sources of variation."

Table V shows (unstandardized) parameter estimates and their likelihood based 95% confidence intervals for GE models with and without sex differences for both

TABLE IV. Reciprocal Causation Models, With and Without Sex Differences[†]

Model	–2lnL	df	Δdf	Δ(2lnL)	*P*
Sex differences					
GCE	2,661.391	963			
GDE	2,661,436	963			
GE	2,664.896	967	4	3.505	ns*
CE	2,678.493	967	4	17.102	<.0025*
No sex differences					
GCE	2,675.596	971			
GDE	2,674.666	971			
GE	**2,675.621**	**973**	**2**	**0.025**	**ns****
CE	2,688.867	973	2	13.271	<.0025**
GE					
Full model	**2,675.621**	**973**			
x = 0	2,688.606	974	1	12.985	<.001***
y = 0	2,680.802	974	1	5.181	<.025***
x & y = 0	2,712.317	975	2	36.696	<.001***

[†]Submodels of the GE model without sex differences, testing whether x or y can be set to zero, are also shown. x = causal path from insulin to glucose; y = causal path from glucose to insulin. Most parsimonious solution is printed in **boldface** type. For further abbreviations see Table III.
*Compared to GCE (sex diff.).
**Compared to GCE (no sex diff.).
***Compared to GE (no sex diff.).

the general bivariate and the reciprocal causation model. Models including C or D were not presented as the hypothesis testing showed (Tables III and IV) that they added very little to the models.

Table VI presents standardized parameter estimates of the best fitting general bivariate and reciprocal causation model. Heritability estimates were similar in both models. As expected from the twin correlations heritabilities for fasting insulin levels were relatively small; 20–25% of the variance in fasting insulin could be explained by genetic factors. Heritabilities for glucose levels were higher (around 50%). In the general bivariate model the covariance between glucose and insulin could be explained by unique environmental factors only; therefore, the genetic correlation between insulin and glucose equaled zero ($r_g = 0$). In the causation model reciprocal paths were of opposite sign, which indicates a negative feedback loop.

The predicted phenotypic correlations between glucose and insulin within the best fitting general bivariate and reciprocal causation model were .26 and .23, respectively. Computed for uncorrected values, this correlation was .15 for males and .10 for females.

DISCUSSION

This study investigated the relative contribution of genes and environment to individual differences in fasting insulin levels, and tested for possible sex differences in estimates of these contributions. A general bivariate and a reciprocal causation model including fasting insulin and fasting glucose were used to analyze the data. Comparison of these models enabled us to test whether the origin of the covariance between fasting glucose and fasting insulin could best be explained by corre-

TABLE V. Parameter Estimates (Unstandardized) and 95% Confidence Intervals for GE Models With and Without Sex Differences for Both the General Bivariate and the Reciprocal Causation Model*

	General bivariate model					Reciprocal causation model		
	Sex difference		No sex	No sex diff		Sex difference		No sex
	Males	Females	difference	and h'_c=0		Males	Female	difference
h_c	0.72	1.07	0.90	0.94	h_{glu}	0.89	1.14	1.03
	(0.34,0.98)	(0.85,1.28)	(0.72,1.07)	(0.77,1.09)		(0.63,1.12)	(0.89,1.38)	(0.86,1.21)
h'_c	–0.23	–0.02	–0.13	fixed to 0	h_{ins}	–0.24	0.83	0.60
	(–0.53,0.01)	(–0.28,0.23)	(–0.37,0.09)			(–0.74,0.76)	(0.35,1.34)	(0.20,0.90)
h_s	0.29	0.77	0.57	0.63	e_{glu}	0.93	0.81	0.88
	(–0.49,0.67)	(0.48,0.98)	(0.20,0.77)	(0.41,0.79)		(0.77,1.15)	(0.60,1.02)	(0.76,1.07)
e_c	1.07	0.84	0.96	0.94	e_{ins}	1.45	1.17	1.31
	(0.90,1.27)	(0.69,1.02)	(0.85,1.10)	(0.83,1.07)		(1.18,1.89)	(0.81,1.49)	(1.09,1.69)
e'_c	0.60	0.41	0.52	0.46	x	0.55	0.62	0.58
	(0.37,0.82)	(0.18,0.64)	(0.34,0.70)	(0.31,0.61)		(0.28,0.87)	(0.33,0.94)	(0.31,0.91)
e_s	1.07	0.90	0.99	1.00	y	–0.35	–0.36	–0.35
	(0.90,1.24)	(0.76,1.08)	(0.88,1.13)	(0.89,1.13)		(–0.81,–0.04)	(–0.75,–0.07)	(–0.81,–0.05)
–2lnL	2666.284		2675.621	2676.874	–2lnL	2,664.896		2,675.621
df	967		973	974	df	967		973

*For abbreviations see Figures 1 and 2 and Table III.

TABLE VI. Standardized Parameter Estimates From the Best Fitting General Bivariate and Reciprocal Causation Model*

	Insulin		Glucose		Covariation (insulin and glucose)	
	h^2	e^2	h^2	e^2		
General bivariate model	0.25	0.75	0.50	0.50	$r_g = 0.00$	$r_e = 0.42$
Reciprocal causation model	0.21	0.79	0.47	0.53	x = 0.57	y = –0.35

*h^2 = additive genetic variance; e^2 = unique environmental variance; r_g = genetic correlation between insulin and glucose; r_e = environmental correlation between insulin and glucose; x = causal path from insulin to glucose; y = causal path from glucose to insulin.

lated latent factors (genetic and/or environmental) or by a reciprocal causation of insulin and glucose (a feedback loop). Before we were able to explore these issues, we had to account for two effects within the model fitting: a truncation effect on fasting insulin and an effect of the assay batch on both fasting glucose and insulin.

Plasma samples were analyzed on 8 different occasions using 8 different assay batches, which had a significant influence on the means of both fasting insulin and glucose. As two members of a twin pair were always measured in the same assay batch, this effect could thus induce a spurious increase in twin correlations. Accounting for the assay batch effect in the model fitting, indeed, decreased twin correlations of especially fasting insulin. For the determination of fasting insulin levels, an assay was used with a lower detection limit of 3.0 mU/L. Thirty males (14.7%) and 53 females (24.3%) had values below this detection limit. Within the quantitative genetic model fitting, the bivariate distribution of glucose and insulin was corrected for this truncation effect. The combination of both the correction for truncation and assay batch turned out to have a considerable impact on the twin correlations of fasting insulin. This suggests that, in case variables are truncated and/or show an assay batch effect, these corrections are highly important in order to obtain unbiased quantitative genetic parameters estimates.

Within the general bivariate as well as the reciprocal model fitting, a model specifying the same additive genetic and unique environmental variance components in males and females (but allowing for a sex difference in the mean of fasting insulin levels) gave the best explanation of the data. Heritability estimates were highly similar in both models: 20–25% of the variance in fasting insulin levels and around 50% of the variance in fasting glucose levels could be attributed to genetic factors. The rest of the variance could be attributed to unique environmental factors. As both the best fitting general bivariate model and its reciprocal causation counterpart contained only two “sources of variation” (G and E), they could not be discriminated on the basis of their fit to the data. A submodel of the general bivariate GE model suggested that the covariance between glucose and insulin has a unique environmental basis. Submodels of the reciprocal causation model showed that neither of the reciprocal paths could be set to zero without a loss in fit. Both causal paths are thus needed to explain the phenotypic correlation between insulin and glucose. Estimates of reciprocal paths were of opposite sign, which indicates that the covariation between insulin and glucose can be described by a negative feedback loop.

The heritability of fasting insulin levels in our study is somewhat lower compared to other studies that reported on the heritability of fasting insulin. Two family

studies in Caucasians observed values of .40 [Iselius et al., 1982] and .44 [Schumacher et al., 1992], whereas family studies in Mexican Americans [Mitchell et al., 1996] and Pima Indians [Sakul et al., 1997] found heritability estimates of 44 and 65%, respectively. Similar values were found in two recently published twin studies. Mayer et al. [1996] reported a heritability of .53 in a study of 178 adult women twin pairs, and Hong et al. [1996] observed a value of .48 for both males and females in a sample of 248 middle-aged and elderly twin pairs. Although the estimate of genetic influence was higher in this last study, the lack of a sex difference in heritability of fasting insulin is in accordance with our findings. Using a more sophisticated method to measure insulin resistance (the hyperinsulinaemic euglycemic clamp) in Pima Indians, Sakul et al. [1997] found heritabilities between 38 and 61% depending on the insulin dose and covariate adjustment. Differences in methodology, subject ascertainment, and ethnicity may explain the larger heritability estimates in above-mentioned studies.

Insulin resistance is more prevalent and may, therefore, be more heritable in specific ethnic subgroups like the Pima Indians and Mexican Americans. Further, Schumacher et al. [1992], only found evidence for a major gene affecting insulin levels if the variance in insulin values attributable to body mass index (BMI) was removed. When segregation analysis was done on the unadjusted fasting insulin values, the data were best explained by an environmental model. The use of a supposedly environmental index in the path model applied by Iselius et al. [1982], may have led to biases in their heritability estimate [Rao and Vogler, 1994; Vogler et al., 1987, 1989], whereas the selection of genetically susceptible subjects through family members with NIDDM may have led to higher heritability estimates in the latter two studies.

Twins in the studies of Mayer et al. [1996] and Hong et al. [1996] were older and less healthy compared to the twins in our study. Both studies, for example, included subjects with NIDDM, whereas our study comprised non-diabetic twins only. This difference in age and health may have increased heritability estimates of fasting insulin in the previous studies. The underlying inherited susceptibility for insulin resistance may be reflected by disproportionally high values of fasting insulin at an older age, when environmental exposures have had the chance to fully induce the expression of the genotype. This would imply that total variance of fasting insulin and its genetic part, expressed as heritability, increases with age. The absence of correlations between age and fasting insulin levels for both males ($r = .004$) and females ($r = -.052$) in our study of relatively healthy middle-aged twins indicates that a rise in insulin levels is more probably due to a deterioration of health with age than to the effect of age alone. This is in accordance with a recent study from Ferrannini et al. [1996] on the association between age and insulin resistance in 1,146 men and women, in which it was concluded that age per se is not a significant cause of insulin resistance in healthy Europeans. Findings from Oppert et al. [1995] offer support for above ideas. In a group of 12 healthy monozygotic twin pairs (mean age 21), a low intraclass correlation of .13 was found for fasting insulin. However, the increase in fasting insulin in response to a long-term overfeeding protocol was highly similar within the pairs (.71), which indicates that the insulin response to this unhealthy environmental change is highly genetically determined.

The above considerations indicate that a low heritability of fasting insulin as found in our study of non-diabetic middle-aged twins, does not neccesarily imply

that heritable influences on insulin resistance are unimportant. Although genes may not explain a large part of the variance of fasting insulin in a relatively healthy population, a subgroup of people may be genetically susceptible to environmental influences, like a high-fat, low-fiber diet [Lovejoy and DiGirolamo, 1992; Sharma, 1992; Mayer et al., 1993], a lack of physical activity [Laws and Reaven, 1991; Lampman and Schteingart, 1991; Mikines, 1992; Perseghin et al., 1996], and smoking [Facchini et al., 1992; Attvall et al., 1993], which are known to trigger development of insulin resistance and subsequent hyperinsulinemia. Our finding that no less than 75–80% of the variance in fasting insulin could be explained by unique environmental factors emphasizes the importance of environmental factors. Whatever the identity of the environmental influences on insulin resistance may be, this study shows that these influences are not shared by family members but are specific to an individual.

Fasting insulin is an adequate surrogate for direct measurement of insulin resistance in persons with normal glucose tolerance only [Laakso, 1993; Anderson et al., 1995]. As our sample consisted of middle-aged twins, it is certainly possible that several subjects may have had impaired glucose tolerance [Mooy, 1995]. Future studies on the genetics of insulin resistance may want to use measures that give a good and consistent approximation of this phenotype, even in people with impaired glucose tolerance [Anderson et al., 1995].

Future data on the genetics of insulin resistance may provide more insight into the causes of two major diseases of the western world: non-insulin-dependent diabetes and coronary heart disease. Evidence for a genetic basis of both NIDDM [Newman et al., 1987; Beck-Nielsen and Groop, 1994; Pierce et al., 1995; Todd, 1996; McCarthy et al., 1994; Hanis et al., 1996] and (risk factors for) CHD [Marenberg et al., 1994; Vogler et al., 1997] is manifold. Carmelli et al. [1994] observed that the common latent factor mediating the clustering of hypertension, NIDDM and obesity was influenced by both genetic (59%) and environmental (41%) effects. Although the identity of this factor could not be determined from the available data, insulin resistance was proposed by Carmelli et al. [1994] as a possible candidate. Both Mitchell et al. [1996] and Hong et al. [1997] analyzed a number of indicators of the insulin resistance syndrome in a multivariate way and concluded that there probably is a common set of genes influencing those traits. However, traditional linkage approaches to locate the underlying genes are inappropriate for a complex trait like insulin resistance [McCarthy et al., 1994]. One solution may be to measure, in addition to fasting insulin, a number of other insulin-resistance-syndrome-traits in a large numbers of unselected sib-pairs or DZ twins for use in a genome screen. The finding that there probably is a common set of genes influencing the multiple traits of the insulin resistance syndrome offers prospects for gene finding as Boomsma [1996] and Martin et al. [1998] have shown that multivariate genetic modeling increases the power to locate pleiotropic quantitative trait loci. If we know which genes underlie the insulin resistance syndrome in the normal population, we might also gain more insight in possible pathophysiological mechanisms that lead to insulin resistance, CHD, and NIDDM.

In conclusion, the low heritability of fasting insulin levels we found need not imply that genetic influences on insulin resistance are unimportant. The importance of genetic influences may be restricted to a subgroup of people with a genetically determined susceptibility to develop insulin resistance and subsequent hyperinsulinemia. Future studies

on the genetics of insulin resistance also have to apply measures that show a greater correspondence to this phenotype or analyze multiple indicators of the insulin resistance syndrome. The substantial influence of unique environmental factors on fasting insulin, may offer a hopeful perspective for the treatment and prevention of insulin resistance. This is all the more important as insulin resistance is regarded a primary factor in the development of both NIDDM and CHD.

ACKNOWLEDGMENTS

This research was funded by the Netherlands Heart Foundation (90.313). The authors thank E. Slagboom (Gaubius Laboratory, TNO-Prevention and Lealth, Leiden, The Netherlands) for DNA fingerprinting and R. Laterveer (Gaubius Laboratory), H.M. de Wolf-Prins (Dept. of Psychophysiology, Vrije Universiteit, Amsterdam, The Netherlands), and K. van der Hei-Steenwijk (Dept. of Psychophysiology, Vrije Universiteit) for skillful technical assistance.

REFERENCES

Aitken AC.1934–35. On least squares and the linear combination of observations. Proc R Soc Edinburgh 55:42–48.

Anderson RL, Hamman RF, Savage PJ, et al. 1995. Exploration of simple insulin sensitivity measures derived from frequently sampled intravenous glucose tolerance (FSIGT) tests. The insulin resistance atherosclerosis study. Am J Epidemiol 142:724–732.

Attvall S, Fowelin J, Lager I, von Schenck H, Smith U.1993. Smoking induces insulin resistance: a potential link with the insulin resistance syndrome. J Intern Med 233:327–332.

Beck-Nielsen H, Groop LC.1994. Metabolic and genetic characterization of prediabetic states. Sequence of events leading to non-insulin-dependent diabetes mellitus. J Clin Invest 94:1714–1721.

Bogardus C, Lillioja S, Nyomba BL, et al. 1989. Distribution of in vivo insulin action in Pima indians as mixture of three normal distributions. Diabetes 38:1423–1432.

Boomsma DI.1996. Using multivariate genetic modeling to detect pleiotropic quantitative trait loci. Behav Genet 26:161–166.

Brück K .1983. Functions of the endocrine system. In: Schmidt RF, Thews G, editors. Human physiology. Berlin: Springer-Verlag, pp 658–686.

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.

Després J-P, Lamarche B, Mauriège P, et al. 1996. Hyperinsulinaemia as an independent risk factor for ischemic heart disease N Engl J Med 334:952–957.

Erikkson J, Franssila-Kallunki A, Ekstrand A, et al. 1989. Early metabolic defects in persons at increased risk for non-insulin-dependent diabetes mellitus. N Engl J Med 321:337–343.

Facchini FS, Hollenbeck CB, Jeppesen J, Chen Y-DI, Reaven GM.1992. Insulin resistance and cigarette smoking. Lancet 339:1128–1130.

Falconer DS.1989. Introduction to quantitative genetics, 3rd ed.. Harlow: Longman, pp 313–335.

Ferrannini E, Haffner SM, Stern MP.1990. Essential hypertension: an insulin-resistant state. J Cardiovasc Pharmacol 15(Suppl 5):S18–S25.

Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U.1996. Insulin action and age. European group for the study of insulin resistance (EGIR). Diabetes 45:947–953.

Flier JS.1992. Lilly lecture: Syndromes of insulin resistance. From patient to gene and back again. Diabetes 41:1207–1219.

Fontbonne A.1993. Epidemiological data on hyperinsulinaemia and vascular disease. Diabetes Metab Rev 9(Suppl 1):13s–17s.

Fontbonne AM, Eschwège EM.1991. Insulin and cardiovascular disease: Paris prospective study. Diabetes Care 14:461–469.

Groop LC, Eriksson JG.1992. The etiology and pathogenesis of non-insulin-dependent diabetes. Ann Med 24:483–489.

Grootenhuis PA.1994. Epidemiological aspects of the insulin resistance syndrome. The Hoorn study. PhD Thesis. Amsterdam: Free University.

Gulli G, Ferrannini E, Stern M, Haffner S, DeFronzo RA.1992. The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. Diabetes 41:1575–1586.

Haffner SM, Stern MP, Hazuda HP, Mitchelll BD, Patterson JK.1988. Increased insulin concentrations in nondiabetic offspring of diabetic parents. N Engl J Med 319:1297–1301.

Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP.1992. Prospective analysis of the insulin-resistance syndrome (Syndrome X). Diabetes 41:715–722

Hanis CL, Boerwinkle E, Chakraborty R, et al. 1996. A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. Nature Genet 13:161–166.

Hansen BC.1993. Genetics of insulin action. Ballière's Clin Endocrinol Metab 7:1033–1061.

Heath AC, Kessler RC, Neale MC, Hewitt JK, Eaves LJ, Kendler KS.1993. Testing hypotheses about direction of causation using cross-sectional family data. Behav Genet 23:29–50.

Hong Y, Pedersen NL, Brismar K, Hall K, DeFaire 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.

Hong Y, Pedersen NL, Brismar K, DeFaire U.1997. Genetic and environmental architecture of the features of the insulin-resistance syndrome. Am J Hum Genet 60:143–152.

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.

Jeffreys AJ, Wilson V, Thein SL.1985. Hypervariable "minisatellite" regions in human DNA. Nature 314:67–73.

Kendall M, Stuart A.1977. The advanced theory of statistics. New York: Macmillan.

Laakso M.1993. How good a marker is insulin level for insulin resistance? Am J Epidemiol 137:959–965.

Lampman RM, Schteingart DE.1991. Effects of exercise training on glucose control, lipid metabolism, and insulin sensitivity in hypertriglyceridemia and non-insulin dependent diabetes mellitus. Med Science Sport Exerc 23:703–712.

Lange K, Westlake J, Spence MA.1976. Extensions to pedigree analysis: III. Variance components by the scoring method. Ann Hum Genet 39:485–491.

Laws A, Reaven GM.1991. Physical activity, glucose tolerance and diabetes in older adults. Ann Behav Med 13:125–132.

Lillioja S, Mott DM, Zawadzki JK, et al. 1987. In vivo insulin action is familial characteristic in nondiabetic Pima indians. Diabetes 36:1329–1335.

Lovejoy J, DiGirolamo M.1992. Habitual dietary intake and insulin sensitivity in lean and obese adults. Am J Clin Nutr 55:1174–1179.

Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U.1994. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 330:1041–1046.

Martin BC, Warram JH, Rosner B, Rich SS, Soeldner JS, Krolewski AS.1992. Familial clustering of insulin sensitivity. Diabetes 41:850–854.

Martin NG, Boomsma DI, Machin G.1998. A twin-pronged attack on complex traits. Nature Genet 17:387–392.

Mayer EJ, Newman B, Quesenberry CP, Selby JV.1993. Usual dietary fat intake and insulin concentrations in healthy women twins. Diabetes Care 16:1459–1468.

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.

McCarthy MI, Froguel P, Hitman GA.1994. The genetics of non-insulin-dependent diabetes mellitus: tools and aims. Diabetologia 37:959–968.

Mikines KJ.1992. The influence of physical activity and inactivity on insulin action and secretion in man. Acta Physiol Scand 146 Suppl 609):5–37.

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.

Mooy JM.1995. Non-insulin-dependent diabetes mellitus in a general caucasian population. The Hoorn study. PhD Thesis. Amsterdam: Free University.

Neale MC.1995. Mx: statistical modeling. Box 710 MCV, Richmond, VA 23298: Department of Psychiatry. 2nd edition.

Neale MC, Cardon LR.1992. Methodology for genetic studies of twins and families. Dordrecht: Kluwer Academic Publishers.

Neale MC, Eaves LJ, Kendler KS, Hewitt JK.1989. Bias in correlations from selected samples of relatives: the effects of soft selection. Behav Genet 19:163–169

Newman B, Selby JV, King M-C, Slemenda C, Fabsitz R, Friedman GD.1987. Concordance for Type 2 .non-insulin-dependent) diabetes mellitus in male twins. Diabetologia 30:763–768.

Nijs HGT, Radder JK, Frohlich M, Krans MJ.1990. In vivo relationship between insulin clearance and action in healthy subjects and IDDM patients. Diabetes 39:333–339.

Oppert J-M., Nadeau A, Tremblay A, et al. 1995. Plasma glucose, insulin, and glucagon before and after long-term overfeeding in identical twins. Metabolism 44:96–105.

Perseghin G, Price TB, Petersen KF, et al. 1996. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. N Engl J Med 335:1357–1362.

Pierce M, Keen H, Bradley C.1995. Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. Diabetes Med 12:6–13.

Raffel LJ, Shohat T, Rotter JI.1994. Diabetes and insulin resistance. In: Goldbourt U, DeFaire U, Berg K, editors. Genetic factors in coronary heart disease. Dordrecht: Kluwer Academic Publishers, pp 203–215.

Rao DC, Vogler GP.1994. Assessing genetic and cultural heritabilities. In: Goldbourt U, DeFaire U, Berg K, editors. Genetic factors in coronary heart disease. Dordrecht: Kluwer Academic Publishers, pp 71–81.

Rao DC, Wette R.1987. Nonrandom sampling in genetic epidemiology: maximum likelihood methods for multifactorial analysis of quantitative data ascertained through truncation. Genet Epidemiol 4:357–376.

Reaven GM.1988. Banting lecture 1988: Role of insulin resistance in human disease. Diabetes 37:1595–1607.

Reaven GM.1990. Role of abnormalities of carbohydrate and lipoprotein metabolism in the pathogenesis and clinical course of hypertension. J Cardiovasc Pharmacol 15(Suppl 5):S4–S7.

Reaven GM.1991. Relationship between insulin resistance and hypertension. Diabetes Care Suppl 4 14:S33–S38.

Reaven GM.1993. Role of insulin resistance in human disease (syndrome X): an expanded definition. Ann Rev Med 44:121–131.

Reaven GM.1994. Syndrome X: 6 years later. J Intern Med 236(Suppl 736):13–22.

Reaven GM, Lithell H, Landsberg L.1996. Hypertension and associated metabolic abnormalities: The role of insulin resistance and the sympathoadrenal system. N Engl J Med 334:374–381.

Rich SS. 1990. Mapping genes in diabetes: genetic epidemiological perspective. Diabetes 39:1315–1376.

Sakul H, Pratley R, Cardon L, Ravussin E, Mott D, Bogardus C.1997. Familiality of physical and metabolic characteristics that predict the development of non-insulin-dependent diabetes mellitus in Pima Indians. Am J Hum Genet 60:651–656.

Schumacher MC, Hasstedt SJ, Hunt SC, Williams RR, Elbein SC.1992. Major gene effects for insulin levels in familial NIDDM pedigrees. Diabetes 41:416–423.

Sharma AM.1992. Effects of nonpharmacological intervention on insulin sensitivity. J Cardiovasc Pharmacol 20(Suppl 11):s27–s34.

Snieder H, van Doornen LJP, Boomsma DI.1995. Genetic developmental trends in blood pressure levels, and blood pressure reactivity to stress. In: Turner JR, Cardon LR, Hewitt JK, editors. Behavior genetic approaches in behavioral medicine. New York: Plenum Press, pp 105–130.

Snieder H, van Doornen LJP, Boomsma DI.1997a. The age dependency of gene expression for plasma lipids, lipoproteins, and apolipoproteins. Am J Hum Genet 60:638–650.

Snieder H, Boomsma DI, van Doornen LJP, de Geus EJC.1997b. Heritability of respiratory sinus arrhythmia: dependency on task and respiration rate. Psychophysiology 34:317–328.

Todd JA.1996. Transcribing diabetes. Nature 384:407–408.

Turner ME, Stevens CD.1959. The regression analysis of causal paths. Biometrics 15:236–258.
Vaag A, Henriksen JE, Beck-Nielsen H.1992. Decreased insulin activation of glycogensynthase in skeletal muscles in young nonobese caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. J Clin Invest 89:782–788.
Van Doornen LJP, Snieder H, Boomsma DI.1998. Serum lipids and cardiovascular reactivity to stress. Biol Psychol 47:279–297.
Vogler GP, Rao DC, Laskarzewski PM, Glueck CJ, Russel JM.1987. Multivariate analysis of lipoprotein cholesterol fractions. Am J Epidemiol 125:706–719.
Vogler GP, Wette R, Laskarzewski PM, et al. 1989. Heterogeneity in the biological and cultural determinants of high-density lipoprotein cholesterol in five North American populations: the lipid research clinics family study. Hum Hered 39:249–257.
Vogler GP, McClearn GE, Snieder H, et al. 1997. Genetics and behavioral medicine: Risk factors for cardiovascular disease. Behav Med 22:141–149.
Wajchenberg BL, Malerbi DA, Rocha MS, Lerario AC, Santomauro MG.1994. Syndrome X: a syndrome of insulin resistance. Epidemiological and clinical evidence. Diabetes Metab Rev 10:19–29.
World Health Organization. 1985. Diabetes Mellitus: report of a WHO study group. Geneva: World Health Organization (Technical report series no. 727).

APPENDIX

The analysis of truncated data requires careful specification of the likelihood [Kendall and Stuart, 1977; Rao and Wette, 1987]. If we assume that both the natural logarithm of insulin (I) and glucose (G) are normally distributed (see, e.g., Ferrannini et al. [1990]), then the likelihood of a pair of twins (1 = twin 1, 2 = twin 2) measured on these two variables may be written as:

$$L(I_1, I_2, G_1, G_2) = \phi(I_1, I_2, G_1, G_2) \tag{1}$$

where is ϕ the multivariate normal probability density function (pdf), given by

$$\phi(x) = \det(2\pi\Sigma)^{-m/2} \exp -.5(x-\mu)' \Sigma^{-1}(x-\mu)$$

in which Σ is the covariance matrix of the vector of m observed variables $\mathbf{x}$, whose mean vector is μ.

The software package Mx [Neale,1995] allows computation of truncated multivariate normal integrals. It also computes the likelihood under the multivariate normal pdf of a vector of observed scores (the normal-theory maximum-likelihood method for rectangular or variable length raw data files). Third, it will compute the moments of the truncated normal distribution. It does not have any direct computation of the above multivariate normal functions truncated and integrated over a subset of the dimensions. In a large sample, such computations would be cpu-intensive, requiring separate integrations for each pair in the sample. Instead, we can rearrange the above expressions to reduce them to forms that can be computed efficiently within Mx.

Pairs concordant for being above the detection limit present no special problem; we can use equation 1 above. In the remaining cases, we would like to find the likelihood of the Glucose measures and any Insulin measures above the detection limit. We can find the conditional distribution of the Glucose observations from the moments of the truncated normal distribution [e.g., Neale et al., 1989]. A computa-

tionally efficient way to obtain this is to compute the moments of the truncated (below limit) Insulin measures, and then use the Pearson-Aitken-Lawley selection formula [Aitken, 1934–35] to obtain the moments of the not-selected variables. The justification for this approach lies in a rearrangement of Bayes' theorem:

$$p(A|B) = \frac{p(A\&B)}{p(B)}$$

so

$$P(A\&B) = p(A|B)p(B) \tag{2}$$

in our case, we seek $p(A\&B)$ where A is the Glucose measures and above-detection-limit Insulin measures and B is the below-detection-limit Insulin measures. The moments of B may be obtained from Mx's momnor function, and the Pearson-Aitken formula gives us the the moments of the conditional distribution $A|B$. We then compute the likelihood in the usual way (a reduced form of equation 1) for the observations under this conditional distribution to obtain $p(A|B)$. The probability of observing B [$p(B)$ in equation 2] is simply the multivariate normal integral from minus infinity to the detection limit over all variables below the limit. Thus in the case where only Insulin on twin 1 is below the limit,

$$p(B) = \int_{-\infty}^{t} \phi(I_1)dI_1$$

and where both twins are below the limit we have:

$$p(B) = \int_{-\infty}^{t}\int_{-\infty}^{t} \phi(I_1, I_2)dI_2dI_1$$

These terms differ according to whether the subjects are MZ or DZ, but otherwise would be expected to be the same for all subjects, in the absence of assay batch effects (see below). Writing the m dimensional integral from $-\infty$ to t as $\int_B$, the log likelihood for all pairs is

$$\log(L) = \sum_{j}^{n}\left(\log(\phi(A|B) + \log(\int_B)\right)$$

where the second term is constant for all pairs of a given zygosity. Thus we have

$$\log(L) = n\log(\int_B) + \sum_{j=1}^{j=n}\log(\phi(A|B))$$

so that only one integration step is required per zygosity group, instead of for every subject.

Assay batch effects on the sample resulted in different means for each batch. These in turn affect the amount of truncation represented by an absolute detection limit of 3 mU/L. Therefore separate terms of the form $\int_{-\infty}^{t} \phi(B_k) d_{Bk}$ are needed for the assay batches.