

# Chapter 4

## The heritability of $\beta$ -cell function parameters in a mixed meal test design

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## Abstract

### *Aims*

Heritability of the individual differences in  $\beta$ -cell function has been estimated in different research settings, but never with the most physiological challenge: the mixed meal test. Here we assessed the heritability of classical and model-derived  $\beta$ -cell function parameters in a mixed meal test design.

### *Methods*

A total of 183 healthy subjects (77 male), recruited from the Netherlands Twin Register, underwent a 4-h mixed meal test. Participants were same-sex twin pairs and their siblings, aged 20-50 years and of European origin. Insulin sensitivity, insulinogenic index, insulin response and post-prandial glycemia were assessed as well as model derived parameters of beta-cell function, in particular  $\beta$ -cell glucose sensitivity and insulin secretion rates. Univariate genetic analyses were used to estimate the heritability of each variable. Multivariate analyses were performed to test overlap in the genetic factors influencing  $\beta$ -cell function, waist circumference and insulin sensitivity.

### *Results*

Significant heritabilities were found for insulinogenic index (63%),  $\beta$ -cell glucose sensitivity (50%), insulin secretion during the first 2 post-prandial hours (42-47%) and post-prandial glycemia (43-52%). Genetic factors influencing  $\beta$ -cell glucose sensitivity and insulin secretion during the first 30 post-prandial minutes showed only negligible overlap with those influencing waist circumference and insulin sensitivity.

### *Conclusions*

The highest heritability for postprandial  $\beta$ -cell function was found for the classical insulinogenic index, but the most specific indices of the heritability of  $\beta$ -cell function appeared to be model-derived  $\beta$ -cell glucose sensitivity and the incremental ISR during the first 30 minutes after a mixed meal.

## Introduction

The pathophysiology of Type 2 diabetes is characterized by insulin resistance, but the development of hyperglycaemia is mainly determined by deterioration of  $\beta$ -cell function (1). Twin and family studies have confirmed a clear cut genetic contribution to type 2 diabetes (2-4). Experimental probing of  $\beta$ -cell function by the intravenous glucose tolerance test (3) and the hyperglycaemic clamp test (5) revealed significant heritability of insulin response after different intravenous secretagogues. Recent genome wide association (GWA) studies have uncovered 26 confirmed gene variants that are associated with a higher risk for the development of type 2 diabetes (6;7) and at least fifteen of these genes affect  $\beta$ -cell function.

Previous findings regarding  $\beta$ -cell function have mostly been based on the calculation of surrogate indices of insulin secretion including fasting- and oral glucose tolerance test (OGTT)-derived plasma insulin and C-peptide levels. The validity of these measures can be expected to be different from the response to a real physiological assessment of the  $\beta$ -cell function as provided by the mixed meal test. This test combines the effect of different natural secretagogues (carbohydrates, proteins and fatty acids) with their contributing effects on gut function, including secretion of incretins and neural signals. To the best of our knowledge no previous study has specifically assessed the heritability of mixed meal test parameters.

The aim of this twin family study is to explore the heritability of classical and model derived  $\beta$ -cell function parameters and of postprandial glycemia indices obtained from a mixed meal test. We used a mathematical model (8;9) that clearly represents different aspects of  $\beta$ -cell function and is frequently applied in intervention studies. The main parameters are insulin secretion rate (ISR), calculated by means of deconvolution of C-peptide levels (10) and  $\beta$ -cell glucose sensitivity, representing the dose-response relation between insulin secretion and glucose concentration. As  $\beta$ -cell function is closely associated with (abdominal) overweight and insulin sensitivity, waist circumference (11) and Oral Glucose Insulin Sensitivity (OGIS)(12), were measured simultaneously. This allowed us to test to which extent genetic factors influencing the most important markers of postprandial insulin secretion (ISR,  $\beta$ -cell glucose sensitivity and insulinogenic index) overlap with those influencing waist circumference and insulin sensitivity.

## Research Design and Methods

### *Participants*

This study used a twin/same-sex sibling design to address genetic and environmental contribution to the variance of  $\beta$ -cell function in Dutch twin families recruited from the Netherlands Twin Register (13) as described previously (14). Mixed meal tests were performed in 183 (77 male) healthy participants of European origin, aged 20 to 49 years. In the weeks prior to these mixed meal tests, the presence of diabetes mellitus was excluded on the basis of a 75 g OGTT. There were 51 MZ twin pairs and 21 same-sex DZ twin pairs from 72 families. Fifteen MZ twin pairs and 8 DZ twin pairs had one additional same-sex sibling. Two MZ twin pairs and one DZ twin pair had 2 additional same-sex siblings. In five more families only one twin of the pair participated together with a same-sex sibling. In total 149 twins and 34 siblings participated. Twin zygosity was determined from DNA polymorphisms. In total 68 dizygotic/sibling pairs could be formed. The mean age difference between twins and their siblings was 3.2 years with a range of 1 to 9 years. Including additional siblings in the classical twin design significantly increases the power to detect the genetic and environmental sources of variation (15). All participants gave written informed consent. The study was approved by the local Ethics Committee and performed in accordance with the Declaration of Helsinki.

### *Meal test*

The nutrient composition of the test meal was calculated from the Dutch Food Composition Table (16) and each portion of the ingredients was weighed before the meal was prepared. The meal for men consisted of 110 g brown bread, 20 g margarine, 25 g fat-rich cheese, 30 g jam, 19 g honey cake and 200cc semi-skimmed milk (721 kcal, 89 g carbohydrates [50% energy], 30 g fat [37% energy] and 24.4 g protein [13% energy]). Women consumed 79% of that meal with the same proportions of nutrients and energy (570 kcal, 71 g carbohydrates, 23 g fat and 19.4 g protein).

After a 12 h fast the mixed meal test procedure was started at the research unit at 08.00 hour. Anthropometric measurements were performed as described previously (5). A cannula was placed retrogradely in a heated dorsal hand vein to obtain arterialized blood. After baseline samples had been taken twice, the test meal was consumed between  $t=0$  and  $t=10$  min. Blood samples for glucose and hormonal levels were drawn at  $t=10, 20, 30, 40,$

50, 60, 90, 120, 150, 180, 210, and 240 min. During the test the participants were confined to bed and were, besides the test meal, only allowed to consume water.

### *Laboratory analysis*

Blood glucose was assessed at bedside using a glucose oxidase method (YSI 2300 Stat plus; Yellow Springs Instruments, Yellow Springs, OH, USA). This device has a within run CV of 2% and a day- to-day CV of 6%. Blood for hormonal levels was centrifuged (1,469 g) at 4<sup>o</sup> C and the serum stored at -80<sup>o</sup> C. All serum specimens were assessed for insulin and C-peptide levels at the VU University Medical Centre (Department of Clinical Chemistry, Amsterdam, The Netherlands) using an immunometric assay luminescence method (ACS: Centaur; Bayer Diagnostics, Mijdrecht, The Netherlands). There was no cross-reactivity with proinsulin or split products. The inter assay CV of insulin and C-peptide measurement was 6.5% and 6% respectively. The intra-assay CV was 4% for both.

### *Classical $\beta$ -cell function parameters*

Fasting and 2-h insulin levels and the insulin IAUC (incremental area under the curve: area under the curve by the trapezium rule minus the fasting level) during the entire 4-h test were measured. The glucose level at 30 min minus fasting level, the glucose level at 120 min and the glucose IAUC during the period from 0 to 120 min and during the entire 4-h test were analyzed. As estimate of early insulin response the insulinogenic index (insulin level t30-t0/glucose level t30-t0) was calculated.

### *Model based $\beta$ -cell function parameters*

ISR and model based  $\beta$ -cell function parameters were calculated using a mathematical model developed by Mari et al.(8;9) with ISR calculated by means of deconvolution of C-peptide level (10). The fasting ISR, the ISR at 4.5 mmol glucose/l and the integral of incremental ISR during three periods: from 0 to 30 min, from 30 to 120 min and during the entire 4-h test were used.

In this model the ISR is composed by the sum of two components that is  $P(t)f(G) + S_d(t)$ , which account for different aspects of the  $\beta$ -cell function.  $f(G)$  is the dose response that represents the static relationship between insulin and glucose concentration during the test. The average slope of this dose-response is denoted as  $\beta$ -cell glucose sensitivity. From

the dose response, insulin secretion at a glucose concentration of 4.5 mmol/l (approximately fasting glucose concentration in the whole group) was also calculated.  $P(t)$  is the potentiation factor which modulates this dose-response relation and has been constrained to have a time average of 1 during the experimental test. It represents a relative potentiation. The second component of insulin secretion ( $S_d(t)$ ) represents the enhancement of insulin secretion proportional to the rate of rising of the plasma glucose concentration and is denoted as rate sensitivity, accounting for an initial fast rise in insulin secretion. Of this model we analyzed rate sensitivity,  $\beta$ -cell glucose sensitivity and the excursions of the potentiation factor using ratios between mean values at different time intervals (e.g.  $P(t100-t120)/P(t0-t20)$  and  $P(t220-t240)/P(t0-t20)$ ).

### *Insulin sensitivity*

Insulin sensitivity (OGIS) was estimated using the meal carbohydrate dose and glucose and insulin levels during the first two hours of the meal test (12).

### *Statistical analysis*

All genetic analyses were carried out in Mx (17), a structural equation modelling program specifically designed for the analysis of twin and family data. In the univariate analyses raw data were used while in the multivariate analyses all variables (waist circumference, OGIS,  $\beta$ -cell glucose sensitivity, insulinogenic index, ISR (0-30) and ISR (30-120) were Z transformed, prior to analysis (mean=0, SD=1) to reduce the large differences in variance across the variables. This transformation does not affect the estimates of familial correlations or heritabilities.

At first, we confirmed that the variances of the variables were comparable for twins and siblings, and that the covariances between DZ twins could be equated to those between a twin and a singleton sibling. The latter allowed us to treat all sibling pairs sharing 50% of their genetic material, whether DZ twin or twin-sibling pair, in the same way.

In the univariate analyses the within-variable cross-person correlations were assessed with age and sex as covariates. The MZ and DZ/sibling correlations describe the resemblance for a variable in MZ twins and in all other pairs of first-degree relatives (DZ twins, twin-sibling, sibling-sibling). These correlations form the basis to estimate the relative contribution of genetic and environmental factors to individual differences in each

variable. MZ twin pairs have all, or nearly all, genes in common, and DZ twin pairs, twin-sibling pairs and sibling-sibling pairs share on average half of their segregating genes. If MZ correlations are twice the DZ/sibling correlations or larger, genetic influences are suggested as the main source of familial resemblance. These genetic influences can be additive (A) or can act in a non-additive manner (D). If the MZ correlations are less than twice the DZ/sibling correlations, common environmental (C) influences shared by family members are suggested in addition to genetic influences. The remaining source of individual differences in the variable are unique environmental influences (E) including measurement error.

In a genetic univariate model (18) the total variance was decomposed into A, C or D, and E components for each trait. We tested if the contributions of these A, C and D factors were all significant using likelihood-ratio tests. The difference in minus two times the log-likelihood (-2LL) between two nested models has a  $\chi^2$  distribution. A corresponding p-value  $> 0.05$  indicated that the more parsimonious model did not fit the data less well than the full model. This procedure was repeated for each variable to arrive at the most parsimonious model that fitted the data. Under this model we estimated the heritability of each variable individually.

Multivariate analyses were performed to assess the phenotypic correlations between selected variables and to reveal the overlapping and separate genetic influences on these variables. The three sets of variables selected were: 1) waist circumference, OGIS and  $\beta$ -cell glucose sensitivity, 2) waist circumference, OGIS, and the insulinogenic index, 3) waist circumference, OGIS, ISR (0-30) and ISR (30-120). All multivariate analyses were performed following the same procedure, based on the results of the univariate analyses. First we estimated the cross variable, within person correlations (e.g. the phenotypic correlations among waist circumference, OGIS and  $\beta$ -cell glucose sensitivity) and then the cross variable cross person correlations in MZ and DZ/sibling pairs for example between the waist circumference of a twin and the OGIS of her co-twin. When the cross-variable cross-person correlation is larger in MZ twin than in DZ/sibling pairs, this indicates that part of the association between the variables is explained by overlapping genetic factors.

Next, a multivariate genetic ACE model was fitted to the data. For instance the full 3 variate models included a set of 3 factors for A, C and E. The first factor influences all 3 variables; the next factor influences 2 variables and the last factor only one variable. We tested if the contributions of these A and C factors were all significant using likelihood-

ratio tests. Next the paths of each A and E factor to the 3 variables were tested for significance (figure 4.1 illustrates this for waist circumference, OGIS and  $\beta$ -cell glucose sensitivity). Under this model we estimated the heritability of each variable individually, and decomposed this heritability into components that are specific to each variable (e.g. waist circumference, OGIS and  $\beta$ -cell glucose sensitivity) and components that overlap (e.g.  $\beta$ -cell glucose sensitivity with waist circumference and OGIS).

Due to the statistical procedure, there may be slight differences in heritability estimates between the univariate and multivariate analyses.

## Results

Sample characteristics are shown in Table 4.1. Waist circumference, weight,  $\beta$ -cell glucose sensitivity, ISR during the first 30 minutes and the insulinogenic index were significantly larger in men; OGIS, ISR at 4.5mmol/l glucose and the IAUC of glucose during the first 2 hours and during the total 4-h mixed meal test were significantly larger in women. The influence of age was significant for waist circumference ( $\beta = +0.51, p=0.003$ ), ISR during the period 30 to 120 min ( $\beta = +0.31, p=0.035$ ) and OGIS ( $\beta = -2.6, p=0.003$ ).

MZ and DZ/sib correlations are shown in Table 4.2, as well as the univariate estimates of A, C and E under the most parsimonious genetic model. Significant heritability was found for 13 variables, and significant contribution of shared environmental factors for 4 variables. No significant family resemblance was found for the potentiation factor ratios and rate sensitivity.

There were significant phenotypic correlations between  $\beta$ -cell glucose sensitivity and waist circumference ( $r = 0.21, p=0.01$ ) and between  $\beta$ -cell glucose sensitivity and OGIS ( $r = -0.33, p < 0.01$ ). Figure 4.1 illustrates the most parsimonious AE model resulting from this 3 variate analysis.

Table 4.1. Sample Characteristics

Variable	total	male	female
Number	183	77	106
Age (years)	31.0 $\pm$ 5.1	30.7 $\pm$ 4.8	31.3 $\pm$ 5.3
Waist circumference (cm)	84.7 $\pm$ 9.8	87.4 $\pm$ 8**	82.8 $\pm$ 10.3
Weight (kg)	72.7 $\pm$ 11.4	79.0 $\pm$ 9**	68.2 $\pm$ 10.6
Oral Glucose Insulin Sensitivity (ml min <sup>-1</sup> m <sup>-2</sup> )	487 $\pm$ 51	471 $\pm$ 50**	498 $\pm$ 50
Model derived $\beta$ -cell function parameters			
$\beta$ -cell glucose sensitivity (pmol min <sup>-1</sup> m <sup>-2</sup> mmol l <sup>-1</sup> )	131 $\pm$ 52	149 $\pm$ 57**	119 $\pm$ 44
Fasting Insulin Secretion Rate (pmol min <sup>-1</sup> m <sup>-2</sup> )	55.3 $\pm$ 16.2	54.6 $\pm$ 17.3	55.8 $\pm$ 15.4
ISR integral of increment (0-30) (nmol m <sup>-2</sup> )	4.83 $\pm$ 2.27	5.37 $\pm$ 2.31*	4.46 $\pm$ 2.18
ISR integral of increment (30-120) (nmol m <sup>-2</sup> )	20.0 $\pm$ 8.8	19.1 $\pm$ 9.1	20.6 $\pm$ 8.6
ISR integral of increment (0-240) (nmol m <sup>-2</sup> )	34.1 $\pm$ 12.9	32.2 $\pm$ 12.6	35.5 $\pm$ 13.0
ISR at 4.5mmol/l glucose (pmol min <sup>-1</sup> m <sup>-2</sup> )	81.8 $\pm$ 34.5	69.3 $\pm$ 29.1*	91.1 $\pm$ 35.4
Potential factor ratio (100-120)/(0-20)	1.26 $\pm$ 0.34	1.25 $\pm$ 0.35	1.27 $\pm$ 0.34
Potential factor ratio (220-240)/(0-20)	1.00 $\pm$ 0.27	1.02 $\pm$ 0.26	0.99 $\pm$ 0.27
Rate sensitivity (pmol min <sup>-1</sup> m <sup>-2</sup> mmol l <sup>-1</sup> )	1135 $\pm$ 607	1137 $\pm$ 657	1135 $\pm$ 573
Classical $\beta$ -cell function parameters			
Insulinogenic index (pmol l <sup>-1</sup> /mmol l <sup>-1</sup> )	171 $\pm$ 88	194 $\pm$ 106*	156 $\pm$ 70
Fasting serum insulin (pmol/l)	34.8 $\pm$ 15.0	33.9 $\pm$ 16.7	35.4 $\pm$ 13.7
Serum insulin at t120 (pmol/l)	20.7 $\pm$ 12.3	19.9 $\pm$ 13.0	21.2 $\pm$ 11.8
Serum insulin IAUC (0-240) (pmol x hr/l)	613 $\pm$ 270	588 $\pm$ 269	630 $\pm$ 271
Glucose t30 minus t0 (mmol/l)	2.08 $\pm$ 0.67	2.11 $\pm$ 0.59	2.05 $\pm$ 0.72
Glucose at t120 (mmol/l)	5.39 $\pm$ 0.65	5.29 $\pm$ 0.55	5.46 $\pm$ 0.71
Glucose IAUC (0-120) (mmol x hr/l)	2.59 $\pm$ 1.05	2.31 $\pm$ 0.96*	2.80 $\pm$ 1.07
Glucose IAUC (0-240) (mmol x hr/l)	3.70 $\pm$ 1.66	2.98 $\pm$ 1.3**	4.23 $\pm$ 1.71

Data are means  $\pm$  SD. \*p<0.05, \*\*p<0.01 vs female; ISR= Insulin Secretion Rate; IAUC= Area Under the Curve minus fasting level.

Table 4.2. Twin/sib correlations and heritability estimates with CI(95%) under the most parsimonious model

variable	Correlation		A: Heritability	C: Shared environment	E: Unique environment
	MZ	DZ/sib			
Waist circumference	0.60	0.30	60 (40, 74)		40 (26, 60)
Weight	0.59	0.25	57 (34, 73)		43 (27, 67)
Oral Glucose Insulin Sensitivity	0.47	0.30	49 (28, 65)		51 (35, 72)
<u>Model derived <math>\beta</math>-cell function parameters</u>					
<u><math>\beta</math>-cell glucose sensitivity</u>	0.51	0.23	50 (26, 68)		50 (32, 74)
Fasting Insulin Secretion Rate	0.43	0.21	43 (21, 61)		57 (39, 79)
ISR integral of increment (0-30)	0.42	0.31	45 (24, 62)		55 (38, 76)
ISR integral of increment (30-120)	0.41	0.25	40 (16, 58)		60 (42, 84)
ISR integral of increment (0-240)	0.43	0.36		40 (21, 56)	60 (44, 79)
ISR at 4.5 mmol/l glucose	0.57	0.45		50 (34, 64)	50 (36, 66)

Potentiation factor ratio $_{(100-120)/(0-20)}$	0.04	0.09	7 (0, 52)	93 (75, 1)
Potentiation factor ratio $_{(220-240)/(0-20)}$	0.20	0.02	15 (0, 39)	85 (61, 1)
Rate sensitivity	0.18	0.16	17 (0, 37)	83 (63, 1)
<hr/>				
Classical $\beta$ -cell function parameters				
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Insulinogenic index	0.63	0.31	63 (43, 77)	37 (23, 57)
Fasting serum insulin	0.37	0.20	38 (11, 59)	62 (41, 89)
Serum insulin at t120	0.26	0.21	24 (4, 43)	76 (57, 96)
Serum insulin IAUC (0-240)	0.45	0.41	44 (25, 59)	56 (41, 75)
Glucose (t30 minus t0)	0.54	0.17	52 (30, 68)	48 (32, 70)
Glucose at t120	0.51	0.13	50 (30, 66)	50 (34, 70)
Glucose IAUC (0-120)	0.58	-0.10	50 (31, 75)	50 (31, 75)
Glucose IAUC (0-240)	0.48	0.02	43 (19, 62)	57 (38, 81)

A = additive genetic influence ; C = shared environmental influence; E = Unique environmental influence; A, C and E values in %.

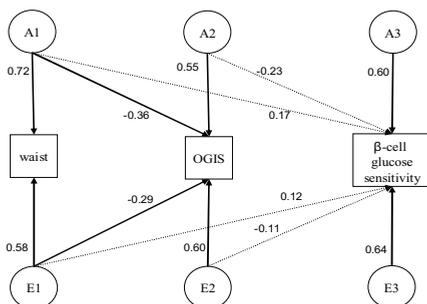


Figure 4.1. Genetic model for waist, OGIS and  $\beta$ -cell glucose Sensitivity with path loadings of observed variables on the latent additive genetic(A) and unique environment (E) factors. Dotted line = non significant.

Table 4.3 shows the decomposition of heritabilities of these three parameters under this model. Out of the 50% of the variance in OGIS due to genetic factors, about one third (15%) is due to the same genetic factors that also influence waist circumference, whereas the remaining two thirds (35%) of the variance is due to genetic factors unique to OGIS. The overlap in the genetic factors influencing  $\beta$ -cell glucose sensitivity and those that influence waist circumference or OGIS, in contrast, is negligible.

Table 4.3 Heritability of waist, OGIS and  $\beta$ -cell glucose sensitivity .

Variable	Heritability			
	Total	Part of the heritability deriving from the genetic factor for		
		waist	OGIS	$\beta$ -cell glucose sensitivity
waist	60 (40, 74)	60		
OGIS	50 (30, 66)	15	35	
$\beta$ -cell glucose sensitivity	50 (27, 68)	3	6	41

Values are per cent (95% CI); OGIS= oral glucose insulin sensitivity.

The phenotypic correlation of insulinogenic index with waist circumference and with OGIS was highly significant ( $r = 0.35$  and  $-0.40$ , respectively, both  $p < 0.01$ ). A second 3-variate analysis (waist circumference, OGIS and insulinogenic index) showed that this phenotypic correlation was mainly based on genetic factors, shared by waist circumference, OGIS and insulinogenic index. Table 4.4 shows the decomposition of heritabilities of these three parameters under the most parsimonious model which was again an AE model. Of the 63% of the variance in the insulinogenic index that was due to genetic factors, about one third

Table 4.4 Heritability of waist, OGIS and insulinogenic index

Variable	Heritability			
	Total heritability	Part of the heritability deriving from the genetic factor for		
		waist	OGIS	insulinogenic index
waist	60 (40, 74)	60		
OGIS	50 (30, 66)	15	35	
insulinogenic index	63 (43, 77)	10	10	43

Values are per cent (95% CI); OGIS= oral glucose insulin sensitivity.

is due to the same genetic factors that also influence waist circumference (10%) or OGIS (10%), whereas the remaining two thirds (43%) of the variance are due to genetic factors unique to the insulinogenic index.

Phenotypic correlations between waist circumference, OGIS, ISR (0-30) and ISR (30-120) are given in Table 4.5 and were all significant. The correlations between waist circumference and insulin secretion during the first two postprandial hours were less than those between OGIS and insulin secretion.

Table 4.5

Phenotypic correlations CI(95%) between waist, OGIS, ISR(0-30) and ISR(30-120)

	waist	OGIS	ISR (0-30)
OGIS	-0.50 (-0.61, -0.36)		
ISR (0-30)	0.13 (-0.03, 0.29)	-0.22 (-0.37, -0.06)	
ISR (30-120)	0.17 (0.01, 0.32)	-0.50 (-0.61, -0.37)	0.41 (0.27, 0.54)

OGIS = oral glucose insulin sensitivity; ISR (0-30) = integral of incremental insulin secretion from 0-30 min; ISR (30-120) = integral of incremental insulin secretion from 30 to 120 min.

According to the results of the 4-variate decomposition shown in Table 4.6 only a very small part of the total heritability of ISR (0-30) is derived from genetic factors shared with waist circumference and OGIS, while nearly one third of the total heritability of ISR (30-120) is derived from genetic factors influencing waist circumference and OGIS.

Table 4.6 Heritability of waist, OGIS, ISR(0-30) and ISR(30-120)

Variable	Heritability		Part of the heritability deriving from the genetic factor for			
	Total Heritability	Heritability				
		waist	OGIS	ISR(0-30)	ISR(30-120)	
waist	62 (42, 75)	62				
OGIS	50 (30, 66)	15	35			
ISR(0-30)	47 (26, 63)	2	3	42		
ISR(30-120)	42 (19, 61)	6	7	8	21	

Values are per cent (95% CI); OGIS= oral glucose insulin sensitivity; ISR (0-30)= integral of incremental insulin secretion from 0 to 30 min; ISR (30-120) = integral of incremental insulin secretion from 30 to 120 min.

## Discussion

This same-sex twin family study is the first that estimated the heritability of  $\beta$ -cell function parameters derived from a mixed meal test, using classical as well as model derived parameters (8).

Our study shows that the model derived  $\beta$ -cell glucose sensitivity has a high heritability (50%) with a negligible overlap with genetic factors for waist circumference and insulin sensitivity. This replicates and extends findings in non genetically related samples (19) that also showed  $\beta$ -cell glucose sensitivity to be largely unrelated to waist circumference and insulin sensitivity. The  $\beta$ -cell glucose sensitivity is the most important parameter of the model because it quantifies the ability of the  $\beta$ -cell to respond to changes in glucose concentration and is a significant independent predictor of glucose intolerance. Recent findings (20;21) suggest a number of variants in several genetic loci (near genes *MTNR1B*, *CDKALI*, *HHEX/IDE* and *TCF7L2*) that can account for the substantial heritability in  $\beta$ -cell glucose sensitivity.

The insulinogenic index is a classically calculated estimate of early insulin response in OGTT, already described in 1967 (22). It is strongly associated with the Acute Insulin Response after intravenous glucose administration (23) and is an independent predictor of worsening of glucose tolerance (24). In this study the insulinogenic index (insulin level t30-t0/glucose level t30-t0) was the postprandial parameter with the largest heritability (63%). This is substantially higher than the 36% heritability found when the insulinogenic index was estimated during an OGTT (25). In spite of this lower heritability, the insulinogenic index as derived from OGTT data has led to the identification of at least sixteen independent loci in genetic association studies (26-30).

The insulinogenic index and  $\beta$ -cell glucose sensitivity have a strong phenotypic correlation (0.68), of which 66% is explained by common genetic factors (data not shown). However, they appear to represent partly different aspects of the beta cell function. Murphy (31) showed that carriers of glucokinase gene mutations have a normal insulinogenic index but reduced  $\beta$ -cell glucose sensitivity with a large right shift. Tura (32) revealed that normal glucose tolerant women, who had previous gestational diabetes, had decreased  $\beta$ -cell glucose sensitivity but a normal insulinogenic index. Mari et al (33) demonstrated that the insulin secretagogue nateglinide improved  $\beta$ -cell glucose sensitivity in patients with type 2

diabetes, while the insulinogenic index did not change significantly. Despite its higher heritability, the insulinogenic index shared more genetic factors with waist circumference and insulin sensitivity than  $\beta$ -cell glucose sensitivity. This makes model derived  $\beta$ -cell glucose sensitivity a more specific genetic marker of the  $\beta$ -cell function.

Although the heritability of fasting insulin levels has been estimated in many studies (ranging from 8% (34) to 54% (35)), to our knowledge the heritability of fasting ISR, which takes insulin clearance into account, has never been assessed. The heritability of the fasting ISR and of the fasting insulin level show the same order of magnitude (43% and 38% respectively) and there is a high correlation between the two insulin measurements (0.80). However, fasting ISR is a better measure of the activity of the  $\beta$ -cell than insulin level, because insulin level is strongly co-determined by insulin clearance. The ISR (0-30) was less correlated with waist circumference and OGIS than the ISR (30-120) and the genetic variation influencing early insulin secretion also overlapped less with that for waist circumference and OGIS than later insulin secretion. This is again compatible with the relatively high number of genetic loci found in GWA studies, that are associated with early insulin secretion (28) while only two genetic loci are found to be significantly associated with reduced insulin secretion during the 2-h OGTT (29;36).

All postprandial glycemia parameters were significantly influenced by genetic factors, with heritability estimates ranging between 43% (4 hours) and 52% (first 30 min). Heritability of the other mixed meal test parameters ISR(0-240), ISR at 4.5 mmol glucose, potentiation factor ratios (100-120)/(0-20) and (220-240)/(0-20), rate sensitivity, serum insulin at t120 and serum insulin IAUC(0-240) was not significant although in many instances the MZ twin pair correlation was larger than the DZ/sib pair correlation. This may reflect the major limitation of the approach used in this study, namely the limited sample size of only 183 participants. Because meal size (37), meal composition (38) and rate of gastric emptying (39) have influence on postprandial insulin secretion and glycemia, a strict protocol was required with adequate trained assistance and researchers. Because our total study design consisted of several tests, it was time consuming and had relatively high costs thereby prohibiting a larger sample size. However, our results show a two-hour meal test to be sufficient for genetic testing, which makes this test really suitable for larger studies.

Up till now GWA studies have only used results of OGTT to test  $\beta$ -cell function and glucose tolerance after a glucose challenge. The advantage of an OGTT test over the mixed meal test is that it is cheaper, it has a simple protocol and it is feasible in the large samples

required for GWA. The disadvantage is that it gives only information of the effect of one secretagogue and is not a reflection of daily life. The mixed meal test is a real physiological challenge with different natural types of secretagogues with influence on incretin secretion and neural activation and in this way will give more relevant information. Moreover, with the same amount of CH intake (40), the  $\beta$ -cell glucose sensitivity and the insulinogenic index are larger in a 2hr mixed meal test than in an OGTT. This makes the mixed meal test a powerful method to study the effects of candidate genetic variants deriving from GWA studies in more detail.

In summary, we find that the highest heritability for postprandial  $\beta$ -cell function was found for the classical insulinogenic index, but the most specific  $\beta$ -cell function parameters appeared to be model derived  $\beta$ -cell glucose sensitivity and the integral of incremental ISR during the first 30 postprandial minutes. We conclude that the mixed meal test provides multiple heritable aspects of the  $\beta$ -cell function that can help us examine the biology underlying the wealth of genetic variants produced by GWA studies.

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