Heritability of Insulin Secretion, Peripheral and Hepatic Insulin Action, and Intracellular Glucose Partitioning in Young and Old Danish Twins

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The etiology of type 2 diabetes is multifactorial, including genetic as well as pre- and postnatal factors that influence several different defects of glucose homeostasis, primarily in muscle, β-cells, and liver. In the present twin study, we report heritability estimates (h^2) for measures of insulin secretion, insulin resistance, hepatic glucose production (HGP), and intracellular glucose partitioning using gold standard methods (euglycemic-hyperinsulinemic clamp technique, tritiated glucose infusion, indirect calorimetry, and intravenous glucose tolerance testing) among 110 younger (22–31 years of age) and 86 older (57–66 years of age) twins. To obtain a valid estimate of β-cell function, insulin secretion was adjusted for the individual degree of insulin action (disposition index). In both age-groups there was a major genetic component in the etiology of insulin secretion that was statistically significantly higher among older twins (young $h^2 = 0.75$ [0.55–0.86] and old $h^2 = 0.84$ [0.69–0.92], $P < 0.05$). The heritability estimates for peripheral insulin sensitivity (young $h^2 = 0.53$ [0.28–0.71] and old $h^2 = 0.55$ [0.20–0.76]) and nonoxidative glucose metabolism (young $h^2 = 0.50$ [0.32–0.64] and old $h^2 = 0.48$ [0.04–0.72]) were similar in younger and older twins, supporting the notion of both genetic and environmental etiological factors in the control of insulin action and nonoxidative glucose metabolism. The results suggested that HGP was predominantly controlled by nongenetic factors in both young and old twins. In conclusion, we provide further evidence for a role of genes in controlling insulin secretion, insulin action, and nonoxidative glucose metabolism. The relative contribution of genes versus environment on in vivo insulin secretion exhibited an age dependency with a slightly greater relative impact of genes among older as compared with younger twins.
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AUC, area under the curve; DZ, dizygotic; HGP, hepatic glucose production; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; MZ, monozygotic; NGT, normal glucose tolerance; NOGM, nonoxidative glucose metabolism; OGTT, oral glucose tolerance test.

Type 2 diabetes is a heterogeneous disease with a multifactorial etiology, including genetic as well as pre- and postnatal environmental factors. A strong genetic influence has been suggested by a greater concordance rate for type 2 diabetes in monozygotic (MZ) compared with dizygotic (DZ) twin pairs (1–5). However, our recent population-based study among older Danish twins demonstrated a relatively stronger environmental influence as opposed to genetics for the development of diabetes per se. The study did, however, also indicate a role for genes in the etiology of abnormal glucose tolerance, including both impaired glucose tolerance (IGT) and type 2 diabetes. Thus, significant heritability estimates were only obtained when twins with IGT were included in the analysis together with twins with overt type 2 diabetes (6).

Impaired insulin secretion, insulin resistance, and increased hepatic glucose production (HGP) are the three major pathophysiological players involved in the development of type 2 diabetes (7). However, it is currently debated which of the defects are primary and perhaps genetically determined and which are secondary to other abnormalities of glucose and lipid metabolism. Patients with overt type 2 diabetes display multiple abnormalities of cellular glucose metabolism, the most important being defective glucose oxidation, glycolytic flux, and nonoxidative glucose metabolism (NOGM) (8). Studies have shown that the genetic component of insulin resistance, as seen in first-degree relatives of type 2 diabetic patients, is almost exclusively explained by defective NOGM (9). Additionally, it is well established that all of the cellular defects of insulin-stimulated glucose metabolism, including nonoxidative glucose uptake in type 2 diabetes, have quantitatively important secondary (nongenetic) components (10).

No major single genes explaining the development of type 2 diabetes have been identified. However, studies have demonstrated associations between various metabolic defects underlying the development of type 2 diabetes and polymorphisms in several susceptibility genes (e.g., *PPARγ* and *PGC-1*) (11,12).

Nongenetic environmental factors include diet, low physical activity, adverse intrauterine environment associated with low birth weight, and age. It may be speculated that the phenotypic expression of an environmentally

induced or possibly inherited defect of a certain biological function may change with age and that such an age dependency could be very important for our understanding of the pathophysiology of type 2 diabetes. In fact, both insulin secretion and to some extent insulin action are known to decrease with age in the general population and in first-degree relatives of type 2 diabetic patients (13).

Few twin studies have addressed the heritability of insulin secretion and/or sensitivity. Most of these studies are based on indirect measures of insulin resistance (fasting hyperinsulinemia [14] and homeostasis model assessment [HOMA] [15]) and insulin secretion (30-min post–oral glucose tolerance test [OGTT] plasma insulin [6]). Similarly, based on studies of a population-based cohort of older twins, we have previously reported heritability estimates of insulin resistance of ~26% and of insulin secretion of ~50% using indirect measures derived from fasting insulin and 30-min post-OGTT insulin concentrations, respectively (16). A recent study (17) in older Finnish twins (aged 54–72 years), using gold standard methods (i.e., euglycemic-hyperinsulinemic clamp and intravenous glucose tolerance test [IVGTT]), demonstrated a high heritability for insulin secretion (55%) and a more modest heritability for insulin resistance (37%). The study did not include younger twins. Furthermore, the study design did not allow estimation of the heritability of HGP and/or intracellular glucose partitioning (17).

In the present study, we report heritability estimates in twins for measures of insulin secretion, insulin resistance, HGP, and intracellular glucose partitioning using gold standard methods (euglycemic-hyperinsulinemic clamp technique, tritiated glucose infusion, indirect calorimetry, and IVGTT) among younger and older twins ascertained from the population-based Danish Twin Registry.

RESEARCH DESIGN AND METHODS

Subjects were identified through the population-based Danish Twin Register (18–20). A random sample of same-sex MZ and DZ twin pairs born in Funen County from 1931 to 1940 (57–66 years of age) and 1966 to 1975 (22–31 years of age) were considered eligible for the study. All potential subjects identified according to these inclusion criteria were contacted and interviewed in order to exclude subjects fulfilling the exclusion criteria. The exclusion criteria were as follows: either twin from the pair not willing to participate; information of pre- or postmaturity birth (birth 3 weeks before or after expected time point); known diabetes; serious heart, liver, or kidney disease; taking medication that influences glucose or lipid metabolism, including oral contraception that could not be withdrawn; and pregnancy/lactation.

A total of 98 twin pairs (33 young MZ, 22 young DZ, 21 older MZ, and 22 older DZ twin pairs) were enrolled in the clinical examination. Among older MZ twins, 76.2% ($n = 32$) had normal glucose tolerance (NGT), 19.0% ($n = 8$) had IGT, and 4.8% ($n = 2$) had previously unknown type 2 diabetes. Among older DZ twins, 72.7% ($n = 32$) had NGT, 25.0% ($n = 11$) had IGT, and 2.3% ($n = 1$) had previously unknown type 2 diabetes. All younger DZ twins had NGT; among younger MZ twins, 97.0% ($n = 64$) were glucose tolerant and 3.0% had IGT ($n = 2$). There was no significant difference in glucose tolerance status between MZ and DZ twins within each age-group. Zygosity was determined by polymorphic genetic markers (21). The study was approved by the regional ethical committees, and the study was conducted according to the principles of the Helsinki Declaration.

Subjects underwent a 2-day clinical examination separated by 1–2 weeks. The two twins in each pair were investigated simultaneously. The subjects were instructed to abstain from strenuous physical activity for 24 h and to perform a 10- to 12-h overnight fast before both examination days.

Day 1 included a standard 75-g OGTT. Peripheral venous blood was taken before oral glucose ingestion and subsequently 30, 60, and 120 min later. Weight and height were measured with the subjects in lightweight clothes with the shoes removed, and BMI (weight [in kilograms] divided by the square of height [in meters]) was calculated. Waist circumference was measured using a soft tape on standing subjects midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteus region, and the waist-to-hip ratio was calculated accordingly. Body composition, i.e., lean body mass and fat mass, was determined by dual-energy X-ray absorptiometry scanning (22).

On day 2, subjects underwent a 2-h hyperinsulinemic-euglycemic clamp (40 $mU \cdot m^{-2} \cdot min^{-1}$) proceeded by a 30-min IVGTT. Polyethylene catheters were placed in the antecubital vein for infusion and in the contra lateral dorsal hand or antecubital vein for blood sampling. This "sampling" hand was placed in a heated Plexiglas box to ensure arterialization of the venous blood sample. A primed-continuous infusion of constant [3-^{3}H]-tritiated glucose (bolus 22 μCi, 0.22 μCi/min) was initiated at 0 min and continued throughout the clinical investigation (basal period 120 min, IVGTT 30 min, and clamp period 120 min). Steady state was defined as the last 30 min of the basal period. After the 2-h basal period, an intravenous glucose bolus (0.3 g/kg body wt, with an upper limit of 25 g glucose) was given over 1 min. Blood samples for glucose and insulin measurements were drawn at 0, 2, 4, 6, 8, 10, 15, 20, and 30 min. After the IVGTT, a primed-continuous insulin infusion (40 $mU \cdot m^{-2} \cdot min^{-1}$) was initiated and continued for 120 min. Steady state was defined as the last 30 min of the 2-h clamp period, when tracer equilibrium (i.e., constant specific activity) was anticipated. A variable infusion of glucose (180 g/l) enriched with tritiated glucose (HOT-GINF) (100 μCi/500 ml) maintained euglycemia during insulin infusion. Plasma glucose concentration was monitored every 5–10 min during the basal and clamp periods using an automated glucose oxidation method (Glucose Analyser 2; Beckman Instruments, Fullerton, CA). Blood samples were drawn for measurements of glucose and insulin every 10–30 min during the basal and clamp steady-state periods.

Indirect calorimetry was performed during both steady-state periods using a computerized flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland). After an equilibrium period of 10 min, the average gas exchange rates recorded over the steady-state periods were used to calculate rates of glucose oxidation and fat oxidation, as previously described (8).

Analytical methods. Plasma glucose concentrations were analyzed by the glucose dehydrogenase oxidation method. Plasma insulin concentrations were measured using a two-site, two-step time-resolved immunofluoremetric assay (Delfia, Turku, Finland), as previously described (23). Crossreactivities with proinsulin, C-peptide, and Des(31,32)-split product in the insulin assay were all <0.4%. Intra- and interassay CVs in the physiological ranges were 3.6–4.3% and 1.7–3.4%, respectively, for plasma insulin. Tritiated water was measured as described by Hother Nielsen et al. (24).

Calculations

Insulin secretion. Insulin response in relation to the glucose concentration (Phi_{IVGTT}) was calculated as the area under the curve (AUC) for insulin divided by the AUC for glucose during the initial 10 min of the IVGTT ($AUC_{ins0-10} \times 10^{-9}/AUC_{glu0-10}$). We calculated disposition indexes (Di_{IVGTT}) in order to estimate the insulin secretion capacity in relation to insulin sensitivity, acknowledging the inverse hyperbolic relationship between insulin secretion and insulin action (insulin secretion [Phi_{IVGTT}] × insulin action [glucose infusion rate during clamp steady state]) (25).

Basal and insulin-stimulated glucose turnover rates. The rate of glucose appearance (R_a), rate of glucose disposal (R_d), and rate of HGP were calculated at 10-min intervals during the steady-state periods using Steele's non–steady-state equations (26). In these calculations, distribution volume of glucose was 200 ml/kg body wt and the pool fraction 0.65. In the calculation of glycolytic flux from the appearance of tritiated water (27), total body water was estimated as 93% of total plasma volume. HGP was calculated by subtracting the rate of the exogenous glucose infusion from the rate of appearance of [3-^{3}H]-tritiated glucose. Exogenous glucose storage was calculated as R_d − glycolytic flux and NOGM as R_d − glucose oxidation, as determined by indirect calorimetry. Glucose turnover rates are expressed as milligrams per kilograms lean body mass per minute and presented as mean values of the 30-min steady-state periods.

Statistical methods. Intraclass correlations and confidence intervals were calculated using the Mx software package. Statistical comparisons of intraclass correlations were made after transformation using the Fisher Z transformation. Raw data for all phenotypes were adjusted for an effect of sex in a regression analysis, and all analyses have been carried out on the residuals. Genetic modeling was conducted separately in the two age-groups using standard Mx scripts. Standard univariate twin modeling based on linear structural equations was used in this study (28). The applied model is based on the assumption that phenotypic variation can be decomposed into additive genetic (A) and genetic dominance (D) or shared environmental (C) and unique environmental effects (E). Additive genetic (A) effects result from single gene effects added over multiple loci, whereas dominant genetic factors (D) refer to genetic interaction within the same locus. Common environment (C) refers to environmental factors shared by twins reared in the same family,

TABLE 1
Clinical and metabolic characteristics among young and older MZ and DZ twins

	Young twins		Older twins	
	MZ	DZ	MZ	DZ
n (M/F)	66 (36/30)	44 (24/20)	42 (22/20)	44 (16/28)
Age (years)	28.2 ± 1.8	27.5 ± 1.9	61.6 ± 2.6	62.0 ± 2.0
Birth weight (g)	2,639.8 ± 90.1	2,561.8 ± 85.2	2,664.4 ± 72.4	2,703.4 ± 103.7
BMI (kg/m^2)	24.3 ± 0.5	23.8 ± 0.6	26.2 ± 0.7	26.1 ± 0.9
WHR	0.85 ± 0.02	0.82 ± 0.02	0.90 ± 0.02	0.87 ± 0.02
FFM (kg)	54.9 ± 1.9	54.3 ± 2.6	51.1 ± 2.7	49.4 ± 2.4
HGP				
Basal	3.10 ± 0.08	3.04 ± 0.08	3.02 ± 0.08	3.10 ± 0.08
Clamp	1.49 ± 0.07	1.49 ± 0.06	1.57 ± 0.09	1.70 ± 0.14
Glucose disposal (R_d)				
Basal	3.10 ± 0.08	3.01 ± 0.07	3.05 ± 0.08	3.14 ± 0.07
Clamp	12.2 ± 0.53	11.0 ± 0.49	8.86 ± 0.59*	10.84 ± 0.57
Glucose oxidation				
Basal	2.17 ± 0.14	2.02 ± 0.15	1.82 ± 0.19	1.75 ± 0.13
Clamp	4.72 ± 0.19	4.54 ± 0.29	3.88 ± 0.26	4.27 ± 0.21
Fat oxidation				
Basal	1.25 ± 0.06	1.32 ± 0.07	1.36 ± 0.08	1.52 ± 0.07
Clamp	0.32 ± 0.05	0.38 ± 0.09	0.56 ± 0.07	0.58 ± 0.08
Glycolytic flux				
Basal	1.62 ± 0.13	2.00 ± 0.19	2.11 ± 0.17	2.28 ± 0.20
Clamp	4.49 ± 0.29	4.37 ± 0.30	3.80 ± 0.30	4.01 ± 0.42
NOGM				
Basal	0.93 ± 0.12	0.99 ± 0.14	1.22 ± 0.17	1.42 ± 0.12
Clamp	7.53 ± 0.46	6.41 ± 0.42	4.99 ± 0.52*	6.67 ± 0.52
Exogenous glucose storage				
Basal	1.48 ± 0.14	1.01 ± 0.20	0.94 ± 0.14	0.86 ± 0.18
Clamp	7.75 ± 0.44	6.59 ± 0.49	5.06 ± 0.53*	6.83 ± 0.55
Insulin secretion (D_i)	2.11E-7 ± 1.14E-7	2.32E-7 ± 1.39E-7	1.10E-7 ± 0.78E-7	1.32E-7 ± 0.92E-7

Data are means ± SE. All metabolic turnover rates are expressed as mg · kg FFM^{-1} · min^{-1}. *$P < 0.05$ for MZ vs. DZ within same age-group. FFM, fat-free mass; WHR, waist-to-hip ratio.

and unique environment (E) represents the environmental experiences that are unique for the individual twin. The fit of each model was assessed by maximum-likelihood methods and resulted in a χ^2 goodness-of-fit index and a probability value, which tested the agreement between the observed and the predicted statistics. In the modeling analysis, we selected the model that best explained the observed covariance matrix (i.e., provided the best χ^2 value separately for the two age-groups). Selecting between non-nested models, the model with the lowest AIC (Akaike′s information criterion) was preferred.

For some of the phenotypes (i.e., basal R_d and NOGM clamp), the variances and/or means across twin pairs and/or zygosity were not equal and therefore did not fulfil the criteria for standard model fitting. To achieve similar means and variances, we performed double entry of the data and adjusted the degree of freedom accordingly, allowing application of the model-fitting analysis on data for basal R_d and the NOGM clamp.

Because MZ twins share their entire genome and DZ twins on average share half of their segregating genes, one may question the supposition that the twins are independent observations. Accordingly, when comparing the phenotypic parameters between MZ and DZ twins, we performed an ANOVA in which we adjusted for the intra–twin pair relationship. The statistical analysis was performed with PROC MIXED of the SAS/STAT system (version 8.2; SAS Institute).

RESULTS

In both age-groups, MZ and DZ twins had similar and birth and adult anthropometry. Furthermore, younger MZ and DZ twins had similar metabolic parameters, whereas older MZ twins had significantly lower insulin-stimulated glucose uptake (R_d), NOGM, and exogenous glucose storage compared with DZ twins (Table 1).

Intraclass correlations were obtained for insulin secretion (D_i), insulin-stimulated glucose uptake (R_d clamp), basal HGP, and basal insulin-stimulated NOGM using Mx in MZ and DZ twin pairs within each age-group, respectively (Table 2 and Figs. 1–3). The data for HGP during the clamp, basal glucose disposal (R_d basal), basal glycolytic flux, and glucose oxidation during both basal and insulin-stimulated steady-state periods (glucose oxidation basal and clamp) did not fulfil the criteria for standard biometric modeling due to differences in mean and/or variance within and between pairs. Nevertheless, when performing double entry of the data, similar means and variances within and between pairs were achieved for basal glucose disposal and NOGM during the clamp period. Negative correlations among either MZ or DZ twins and/or a higher correlations among DZ compared with MZ were seen in the data on glycolytic flux during the clamp period, exogenous glucose storage, and fat oxidation during both steady-state periods; therefore, biometric modeling could not be performed on these data.

Biometric models were calculated for insulin secretion (D_i), basal HGP, glucose disposal during both steady-state periods (R_d basal and R_d clamp), and insulin-stimulated NOGM clamp, which were the only metabolic parameters fulfilling the criteria for biometric modeling (Table 3). In both young and old twins, the best fitting model for insulin secretion was an AE model pointing toward a major genetic component that was statistically significantly higher among older ($a^2 = 0.84$) compared with younger ($a^2 = 0.75$) twins ($P < 0.01$). Basal HGP data in the old twins fitted a model comprising only environmental components

TABLE 2
Intraclass correlations for metabolic variables in MZ and DZ young and older twins

	Young			Old		
	MZ	DZ	*P*	MZ	DZ	*P*
Insulin secretion (D_i)	0.75 (0.56–0.86)	0.30 (−0.10–0.59)	<0.001	0.84 (0.67–0.92)	0.60 (0.27–0.79)	<0.01
HGP						
Basal	0.29 (−0.01 to 0.53)	0.07 (−0.45 to 0.53)	0.25	0.55 (0.16–0.77)	0.53 (0.19–0.74)	0.89
Clamp*	—	—	—	—	—	—
Glucose disposal (R_d)						
Basal†	0.27 (0.06–0.44)	0.13 (−0.35 to 0.50)	0.47	0.67 (0.47–0.80)	0.34 (0.08–0.55)	0.02
Clamp	0.53 (0.26–0.71)	0.31 (−0.21 to 0.64)	0.18	0.56 (0.17–0.77)	0.24 (−0.17 to 0.55)	0.054
Glycolytic flux						
Basal*	—	—	—	—	—	—
Clamp	0.28 (−0.02 to 0.53)	0.58 (0.13–0.78)	—	0.31 (−0.46 to 0.69)	−0.18 (−0.46 to 0.14)	—
Glucose oxidation						
Basal*	—	—	—	—	—	—
Clamp*	—	—	—	—	—	—
NOGM						
Basal*	−0.27 (−0.53 to 0.07)	−0.16 (−0.53 to 28)	—	0.04 (−0.32 to 0.38)	−0.33 (−0.65 to 0.17)	—
Clamp†	0.49 (0.22–0.69)	0.31 (−0.22 to 0.64)	0.28	0.48 (0.02–0.73)	0.10 (−0.29 to 0.44)	0.03
Exogenous glucose storage						
Basal	0.14 (−0.24 to 0.46)	0.24 (−0.16 to 0.54)	—	−0.18 (−0.58 to 0.35)	−0.05 (−0.38 to 0.30)	—
Clamp	0.26 (−0.07 to 0.53)	0.33 (−0.13 to 0.63)	—	0.62 (0.25–0.80)	0.26 (−0.13 to 0.57)	0.02
Fat oxidation						
Basal*	−0.63 (−0.78 to −0.37)	−0.26 (−0.58 to 0.15)	—	−0.22 (− 0.55 to 0.20)	−0.41 (−0.68 to 0.02)	—
Clamp	−0.24 (− 0.54 to 0.14)	0.31 (−0.06 to 0.59)	—	−0.01 (−0.41 to 0.39)	−0.44 (−0.69 to −0.06)	—

Data are correlation coefficients (95% CI) adjusted for sex. *P* value expresses the significance level for comparisons of MZ and DZ twin within each age-group. *Do not fulfill conditions for biometric modeling; †double entry of data.

(CE model: $c^2 = 0.54$ and $e^2 = 0.46$), whereas the data in the young twins fitted a DE model comprising a dominant genetic component ($d^2 = 0.29$) and unique environmental component ($e^2 = 0.71$). The best fitting model for glucose disposal during the basal state (R_d basal) was an AE model in both age-groups. In the younger twins there was a major unique environmental component ($a^2 = 0.27$ and $e^2 = 0.73$). Conversely, there was a major and significantly higher genetic component in the older twins ($a^2 = 0.67$ and $e^2 = 0.33$). Insulin-stimulated glucose uptake (R_d clamp) fitted an AE model in both young ($a^2 = 0.53$ and $e^2 = 0.47$) and older twins ($a^2 = 0.55$ and $e^2 = 0.45$) with a similar distribution of etiological components in both age-groups. Insulin-stimulated NOGM fitted an AE model in the young twins ($a^2 = 0.50$ and $e^2 = 0.50$), whereas among the older twins a DE model was best fitting ($d^2 = 0.48$ and $e^2 = 0.52$) (Table 3).

DISCUSSION

In this study, we report for the first time simultaneously the heritability estimates for the three major pathophysiological mechanisms of relevance in type 2 diabetes (i.e., insulin sensitivity, insulin secretion, and HGP) among young and older twins, disclosing a potential age dependency in the relative importance of genetic versus envi-

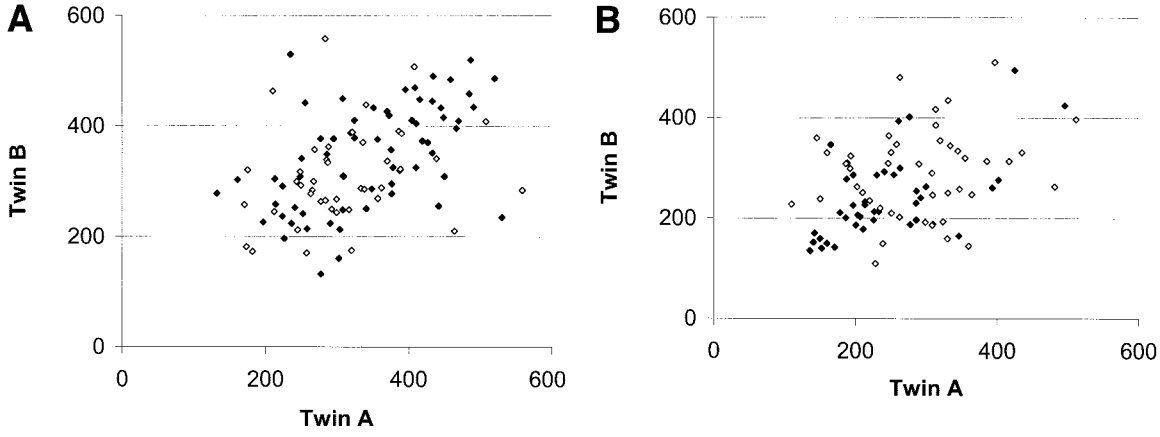

FIG. 1. *A* and *B*: Insulin-stimulated glucose disposal (R_d) in twin A in relation to twin B within each pair of younger (*A*) and older (*B*) MZ (♦) and DZ (◇) twins. The intraclass correlations were $r = 0.53$ for MZ and $r = 0.31$ for DZ younger twin pairs and $r = 0.56$ for MZ and $r = 0.24$ for DZ older twin pairs.

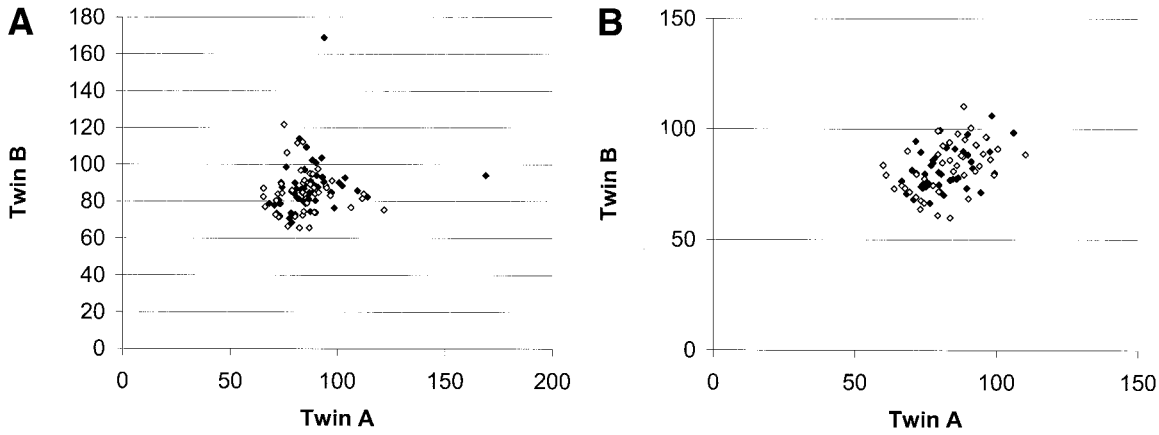

FIG. 2. *A* **and** *B*: **Basal HGP in twin A in relation to twin B within each pair of younger (*A*) and older (*B*) MZ (♦) and DZ (◇) twins. The intraclass correlations were $r = 0.29$ for MZ and $r = 0.07$ for DZ younger twin pairs and $r = 0.55$ for MZ and $r = 0.53$ for DZ older twin pairs.**

ronmental factors. The experimental design using gold standard methods enabled us to distinguish between different pathways (i.e., oxidative and nonoxidative) in glucose metabolism, permitting a more precise estimation of which metabolic defects are primary and presumably genetically determined and which are secondary to other abnormalities of glucose and lipid metabolism.

The heritability estimates (i.e., the proportion of total variance attributable to genetic variance) demonstrated a major genetic component in insulin secretion (expressed in relation to insulin resistance as disposition indexes) among both young and older twins, indicating a role for genes in the etiology and control of insulin secretion. Furthermore, the heritability of insulin secretion was slightly, though significantly, higher among older twins, indicating an influence of age (or cohort) on the relative importance of genes versus environmental factors on insulin secretion. We have previously reported a genetic component ($h^2 = 0.50$) in the etiology of insulin secretion, as determined indirectly by the 30-min post-OGTT plasma insulin concentration and without correction for insulin action in older twins (6). In line with our present findings, Lehtovirta et al. (17) found a significant intraclass correlation for first- and second-phase insulin response to intravenous glucose administration among older MZ twin pairs with heritability estimates of 0.55 and 0.58, respectively. Using insulin secretion rates corrected for the degree of insulin action, and thereby a more precise measure of β-cell function, we confirm and expand this finding to both young and older twins. Few studies have demonstrated a defective insulin secretion among particularly older first-degree relatives of type 2 diabetic subjects (13). Nevertheless, some of the strongest evidence for a genetically determined defect of insulin secretion in the pathophysiology of type 2 diabetes comes from our previous study of MZ twins discordant for type 2 diabetes, demonstrating an impaired insulin secretion among the nondiabetic cotwins of MZ twins with type 2 diabetes as compared with matched NGT control subjects without a family history of type 2 diabetes (9). The present study confirms the notion of an important impact of genetics for the insulin secretion capacity in a larger group of both younger and older nondiabetic twins, supporting the idea that genetic defects could contribute to the impaired insulin secretion as seen in type 2 diabetes.

Interestingly, we found some indication of an age dependency in the relative contribution of genetic versus environmental factors in the etiology and control of insulin secretion, with a heritability estimate of insulin secretion of 0.75 among younger and 0.84 among older twins. Nevertheless, the absolute additive genetic variance decreased with age with a magnitude almost similar to the

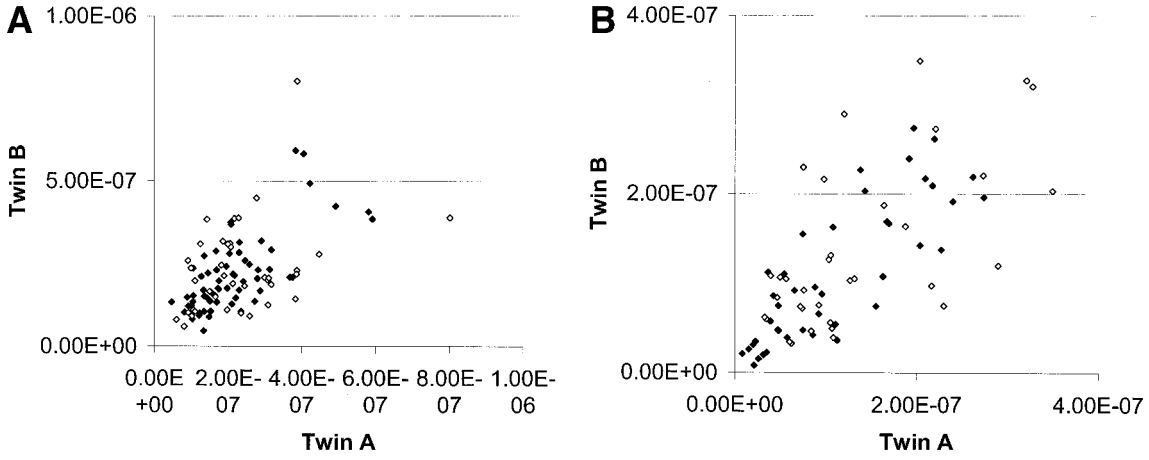

FIG. 3. *A* **and** *B*: **Insulin secretion (D_i) in twin A in relation to twin B within each pair of younger (*A*) and older (*B*) MZ (♦) and DZ (◇) twins. The intraclass correlations were $r = 0.75$ for MZ and $r = 0.30$ for DZ younger twin pairs and $r = 0.84$ for MZ and $r = 0.60$ for DZ older twin pairs.**

TABLE 3
Best fitting biometric models for metabolic variables in young and older twins

	Components of variance				Goodness of fit tests		
	Additive genetic (a^2)	Dominant genetic (d^2)	Common environment (c^2)	Unique environment (e^2)	χ^2	P	AIC
Insulin secretion							
Young							
AE	0.75 (0.55–0.86)*	—	—	0.25 (0.14–0.45)*	0.0	1.00	−2.0
Older							
AE	0.84 (0.69–0.92)	—	—	0.16 (0.08–0.31)	1.4	0.24	−0.6
HGP							
Young							
DE	—	0.29 (0.08–0.47)	—	0.71 (0.53–0.92)	0.0	1.00	−2.0
Older							
CE	—	—	0.54 (0.29–0.72)	0.46 (0.28–0.71)	0.0	0.90	−2.0
Basal glucose uptake							
Young							
AE	0.27 (0.07–0.44)*	—	—	0.73 (0.56–0.93)*	0.0	1.00	−2.0
Older							
AE	0.67 (0.39–0.83)	—	—	0.33 (0.17–0.61)	0.0	0.98	−2.0
Insulin-stimulated glucose uptake							
Young							
AE	0.53 (0.28–0.71)	—	—	0.47 (0.29–0.72)	0.0	0.84	−2.0
Older							
AE	0.55 (0.20–0.76)	—	—	0.45 (0.24–0.80)	0.0	1.00	−2.0
NOGM							
Young							
AE	0.50 (0.32–0.64)	—	—	0.50 (0.36–0.68)	0.16	0.69	−1.8
Older							
DE	—	0.48 (0.04–0.72)	—	0.52 (0.28–0.96)	0.0	1.00	−2.0

Data are proportion of total variance (95% CI) adjusted for sex. *$P < 0.05$ for young vs. old. AIC, Akaike's information criterion.

decrease in total variance of insulin secretion. Because heritability expresses the ratio between additive genetic variance and total variance, the apparently increased heritability of insulin secretion in the older twins may to some extent be explained by the high total variance of insulin secretion in young versus older twins. We speculate that the greater total variance of insulin secretion in young twins may be due to a more diverse level of physical activity, and perhaps diet, as compared with older twins. Indeed, these age differences, or dependencies, of both genetic and nongenetic factors on insulin secretion may somehow be related to the crucial role of age for the development type 2 diabetes. A high level of physical activity, or perhaps other healthy lifestyle factors, may protect some individuals with a genetically predisposed low insulin secretion from type 2 diabetes while they are young, whereas they are less able to escape their genetic potential to develop type 2 diabetes with age.

Peripheral insulin resistance represents an additional major defect in type 2 diabetes. Several studies have reported insulin resistance in nondiabetic first-degree relatives of type 2 diabetes subjects, indicating a genetic component (29). In the present twin study, insulin sensitivity, expressed as insulin-stimulated glucose uptake (R_d clamp) during the euglycemic-hyperinsulinemic clamp steady-state period, had a similar heritability of 0.53 and 0.55 among both young and older twins, respectively, indicating the same relative importance of genes and environment in both age-groups. All previous twin studies (14–16), except for one (17), have used indirect measures of insulin sensitivity (i.e., fasting plasma insulin and HOMA) in the assessment of heritability. The recent Finnish study using the clamp method demonstrated a relatively low heritability for insulin-stimulated glucose uptake of 0.37 (17). Among a larger population-based cohort of older twins, we have previously shown a heritability estimate of 0.26 for fasting plasma insulin concentrations, indicating a major environmental factor on this indirect measure of insulin sensitivity (16). Similarly, other twin studies have previously reported a heritability of 0.47 for fasting insulin concentrations (14) and a heritability of 0.39 for insulin resistance estimated by the HOMA method (15). Thus, using the gold standard euglycemic-hyperinsulinemic clamp technique, the present study confirms the results from other previous twin studies using more indirect measures of insulin action.

We have performed an IVGTT before the clamp, which may in theory have primed the peripheral tissues, thereby potentially increasing glucose disposal and cellular glucose metabolism. However, the arguments for using this particular design were several fold. First, the order of events (bursts of glucose and insulin, followed by glucose uptake) resembles the daily-life meal situation. Second, we wanted to assess independent measures of insulin sensitivity and insulin secretion on the same day in order to calculate the disposition index. And third, we wanted to reduce the number of study days and interventions. Regardless, all study subjects were treated equally, and glucose metabolism was assessed during similar steady-state plasma glucose and insulin concentrations, which is

the most important advantage of the euglycemic-hyperinsulinemic clamp. Two studies have performed tests comparing a clamp with and without an IVGTT and found that glucose disposal from the two clamps correlated strongly ($r = 0.95, P < 0.005$) independent of glucose tolerance (30) and found no differences in nondiabetic offspring of either type 2 diabetic patients or control subjects (31).

The major defect of intracellular glucose metabolism in insulin-resistant subjects is in the pathway of NOGM, primarily accounting for glycogen synthesis in skeletal muscle. Studies in first-degree relatives have indicated a genetic origin of the defective insulin activation of muscle glycogen synthase (29). Conversely, studies in MZ twins discordant for type 2 diabetes have demonstrated that the defective glycogen activation in type 2 diabetes to some extent also has a nongenetic origin (10). The present study is the first classic twin study in which the methodological design permits distinguishing between the heritability of different pathways of glucose metabolism. We found that insulin-stimulated NOGM, like total glucose disposal, was best explained by models in which genetic and unique environmental factors each accounted for ~50% of the variance. Thus, our data on NOGM are in line with our results on total insulin action and further support the results from previous studies of first-degree relatives and nondiabetic cotwins of type 2 diabetic patients, indicating that the defective NOGM in overt type 2 diabetes is explained by both genetic as well as environmental factors. Interestingly, the genetic component in the older twins was of nonadditive genetic origin (dominance), suggesting an interaction between alleles at the same loci. The explanation for and impact of this finding is unknown, as well as it is uncertain whether this finding represents a true age effect, a cohort effect, or perhaps even a random finding. The different model fits may to some extent relate to the age-dependent impact of zygosity status particularly on NOGM and thereby indicate that age-dependent differences in the relative contribution of both pre- and postnatal environmental factors may interact with and/or modify the apparent mode of inheritance. Nevertheless, this does not change the overall conclusion that NOGM is influenced by genetic factors to the same overall extent in both young and older twins.

We have previously demonstrated a similar basal and insulin-stimulated HGP among genetically identical older nondiabetic cotwins (to diabetic twins) compared with matched control subjects, indicating that hepatic glucose processing and the increased HGP in type 2 diabetes is primarily controlled by nongenetic factors (9). No classic twin studies have previously addressed the heritability of HGP and hepatic insulin action. Although biometric modeling suggested an impact of a genetically dominant effect on basal HGP in young twins, this effect was quantitatively minor as compared with the impact of environmental factors and was not seen in the older twins. Accordingly, our data support the notion that HGP is primarily controlled by nongenetic factors in both diabetic subjects as well as young and older nondiabetic subjects.

While skeletal muscle is responsible for the majority of tissue glucose uptake during insulin infusion and insulin resistance in type 2 diabetes, other tissues including the brain account for the majority of glucose utilization in the fasting state (7). We found a marked age dependency of heritability estimates for basal R_d with a low heritability among young twins of 27% and a statistical significantly higher heritability among older twins of 67%. The significance of this finding in understanding the regulation of glucose homeostasis and type 2 diabetes is not known. Thus, the current view is that defective basal glucose uptake does not contribute to the pathophysiology of type 2 diabetes (7–9).

Some distinct metabolic parameters (i.e., glycolytic flux, glucose oxidation, and insulin-stimulated HGP) did not fulfill the conditions for application of biometric modeling (i.e., equal mean values and similar variances within and between twin pairs). Furthermore, exogenous glucose storage and fat oxidation exhibited negative intraclass correlations and/or higher intraclass correlations among DZ compared with MZ twins, which is why application of biometric modeling was not appropriate. However, these findings by themselves and in different ways indicate major roles of environmental factors on these distinct metabolic parameters. For instance, for some of the metabolic parameters (i.e., exogenous glucose storage and fat oxidation), intraclass correlations were higher for DZ as compared with MZ twins. This can be explained by a major role of secondary nongenetic factors, including both the pre- and postnatal environment, but may also be due to random variation in the sample.

The validity of twin studies has previously been challenged by the putative different impact of the intrauterine environment in MZ and DZ twins. Phillips (32) postulated that the measures of similarity (i.e., intraclass correlation and heritability) among MZ twins not only express genetic factors but may also express common fetal environmental factors due to the fact that twins share the same uterine environment. It is generally assumed that MZ twins experience a more adverse intrauterine environment and a more similar ("concordant") environment compared with DZ twins because of the placental circumstances characterizing MZ pregnancies. Indeed we have previously shown that older MZ twins are more insulin resistant compared with DZ twins (20). This difference in insulin action was also seen in this study (Table 1) and was furthermore shown to be accounted for by a difference in NOGM alone. However, the absolute values of birth weight in all twin groups were similar and did not correlate to the metabolic parameters, so adjustment for this parameter was not performed. Furthermore, birth weight discordance by means of intrapair birth weight differences in MZ and DZ twins were similar within each age-group. Although these data of course do not disclose an important impact of the intrauterine environment on insulin secretion and/or action, they do provide some validation of the calculated heritability estimates in this study.

Our finding of a higher degree of insulin resistance in MZ compared with DZ older twins may challenge the assumption of environmental similarity between MZ and DZ twins. MZ and DZ twins were selected in a similar manner. The relatively small sample size could account for the differences in means. However, the present differences in insulin sensitivity among the elder MZ and DZ twins are consistent with our previous findings in a larger twin population (33), so we believe that these differences are

true zygosity effects. Whether these effects originate from prenatal or postnatal life factors is currently unknown. Furthermore, the finding of unequal variance between MZ and DZ twins for some of the metabolic parameters in the present study (i.e., glucose oxidation and insulin-stimulated HGP) could be explained by sibling interactions (i.e., effects of cooperation/competition) regarding, e.g., diet and exercise, parameters with a known effect on these metabolic pathways. These sibling interactions can arise equally well in prenatal life, during childhood, or during adulthood. Preferably, the variance differences could be included in the biometric models. However, the relatively small sample size did not enable us to do this in the present study.

A potential limitation of the results of this study relates to the inclusion of only twins without "known diabetes." Although we did include a few twin pairs with previously unknown mild glucose intolerance and type 2 diabetes, as well as twins with a family history of diabetes, the exclusion of twins with known diabetes may have selected a twin population that had been somewhat "diluted" for type 2 diabetes susceptibility genes, in particular in the group of older twins. The rationale for this selection procedure was to eliminate the known (nongenetic) influence of diabetes diet and other treatments and to minimize the secondary effects of hyperglycemia (glucose toxicity) and elevated fatty acids (lipid toxicity) on the phenotype in question.

Given that the heritability estimates were qualitatively similar in the young and older twins and the fact that type 2 diabetes susceptibility genes are thought to be common in the general population, we do not think that the applied selection procedure has flawed the results to any significant degree or that it has limited the implication of our results for the understanding of the etiology of the major defects of metabolism in patients with type 2 diabetes. Of note, our major results and conclusions are consistent with those from previous studies (8–10) of first-degree relatives of type 2 diabetic patients as well as with studies of twins discordant for type 2 diabetes.

Finally, it should be noted that the applied univariate assessment of the degree of heritability of each of the major metabolic defects in type 2 diabetes does not exclude the possibility that one or more of the distinct defects of metabolism may be influenced by the same genetic or environmental factors.

In conclusion, using the classic twin approach and gold standard methods, we provide further support for a role of genes controlling insulin secretion and insulin sensitivity in vivo. The relative contribution of genes and environment may to some extent exhibit an age dependency with a relatively larger heritability for insulin secretion in older compared with younger twins. Conversely, we demonstrated a near equal impact of genes versus environmental factors on insulin sensitivity (and in particular on the nonoxidative pathway) among older compared with younger twins. In contrast, no major genetic contribution to the variation of HGP was indicated among either younger or older twins. Although the small sample size in this study only allows cautious conclusions, these findings obtained with state-of-the-art techniques may be important in relation to the ongoing search for genes responsible for the development of type 2 diabetes and associated defects of glucose metabolism (i.e., insulin secretion, insulin resistance, and HGP).

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