GLP-1 Responses Are Heritable and Blunted in Acquired Obesity With High Liver Fat and Insulin Resistance

Niina Matikainen,[1,2,3] Leonie H. Bogl,[5] Antti Hakkarainen,[3,4] Jesper Lundbom,[3,4] Nina Lundbom,[3,4] Jaakko Kaprio,[5,6,7] Aila Rissanen,[2,8] Jens J. Holst,[9] and Kirsi H. Pietiläinen[1,2,6]

OBJECTIVE

Impaired incretin response represents an early and uniform defect in type 2 diabetes, but the contributions of genes and the environment are poorly characterized.

RESEARCH DESIGN AND METHODS

We studied 35 monozygotic (MZ) and 75 dizygotic (DZ) twin pairs (discordant and concordant for obesity) to determine the heritability of glucagon-like peptide 1 (GLP-1) responses to an oral glucose tolerance test (OGTT) and the influence of acquired obesity to GLP-1, glucose-dependent insulinotropic peptide (GIP), and peptide YY (PYY) during OGTT or meal test.

RESULTS

The heritability of GLP-1 area under the curve was 67% (95% CI 45–80). Cotwins from weight-concordant MZ and DZ pairs and weight-discordant MZ pairs but concordant for liver fat content demonstrated similar glucose, insulin, and incretin profiles after the OGTT and meal tests. In contrast, higher insulin responses and blunted 60-min GLP-1 responses during the OGTT were observed in the heavier as compared with leaner MZ cotwins discordant for BMI, liver fat, and insulin sensitivity. Blunted GLP-1 response to OGTT was observed in heavier as compared with leaner DZ cotwins discordant for obesity and insulin sensitivity.

CONCLUSIONS

Whereas the GLP-1 response to the OGTT is heritable, an acquired unhealthy pattern of obesity characterized by liver fat accumulation and insulin resistance is closely related to impaired GLP-1 response in young adults.

Diabetes Care 2014;37:242–251 | DOI: 10.2337/dc13-1283

The insulinotropic response to intraluminal nutrients is mediated through secretion of gut incretins, mainly glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). In healthy individuals, this incretin effect accounts for up to 70% of insulin secretion in an oral glucose tolerance test (OGTT) (1). In their classic study, Nauck et al. (2) demonstrated that compromised incretin effect is a central pathological feature of type 2 diabetes.

The inherent nature of the incretin defect in type 2 diabetes has raised speculations on whether altered secretion or action of incretins represents a primary, genetically

[1]Department of Medicine, Division of Endocrinology, University of Helsinki, Helsinki, Finland
[2]Diabetes and Obesity, Research Programs Unit, University of Helsinki, Helsinki, Finland
[3]Heart and Lung Center, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland
[4]Department of Radiology, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland
[5]Department of Public Health, Hjelt Institute, University of Helsinki, Helsinki, Finland
[6]FIMM Institute for Molecular Medicine, University of Helsinki, Helsinki, Finland
[7]National Institute for Health and Welfare, Department of Mental Health and Substance Abuse Services, Helsinki, Finland
[8]Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland
[9]The Novo Nordisk Foundation Center for Basic Metabolic Research, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Corresponding author: Kirsi H. Pietiläinen, kirsi.pietilainen@helsinki.fi.

Received 30 May 2013 and accepted 19 August 2013.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc13-1283/-/DC1.

determined defect predisposing to diabetes. Genetic factors are difficult to isolate in demanding metabolic studies, and thus the genetic influence on the incretin response remains poorly characterized. Although several polymorphisms in the GIP receptor (GIPR) have been associated with both obesity and glycemia (3,4), genetic loci predisposing to type 2 diabetes identified so far do not directly involve *GLP-1* or *GIP* genes (5,6). Besides GLP-1, intestinal L cells secrete peptide YY (PYY), an anorexigenic hormone, in response to nutrient stimuli. Polymorphisms in the *PYY* gene may contribute to obesity and diabetes risk (7,8). Therefore, it is relevant to study whether incretin responses to oral nutrients impaired in obesity are accounted for by genetic variation or due to acquired factors.

Monozygotic (MZ) and dizygotic (DZ) twins offer a study design to estimate the relative contribution of genes and environment to various traits. Closer resemblance of MZ twins as compared with DZ twins suggests the importance of genetic factors to the trait under study. Phenotypically discordant MZ twins are of special interest as the two extremes of the phenotype do not differ in their genomic sequence but all of the variability in the phenotype is accounted for by variations in the environmental factors or gene-environment interaction (9).

The aims of our study were to investigate the heritability of GLP-1, insulin, and glucose response after an OGTT in young-adult twin pairs. In addition, GLP-1 and GIP responses after an OGTT and GLP-1 and PYY after a mixed meal were studied in more detail in MZ twin pairs who are discordant only for weight or discordant for weight, liver fat, and insulin sensitivity, i.e., present a pattern of metabolically healthy or unhealthy obesity.

RESEARCH DESIGN AND METHODS

Subjects

The current study consists of 110 twin pairs, 35 MZ (17 male and 18 female) and 75 DZ (43 male and 32 female) pairs, aged 22.8–33.1 years, identified from Finn Twin16 and Finn Twin12 cohorts (n = 5,417 families) (10,11). Twenty-three MZ twins have detailed measurements of adiposity, and their initial data have been published previously (12). Fourteen of the MZ pairs and 40 of the DZ pairs were defined as discordant for BMI (intrapair difference, ΔBMI $\geq$3 kg/m^2), and the rest were concordant (ΔBMI $<$3 kg/m^2). All pairs were Caucasian of Finnish ancestry. One MZ twin had an inactive ulcerative colitis and used mesalazine and azathioprine. All other subjects were healthy and normotensive and did not take any medications except for oral contraceptives. The study protocols were approved by the ethical committee of the Hospital District of Helsinki and Uusimaa, Finland. Written informed consent was obtained from all participants.

Body Composition

Weight, height, whole-body fat (dual-energy X-ray absorptiometry); abdominal, subcutaneous, and intra-abdominal fat (magnetic resonance imaging); and liver fat (magnetic resonance spectroscopy) were measured as described previously (13). The magnetic resonance imaging/magnetic resonance spectroscopy measurements were performed for the intensive MZ subsample (23 MZ twin pairs: 14 discordant and 9 concordant).

Glucose, Insulin, and Incretins During the OGTT and Meal Test

The 75-g OGTT was performed after a 12-h overnight fast with measurements of plasma glucose, serum insulin, plasma GLP-1, and plasma GIP at 0, 30, 60, and 120 min. The meal test with a standardized McDonald's Big Mac Meal (hamburger, 100 g French fries, and 400 g sucrose-sweetened Coca Cola) containing 979 kcal (123 g carbohydrates, 40 g fat, 32 g protein) was performed ~3 weeks after the OGTT, after a 12-h overnight fast with measurements of plasma glucose, serum insulin, plasma GLP-1, and plasma PYY at 0, 30, 60, and 120 min. The meal test was performed only for the intensive subsample.

HOMA-IR (14) and Matsuda index (15) were calculated using glucose and insulin measurements from the OGTT measurements. GLP-1, GIP, and PYY area under the curves (AUCs) were calculated using the trapezoid rule.

Analytical Measures

Plasma glucose and serum insulin were measured as previously described (12). GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (volume/volume, final concentration). For the GIP radioimmunoassay (16) we used the COOH terminus–directed antiserum code 867, which was raised against a synthetic peptide corresponding to the COOH terminus of human GIP. It does not cross-react with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion is uncertain. It reacts fully with the primary metabolite GIP 3-42. Human GIP and ^{125}I human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured (17) against standards of synthetic GLP-1 7-36–amide using antiserum code 89390, which is specific for the amidated COOH terminus of GLP-1 and therefore does not react with GLP-1–containing peptides from the pancreas. The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite GLP-1 9-36–amide, into which GLP-1 is rapidly converted. For both assays, sensitivity was $<$1 pmol/L, intra-assay CV $<$6% at 20 pmol/L, and recovery of standard, added to plasma before extraction, ~100% when corrected for losses inherent in the plasma extraction procedure.

Statistical Analyses

Statistical analyses were performed with Stata statistical software (release 11.0; Stata Corp., College Station, TX) and Mx, a software designed for the analysis of twin data (18). Results are expressed as mean ± SE unless otherwise informed. Comparisons between the cotwins were made by matched-pairs Wilcoxon rank sum tests. Comparisons between lean-to-lean and obese-to-obese cotwins in two phenotypically different MZ discordant groups were made by Mann-Whitney U test. Sex distributions between the groups were tested by χ^2 test. Within-pair differences (Δ) were calculated by

subtracting the leaner cotwin's value from the heavier cotwin's values. Intraclass correlation coefficients (ICCs) were computed for each variable and zygosity group. A higher within-pair resemblance in MZ twins as compared with DZ twins is suggestive of potential genetic influences in the traits.

Quantitative genetic analyses were then conducted by the method of maximum likelihood to quantify the genetic and environmental influences on the variables. In this analysis, the phenotypic variances are decomposed into additive genetic effects of individual alleles (A), dominant genetic effects by allelic interactions inside a locus (D), environmental effects shared by the cotwins (C), and unique (nonshared) environmental effects unique to each twin (E). The estimates of the variance components were calculated as the proportion of variance divided by the total variance. The proportion of A in relation to total variance (percent) is called heritability, expressed as h^2. Heritability is a population-specific characteristic that has no interpretation on the level of the individual or family. One can fit models based on the different combinations of these parameters (ADE, ACE, AE, and CE), but effects due to dominance and shared environmental effects cannot be simultaneously modeled with data limited to that from twins reared together. Also, DE models, in which the effects of D and E are estimated but the additive genetic effects are fixed to zero were not fitted because such models are biologically implausible (19). The ACE and ADE models were compared with one another using the Akaike information criterion (AIC) (20). The model with the lower AIC was used as a starting point of the modeling, from which the significance of variance components was tested by removing them sequentially in nested submodels. Model fit was assessed using the −2 log likelihood. Submodels were compared with the full models by use of hierarchical χ^2 tests. The best model fit was evaluated according to the principle of parsimony, in which models with fewer parameters were considered preferable if the removal did not result in a significant degradation of model fit. The goodness of fit of the submodels was also evaluated by the AIC. Classical twin models assume that MZ and DZ pairs share common environmental factors to the same extent, no interaction between genes, and no gene-environment interactions (20). Saturated models were used to test the basic assumptions of twin modeling (equal means and variances for twin 1 and twin 2 and for MZ and DZ pairs). No age effect was seen in the saturated models. For the estimation of variance components and ICCs, log-transformed and sex-adjusted values were used.

RESULTS

Heritability of GLP-1 and Insulin Sensitivity Measures

The means (SE), ICCs, and heritability estimates of the best-fitting models of GLP-1 and insulin sensitivity measures are given in Table 1. There were no significant differences between the MZ and DZ twins in the means of HOMA index, Matsuda index, fasting GLP-1, and AUCs for glucose, insulin, and GLP-1. Therefore, the assumption of the twin method that the trait means do not differ between MZ and DZ twins was fulfilled. The ICCs were higher for MZ than DZ pairs for all measures, except for fasting GLP-1, suggesting the presence of genetic variance for most variables. The results from univariate model fitting analysis and fit statistics for the full and reduced models are shown in Supplementary Table 1. For HOMA index, Matsuda index, AUC insulin, AUC glucose, and AUC GLP-1, the AE model fitted the data better than the ACE or the ADE model. The AE model was also superior to the E model. Hence, the best-fitting model contained only additive genetic and unique environmental components (AE model). For AUC insulin, the ADE model had a slightly lower AIC than the AE model (AIC: −101.751 for ADE and −101.114 for AE). However, in the ADE model, all of the genetic influence was placed on the D effect. Since a model in which all the variance is due to dominance and none is additive is biologically implausible, AE models were chosen as the best-fitting model. The heritability of variables for insulin sensitivity/resistance ranged from 52 to 60%, and that of GLP-1 AUC was 67%. The remaining variance was explained by unique environmental factors. In contrast, for fasting GLP-1, the genetic contribution was not significant, and the variance in fasting GLP-1 was explained solely by common (58% [95% CI 44–69]) and unique (42% [31–56]) environmental factors (Table 1).

MZ and DZ Pairs Discordant for Obesity

Adiposity and metabolic measures for MZ and DZ twin pairs are summarized in Table 2. In the MZ obesity-discordant pairs, the average intrapair difference (Δ) of BMI was 5.7 kg/m^2, in the MZ concordant pairs 1.3 kg/m^2, in the DZ discordant pairs 6.2 kg/m^2, and in the DZ concordant pairs 1.6 kg/m^2. The MZ

Table 1—Mean values, ICCs, and heritability estimates for OGTT-derived traits in 35 MZ and 75 DZ twin pairs adjusted for sex

	Mean ± SE		ICC (95% CI)		
	MZ	DZ	MZ	DZ	Heritability (95% CI)
HOMA index	1.50 ± 0.14	1.45 ± 0.09	0.58 (0.35–0.80)	0.16 (0.00–0.39)	0.52 (0.35–0.71)
Matsuda index	8.41 ± 0.76	9.00 ± 0.43	0.67 (0.47–0.87)	0.18 (0.00–0.41)	0.60 (0.34–0.77)
AUC glucose (mmol · h · L^{-1})	13.5 ± 0.43	12.9 ± 0.25	0.56 (0.32–0.80)	0.22 (0.00–0.44)	0.52 (0.28–0.69)
AUC insulin (mU · h · L^{-1})	89.27 ± 8.06	85.55 ± 4.33	0.69 (0.50–0.87)	0.12 (0.00–0.36)	0.59 (0.33–0.67)
AUC GLP-1 (pM · h · L^{-1})	25.38 ± 1.5	28.34 ± 1.36	0.61 (0.39–0.83)	0.37 (0.16–0.57)	0.67 (0.45–0.80)
Fasting GLP-1 (pmol/L)	7.47 ± 0.43	8.12 ± 0.45	0.47 (0.20–0.73)	0.64 (0.50–0.77)	0 (0–0)

No significant difference in means between MZ and DZ twins using the Wald test for equality of means.

Table 2—Clinical characteristics of the MZ and DZ twin pairs by concordance and discordance for BMI

	MZ discordant pairs (ΔBMI ≥3 kg/m^2)									
	Group 1 with Δliver fat <2.5%		Group 2 with Δliver fat ≥2.5%		MZ concordant pairs (ΔBMI <3 kg/m^2)		DZ discordant pairs (ΔBMI ≥3 kg/m^2)		DZ concordant pairs (ΔBMI <3 kg/m^2)	
	Leaner (*n* = 7)	Heavier (*n* = 7)	Leaner (*n* = 7)	Heavier (*n* = 7)	Leaner (*n* = 21)	Heavier (*n* = 21)	Leaner (*n* = 40)	Heavier (*n* = 40)	Leaner (*n* = 35)	Heavier (*n* = 35)
Age (years)	27.0 ± 1.5		27.2 ± 1.6		29.2 ± 0.5		28.0 ± 0.3		28.4 ± 0.3	
Sex (male/female)	2/5		3/4		12/9		24/16		19/16	
Height (cm)	171.3 ± 3.8	171.3 ± 3.7	175.3 ± 4.8	175.4 ± 4.3	172.4 ± 1.9	172.8 ± 1.8	173.6 ± 1.2	174.9 ± 1.5	173.2 ± 1.6	171.6 ± 1.4
Weight (kg)	69.2 ± 5.7	86.6 ± 6.4*	81.6 ± 8.3	98.0 ± 7.7*	74.8 ± 3.4	79.0 ± 3.0***	65.6 ± 1.4	75.3 ± 2.1***	70.3 ± 2.5	73.7 ± 2.6**
BMI (kg/m^2)	23.3 ± 1.2	28.6 ± 1.1*	26.2 ± 1.5	31.7 ± 1.7*	25.1 ± 0.8	26.4 ± 0.8***	21.7 ± 0.4	27.9 ± 0.6***	23.2 ± 0.6	24.9 ± 0.7***
Body fat (%)	33.3 ± 2.7	42.6 ± 2.7*	32.5 ± 4.3*	40.5 ± 3.0*	27.9 ± 2.1	30.2 ± 1.9**	21.7 ± 1.7	33.2 ± 1.6***	26.6 ± 1.4	27.8 ± 1.6
Subcutaneous fat (dm^3)†	3.3 ± 0.4	5.9 ± 0.6*	4.1 ± 0.8	6.1 ± 0.8*	3.6 ± 0.4	3.9 ± 0.5	NA	NA	NA	NA
Intra-abdominal fat (dm^3)†	0.4 ± 0.09	0.8 ± 0.09	0.7 ± 0.2	1.4 ± 0.2*#	1.0 ± 0.2	1.0 ± 0.2	NA	NA	NA	NA
Liver fat (%)†	0.5 ± 0.03	0.7 ± 0.1	1.1 ± 0.4	6.0 ± 0.9*##	1.3 ± 0.3	2.6 ± 1.2	NA	NA	NA	NA
OGTT										
HOMA index	1.1 ± 0.2	1.4 ± 0.3	1.5 ± 0.3	2.8 ± 0.6*	1.4 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	1.9 ± 0.2***	1.4 ± 0.1	1.6 ± 0.2
Matsuda index	8.7 ± 1.3	8.1 ± 0.9	7.8 ± 2.0	3.7 ± 0.4*	9.7 ± 1.3	8.2 ± 1.1	10.9 ± 0.9	7.3 ± 0.7**	8.6 ± 0.7	8.6 ± 0.8
AUC glucose (mmol · h · L^{-1})	11.7 ± 0.9	12.1 ± 0.9	13.0 ± 0.7	15.9 ± 0.8*	13.5 ± 0.7	13.5 ± 0.7	12.5 ± 0.5	13.3 ± 0.5	12.8 ± 0.4	13.0 ± 0.4
AUC insulin (mU · h · L^{-1})	82.3 ± 13.1	87.6 ± 16.6	77.4 ± 9.3	129.2 ± 11.2*	88.6 ± 17.5	83.0 ± 11.4	79.5 ± 6.9	103.0 ± 10.0	76.7 ± 6.2	85.3 ± 10.8
Fasting GLP-1 (mmol/L)	7.8 ± 0.7	8.5 ± 1.1	6.7 ± 0.9	5.7 ± 1.1	7.6 ± 0.7	7.8 ± 0.8	8.6 ± 0.7	7.7 ± 0.6	8.1 ± 0.7	8.2 ± 0.9
AUC GLP-1 (pM · h · L^{-1})	29.3 ± 5.9	30.9 ± 5.6	28.3 ± 2.1	22.9 ± 3.7	26.0 ± 2.0	23.6 ± 1.8	31.6 ± 2.4	25.6 ± 1.9*	29.2 ± 2.9	27.6 ± 2.4
AUC GIP (pg · h · mL^{-1})	34.6 ± 8.8	26.8 ± 6.1	40.3 ± 6.5	57.7 ± 9.6	47.2 ± 4.2	43.9 ± 9.0	NA	NA	NA	NA
Meal test							NA	NA	NA	NA
AUC glucose (mmol · h · L^{-1})	11.9 ± 1.0	12.7 ± 0.9	14.5 ± 0.6	15.5 ± 0.8	14.0 ± 0.6	13.9 ± 0.7	NA	NA	NA	NA
AUC insulin (mU · h · L^{-1})	99.5 ± 22.6	109.6 ± 24.2	99.7 ± 12.1	150.2 ± 29.1*	95.2 ± 14.1	93.2 ± 13.1	NA	NA	NA	NA
AUC GLP-1 (pM · h · L^{-1})	26.5 ± 1.8	32.8 ± 7.1	31.8 ± 7.2	25.4 ± 7.7	32.7 ± 5.6	30.5 ± 3.9	NA	NA	NA	NA

Data are mean ± SE. Only significant differences are shown. NA, not assessed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for differences between leaner vs. heavier cotwin (paired Wilcoxon test). #$P < 0.05$, ##$P < 0.001$ for differences between heavier cotwins between group 1 and group 2 discordant MZ pairs. †*n* = 11 concordant pairs

obesity-discordant pairs could be further divided to liver fat–discordant or –concordant groups, based on a cutoff of 2.5%, the mean ΔLiver fat within all MZ discordant pairs. In group 1, all obese and nonobese cotwins had low liver fat contents (range 0.3–0.8%, mean ΔLiver fat 0.04%), whereas in group 2, the liver fat in the obese (range 3.8–9.4%) was on average 4.9 percentage points higher than in the nonobese cotwins (range 0.4–3.5%). Group 1 (Δ5.8 kg/m^2) and group 2 (Δ5.4 kg/m^2) had equally large BMI differences. The obese twins in group 2 had significantly higher liver fat content (P = 0.0012) and intra-abdominal fat volume (P = 0.037) than obese twins in group 1, but the other adiposity measures did not differ. Lean cotwins between the groups did not differ on any adiposity measures or sex.

The MZ obesity-discordant groups 1 and 2 differed significantly for metabolic health. Whereas the obese twins in group 1 did not differ from their lean cotwins for HOMA in fasting (P = 0.50) or Matsuda index during the OGTT (P = 0.69), the obese twins in group 2 had significantly higher HOMA (P = 0.018) and lower Matsuda (0.028) than the lean twin pair members. In the DZ pairs, liver fat was not measured and groupings for liver fat could not be done. In the whole DZ obesity-discordant group, HOMA (P = 0.0022) and Matsuda indexes (P = 0.0023) revealed significantly poorer insulin sensitivity in the obese as compared with the lean cotwins. Concordant MZ and DZ twin pairs had statistically significant adiposity differences between leaner and heavier cotwins, but metabolically the cotwins resembled each other within pairs.

Effects of Acquired Obesity on Glucose, Insulin, and Incretin Responses

In the OGTT, glucose, insulin, and GLP-1 responses did not differ within group 1 obesity-discordant MZ pairs (Fig. 1 and Table 2), suggesting that obese individuals who remain insulin sensitive do not have altered GLP-1 secretion. In contrast, within group 2 obesity-discordant MZ pairs, GLP-1 was blunted at 60 min in every obese cotwin as compared with their leaner counterparts (P = 0.022). Both AUC glucose (P = 0.028) and AUC insulin (P = 0.028) were significantly higher in group 2 obese than nonobese cotwins. Similar tendencies were observed for the meal test, although they were significant only for the AUC insulin, which was increased in group 2 obese as compared with the nonobese cotwins (P = 0.047).

In the DZ obesity-discordant pairs, the AUC GLP-1 during the OGTT was significantly lower in the obese than in the nonobese cotwins (P = 0.029). The obese cotwins also had higher glucose and insulin values at 0 and 120 min ($P < 0.05$).

GIP was measured in MZ pairs during the OGTT. Neither the group 1 nor group 2 obesity-discordant pairs revealed significant differences between the cotwins. However, it is of note that the GIP response was not lower (rather the opposite) in the insulin-resistant group 2 obese twins (Fig. 1 and Table 2).

In MZ and DZ concordant pairs, all incretin, glucose, and insulin measures were the same for all cotwins within pairs (Figs. 1 and 2 and Table 2).

Effects of Obesity on the Relative Responses of Incretins to Glucose and Insulin

The ratios of GLP-1 or GIP AUC to glucose and insulin AUC are shown in Fig. 3. Relative to the prevailing glucose concentration in the OGTT, the GLP-1 secretion was blunted in group 2 obese MZ twins (P = 0.028), whereas in group 1, the obese and nonobese twins had similar GLP-1/glucose AUC ratios. The same applied to GLP-1/insulin AUC, which was lower in group 2 obese relative to nonobese cotwins both in OGTT (P = 0.018) and the meal test (P = 0.046). Similar low GLP-1/glucose and GLP-1/insulin AUC ratios were observed for obese cotwins within the DZ obesity-discordant pairs. Neither MZ nor DZ concordant twins differed from each other in these measures.

PYY3-36

PYY3-36 was measured during the meal test in MZ twins. It did not differ between the group 1 and group 2 obesity-discordant groups (data not shown). PYY3-36 AUC in the leaner versus heavier cotwins was 108.9 ± 7.7 vs. 119.6 ± 10.8 pg/mL (P = 0.18) in the discordant and 104.4 ± 15.8 vs. 105.3 ± 25.4 pg/mL (P = 0.71) in the concordant twin pairs.

CONCLUSIONS

We studied the contribution of genes and environment to incretin response after an OGTT in a population of healthy MZ and DZ twin pairs using a classic twin design. Based on measurements of the major incretin, GLP-1 after an OGTT, we estimate the heritability of GLP-1 response to be 67%. Furthermore, we demonstrate in a unique sample of weight- and liver fat–discordant twin pairs that defective incretin response to OGTT or mixed meal associates with features of high liver fat and insulin resistance.

Because incretin defect in type 2 diabetes appears early and apparently uniformly, the question under study has long been if altered secretion of incretins represents a primary genetic defect (21). To our knowledge, our study is the first one to address the question of whether the incretin defect is genetic or associated with acquired obesity. We found that the response of GLP-1 after OGTT is highly correlated within MZ and to a lesser extent within DZ twin pairs, and that genetic factors explain a large part of the variance in GLP-1 in young adulthood. Heritability estimates for HOMA index, Matsuda index, AUC insulin, and AUC glucose were generally in the same range as those reported for OGTT-derived measures of glucose tolerance and indices of insulin sensitivity and secretion in a large population–based sample of adult female and male twin pairs free of diabetes or cardiovascular disease (22).

An acquired "healthy" pattern of obesity determined by low liver fat and/or normal HOMA and Matsuda indexes was not associated with an altered incretin response. Further, the PYY-36 and GIP responses to oral nutrients were not altered in acquired obesity per se. Previous data on healthy subjects demonstrate that the incretin response is independent of prevailing glucose concentration and that GLP-1 response is rather reproducible among individuals (23). In line with our results, no incretin defect was demonstrated in the first-degree relatives of patients with type 2

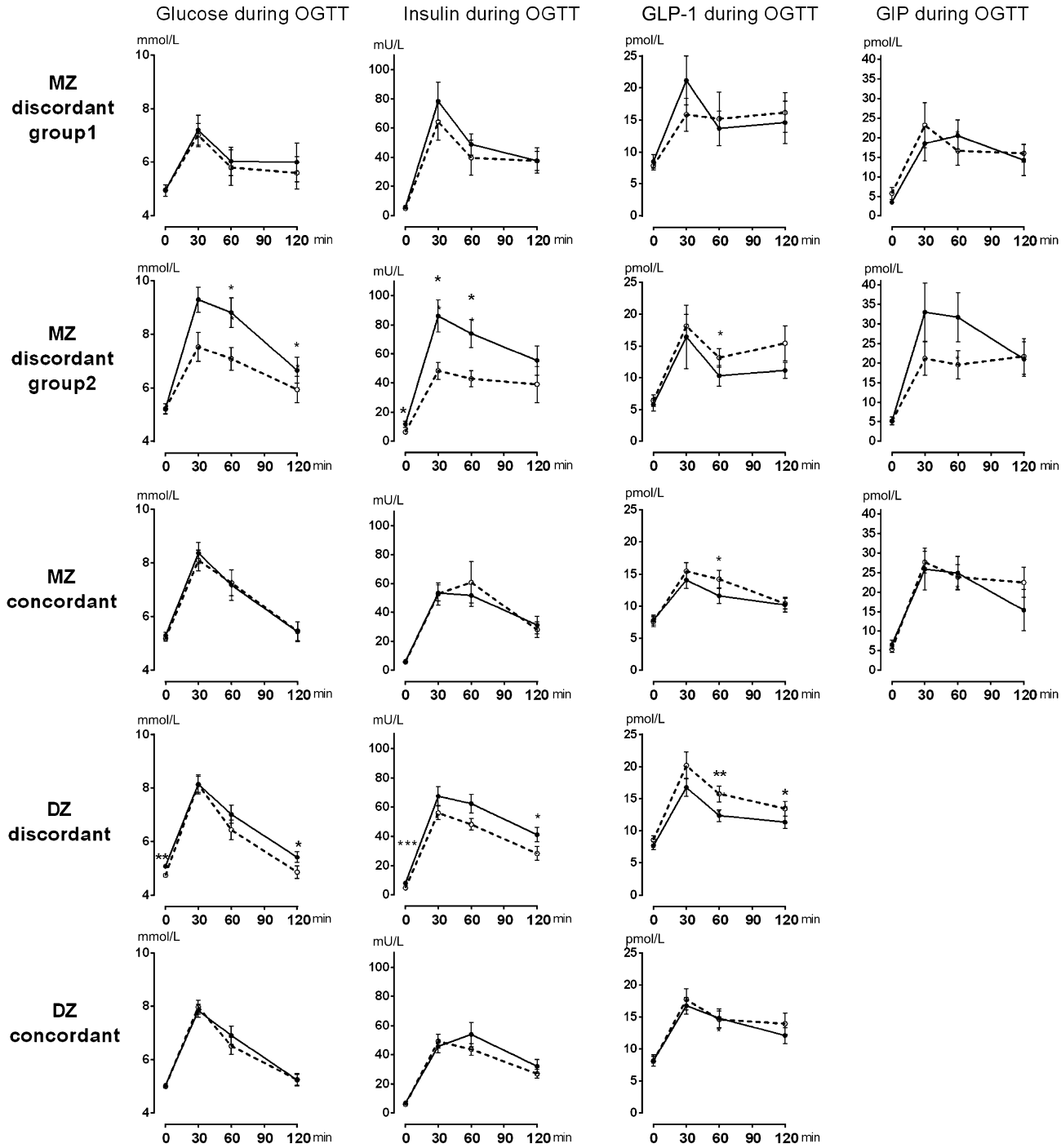

Figure 1—The responses of glucose, insulin, GLP-1, and GIP after OGTT in MZ and DZ twin pairs either discordant or concordant for BMI. Dashed lines represent leaner cotwins and solid lines heavier cotwins. Group 1 is MZ twin pairs discordant for BMI but not for liver fat. Group 2 is MZ twin pairs discordant for both BMI and liver fat. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ for differences between leaner vs. heavier cotwin (paired Wilcoxon test).

diabetes as compared with healthy control subjects with comparable insulin sensitivity (24) or in women with a history of gestational diabetes (25). These studies support our findings that obese cotwins with preserved insulin sensitivity had normal GLP-1, GIP, insulin, and glucose concentrations after OGTT and meal test.

We could identify an extremely informative subset of MZ twin pairs who are discordant for obesity, liver fat, and insulin resistance, i.e., each pair includes a metabolically healthy lean versus an unhealthy obese twin with identical genotype. The heavier, more insulin-resistant cotwin group with higher liver fat showed blunted GLP-1 response to both oral challenges. That finding was confirmed among weight-discordant DZ twin pairs, among whom

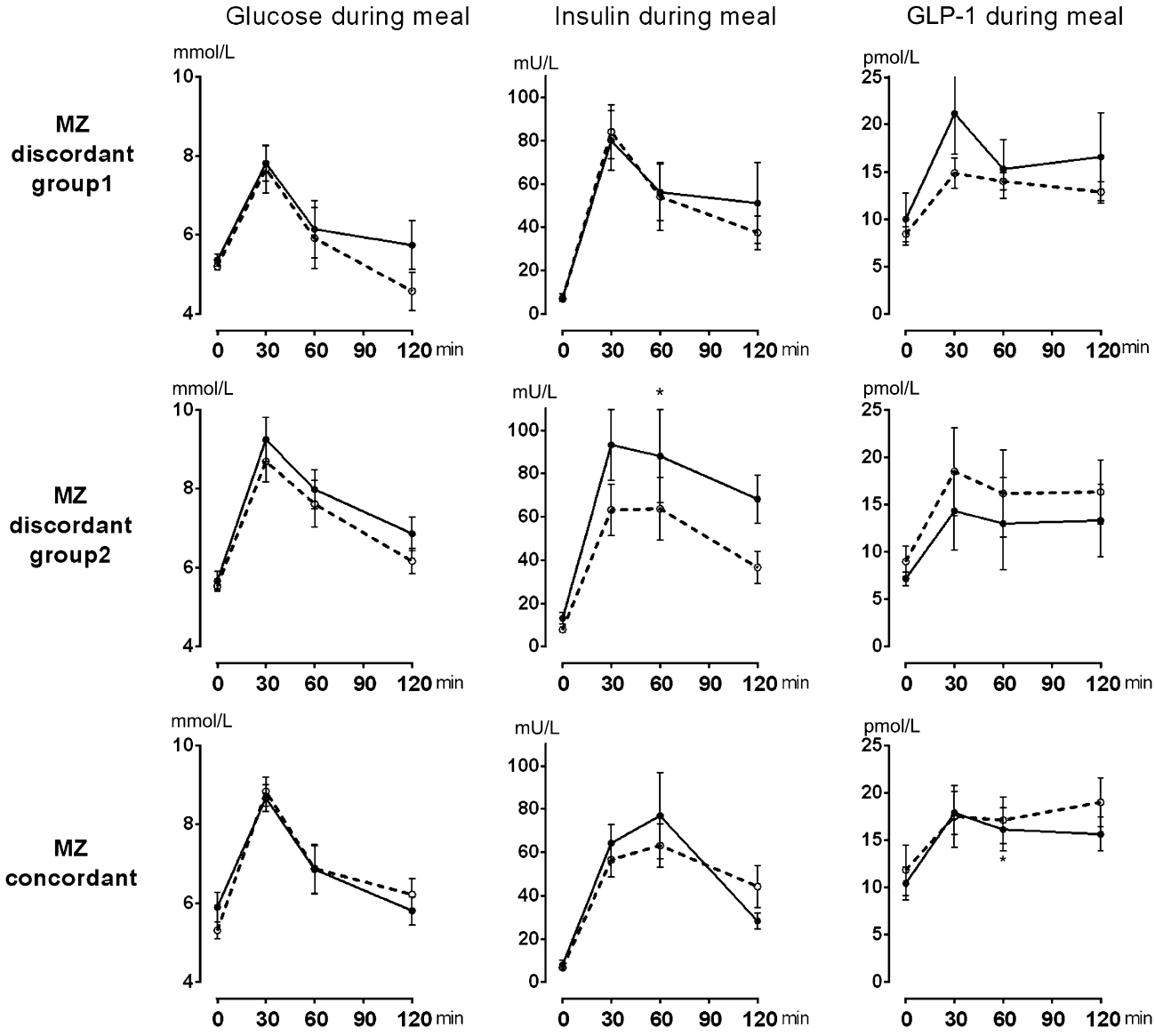

Figure 2—The responses of glucose, insulin, and GLP-1 after mixed meal in MZ and DZ twin pairs either discordant or concordant for BMI. Dashed lines represent leaner cotwins and solid lines heavier cotwins. Group 1 is MZ twin pairs discordant for BMI but not for liver fat. Group 2 is MZ twin pairs discordant for both BMI and liver fat. *$P < 0.05$ for differences between leaner vs. heavier cotwin (paired Wilcoxon test).

the heavier cotwin group also displayed blunted GLP-1 and higher insulin response to OGTT as compared with the leaner cotwin group. It must be noted that the DZ weight-discordant twin pairs did not undergo liver fat measurement but as a group they clearly represent the unhealthy obesity pattern based on significantly higher HOMA-IR and lower Matsuda indices. Most of the previous data also support our view that impaired incretin response develops concomitantly with the features of insulin resistance rather than represents a primary genetic trait that drives the progress to type 2 diabetes (1,26,27). In the current study, a clustering of metabolic risk factors with increased liver fat and insulin resistance was required on top of simple obesity for impaired incretin response to develop. However, whether the metabolically unhealthy obesity pattern is acquired or represents interactions between susceptibility genes and environment remains to be determined.

Single nucleotide polymorphisms affecting diabetes risk that are associated with incretin secretion or action are surprisingly few and do not reveal if incretin defect precedes diabetes or requires insulin resistance to develop. So far, identified genes include *TCF7L2*, *GIPR*, *WSF1*, and *KCNQ1*, which mostly regulate the incretin effect on islet cells rather than concentrations of GLP-1 and GIP (3,4,6). Carriers of the T allele of TCLF7 variant rs7903146 demonstrate 30% loss of the incretin effect, suggesting impaired β-cell capacity to respond to incretin stimulus (28). Also GIPR and WSF1 affect the pancreatic incretin-dependent insulin signaling pathways. Only KCNQ1 single nucleotide polymorphisms theoretically

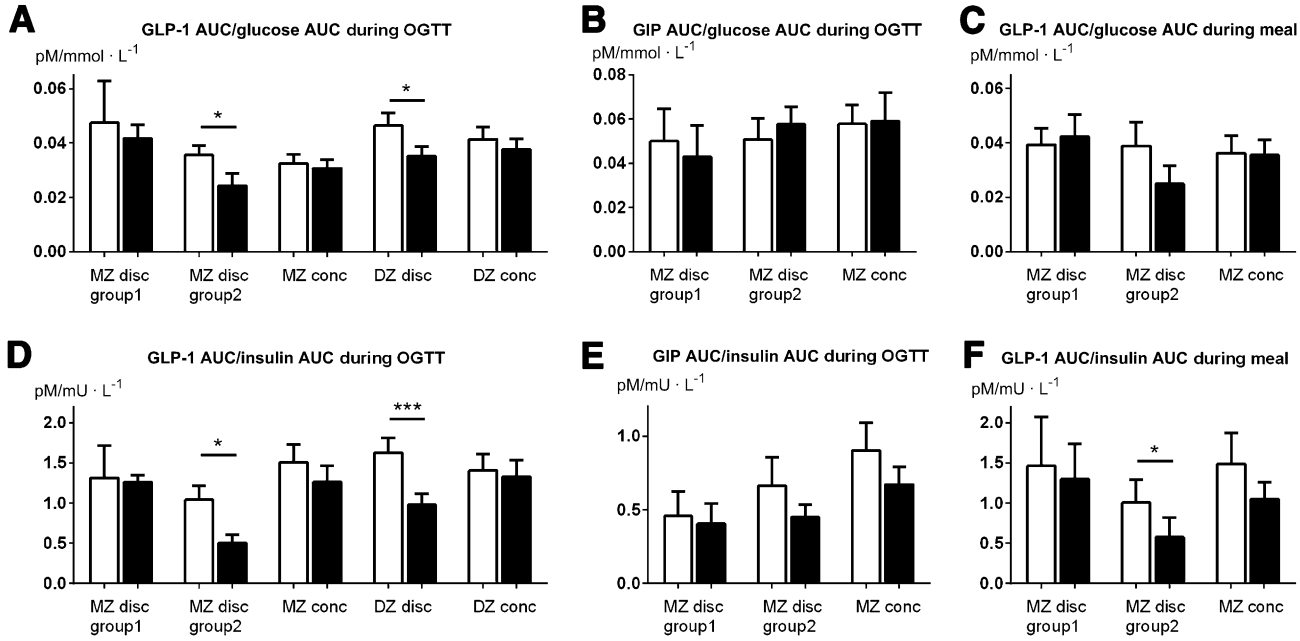

Figure 3—The relationships between area responses of GLP-1 to glucose and GLP-1 to insulin after OGTT (*A* and *D*) and meal test (*C* and *F*) and the relationships between area responses of GIP to glucose and GIP to insulin after OGTT (*B* and *E*) in MZ and DZ twin pairs either discordant or concordant for BMI. Group 1 is MZ twin pairs discordant for BMI but not for liver fat. Group 2 is MZ twin pairs discordant for both BMI and liver fat. Open bars, leaner cotwins; black bars, heavier cotwins. *$P < 0.05$, ***$P < 0.001$ for differences between leaner vs. heavier cotwin (paired Wilcoxon test).

may impair ileal incretin secretion (6). Recently, Smushkin et al. (29) reported in healthy individuals that *TCF7L2*, *WFS1*, and *KCNQ1* genotypes had no effect on GLP-1 concentrations after an OGTT, or on β-cell responsiveness to hyperglycemic clamp and GLP-1 infusion. Based on these findings, we assume that the β-cells of the healthy subjects in our study respond normally to GLP-1. Furthermore, we found that the heavier MZ weight-discordant twins with low liver fat were able to even increase their GLP-1 secretion during OGTT and mixed meal at 30 min with simultaneous increase in insulin secretion. This resulted in maintenance of identical glucose levels, as observed in their leaner twin pairs. Our data suggest that the incretin response to oral nutrients is well preserved and may even compensate for the increased insulin requirements in metabolically healthy obese subjects. Instead, the heavier MZ weight-discordant twins with high liver fat showed slightly decreased GLP-1 levels and clearly elevated insulin and glucose levels during the OGTT and mixed meal. The data suggest that the enhanced insulin response in the metabolically unhealthy, heavier MZ is not accounted for by GLP-1 secretion but presumably by direct effects of elevated glucose concentrations.

A severely reduced incretin response is a uniform finding in type 2 diabetes, in which the β-cell insulin secretion due to incretins may be reduced to <20% from that of healthy subjects (2). The data in subjects with diabetes on GLP-1 and GIP plasma levels are conflicting with studies reporting especially reduced, unaltered, and increased GIP concentrations after OGTT (2,30–33). It must be noted that these studies include subjects from mixed populations with variable genetic background and duration of full-blown diabetes in which both reduced secretion and impaired action of incretins have been suggested as the culprit. Our young and healthy study population, on the other hand, does not have diabetes, originates from a genetically homogeneous Finnish population, and is perfectly matched for genes between the obese and nonobese subjects. Therefore, one can assume that the differences in GLP-1 and GIP responses relative to insulin and glucose responses detected between weight- and liver fat–discordant MZ twin pairs are representing the true impairment in GLP-1 metabolism and relatively more preserved GIP response elicited by the phenotype. Because of our study design, we are not able to estimate if insulinotropic action of GIP on β-cells is impaired or not in the metabolically unhealthy pattern of obesity.

Our finding that leaner and heavier cotwins of obesity-discordant MZ pairs demonstrate a similar AUC for PYY levels is in contradiction with reports of decreased PYY secretion after meal in obesity in individuals (34,35). This finding suggests that common genetic or environmental factors may underlie obesity and PYY response to oral nutrients. Also, our subjects were young with a very homogeneous background and only a moderate degree of obesity, which may also account for discrepancies between findings.

Young-adult obesity-discordant MZ twins with no major confounding factors such as medications and other diseases are extremely rare, and despite screening of 10 yearly cohorts with well over 5,000 twin pairs, the final sample size was quite small and therefore potentially underpowered. However, this selection procedure provided a highly informative sample for studying

the association between acquired obesity and incretin response. Regarding the representativeness of the twin model estimates from this selected sample, it has been shown with simulated twin data that the bias resulting from the extreme concordant and discordant design is minimal (36). Other limitations include lack of measurement of glucagon, which has been linked to impaired incretin response, and not performing laborious clamp studies, which would have directly measured insulin sensitivity and the β-cell incretin responses (37). However, the use of HOMA and Matsuda indices has been previously well validated in healthy subjects (38).

In conclusion, in the present twin study, the estimated heritability of GLP-1 response to OGTT is 67%. The incretin responses of GLP-1 and GIP as well as PYY to oral nutrients do not differ between cotwins who are concordant for obesity or discordant for obesity but metabolically healthy. A defective GLP-1 response emerges concomitantly with increase in liver fat content and impairment of insulin sensitivity as an early sign of unhealthy obesity. Whether such a pattern is determined by susceptibility genes or depends on lifestyle choices over and above obesity remains to be studied. Our findings suggest that GLP-1–based therapies may be widely applicable treatment options in the unhealthy pattern of obesity.

Funding. This work was supported by grants from the Finnish Diabetes Research Foundation (N.M. and K.H.P.), the Novo Nordisk Foundation (K.H.P.), the Juho Vainio Foundation (L.H.B.), the Jenny and Antti Wihuri Foundation (L.H.B.), the Biomedicum Helsinki Foundation (L.H.B. and K.H.P.), the Danish Medical Research Council (J.J.H.), and the Academy of Finland (grants 141054, 265240, and 263278 to J.K.).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. N.M. and L.H.B. wrote the manuscript and researched data. A.H. and J.L. researched data. N.L. and J.J.H. researched data and reviewed the manuscript. J.K. and A.R. contributed to the study design and reviewed the manuscript. K.H.P. wrote the manuscript, researched data, and contributed to the study design. K.H.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Meier JJ. The contribution of incretin hormones to the pathogenesis of type 2 diabetes. Best Pract Res Clin Endocrinol Metab 2009;23:433–441
2. Nauck M, Stöckmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia 1986;29:46–52
3. Speliotes EK, Willer CJ, Berndt SI, et al.; MAGIC; Procardis Consortium. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 2010;42:937–948
4. Saxena R, Hivert MF, Langenberg C, et al.; GIANT consortium; MAGIC investigators. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet 2010;42:142–148
5. Nauck M, Hahn S, Sauerwald A, Schmiegel W. Lack of germline mutations in the preproglucagon gene region coding for glucagon-like peptide 1 in type 2 diabetic (NIDDM) patients. Exp Clin Endocrinol Diabetes 2000;108:72–75
6. Müssig K, Staiger H, Machicao F, Häring HU, Fritsche A. Genetic variants affecting incretin sensitivity and incretin secretion. Diabetologia 2010;53:2289–2297
7. Yeung EH, Zhang C, Chen J, et al. Polymorphisms in the neuropeptide Y gene and the risk of obesity: findings from two prospective cohorts. J Clin Endocrinol Metab 2011;96:E2055–E2062
8. Campbell CD, Lyon HN, Nemesh J, et al. Association studies of BMI and type 2 diabetes in the neuropeptide Y pathway: a possible role for NPY2R as a candidate gene for type 2 diabetes in men. Diabetes 2007;56:1460–1467
9. van Dongen J, Slagboom PE, Draisma HH, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. Nat Rev Genet 2012;13:640–653
10. Kaprio J. Twin studies in Finland 2006. Twin Res Hum Genet 2006;9:772–777
11. Tyynismaa H, Raivio T, Hakkarainen A, et al. Liver fat but not other adiposity measures influence circulating FGF21 levels in healthy young adult twins. J Clin Endocrinol Metab 2011;96:E351–E355
12. Granér M, Seppälä-Lindroos A, Rissanen A, et al. Epicardial fat, cardiac dimensions, and low-grade inflammation in young adult monozygotic twins discordant for obesity. Am J Cardiol 2012;109:1295–1302
13. Lundbom J, Hakkarainen A, Fielding B, et al. Characterizing human adipose tissue lipids by long echo time ^{1}H-MRS in vivo at 1.5 Tesla: validation by gas chromatography. NMR Biomed 2010;23:466–472
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412–419
15. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–1470
16. Krarup T, Madsbad S, Moody AJ, et al. Diminished immunoreactive gastric inhibitory polypeptide response to a meal in newly diagnosed type I (insulin-dependent) diabetics. J Clin Endocrinol Metab 1983;56:1306–1312
17. Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 1994;43:535–539
18. Neale MC, Boker SM, Xie G, Maes HH. *Mx: Statistical Modeling.* 6th ed. Richmond, VA, Virginia Commonwealth University Department of Psychiatry, 2003
19. Falconer DS, Mackay TFC. *Introduction to Quantitative Genetics*. 4th ed. Harlow, Essex, UK, Longmans Green, 1996
20. Neale MC, Cardon LR. *Methodology for Genetic Studies of Twins and Families*. Dordrecht, The Netherlands, Kluwer Academic Publications, 1992
21. Knop FK, Aaboe K, Vilsbøll T, et al. Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. Diabetes Obes Metab 2012;14:500–510
22. Schousboe K, Visscher PM, Henriksen JE, Hopper JL, Sørensen TI, Kyvik KO. Twin study of genetic and environmental influences on glucose tolerance and indices of insulin sensitivity and secretion. Diabetologia 2003;46:1276–1283
23. Salehi M, Aulinger B, D'Alessio DA. Effect of glycemia on plasma incretins and the incretin effect during oral glucose tolerance test. Diabetes 2012;61:2728–2733
24. Nauck MA, El-Ouaghlidi A, Gabrys B, et al. Secretion of incretin hormones (GIP and GLP-1) and incretin effect after oral glucose in first-degree relatives of patients with type 2 diabetes. Regul Pept 2004;122:209–217
25. Meier JJ, Gallwitz B, Askenas M, et al. Secretion of incretin hormones and the insulinotropic effect of gastric inhibitory polypeptide in women with a history of gestational diabetes. Diabetologia 2005;48: 1872–1881
26. Vaag AA, Holst JJ, Vølund A, Beck-Nielsen HB. Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM)—evidence for

decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins. Eur J Endocrinol 1996;135:425–432

27. Rask E, Olsson T, Söderberg S, et al.; Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA). Impaired incretin response after a mixed meal is associated with insulin resistance in nondiabetic men. Diabetes Care 2001;24:1640–1645
28. Villareal DT, Robertson H, Bell GI, et al. TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. Diabetes 2010;59:479–485
29. Smushkin G, Sathananthan M, Sathananthan A, et al. Diabetes-associated common genetic variation and its association with GLP-1 concentrations and response to exogenous GLP-1. Diabetes 2012;61:1082–1089
30. Ross SA, Brown JC, Dupré J. Hypersecretion of gastric inhibitory polypeptide following oral glucose in diabetes mellitus. Diabetes 1977;26:525–529
31. Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes 2001;50:609–613
32. Toft-Nielsen MB, Damholt MB, Madsbad S, et al. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. J Clin Endocrinol Metab 2001;86:3717–3723
33. Muscelli E, Mari A, Casolaro A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes 2008;57:1340–1348
34. Nguyen AD, Herzog H, Sainsbury A. Neuropeptide Y and peptide YY: important regulators of energy metabolism. Curr Opin Endocrinol Diabetes Obes 2011;18:56–60
35. Ashby D, Bloom SR. Recent progress in PYY research—an update report for 8th NPY meeting. Peptides 2007;28:198–202
36. Derks EM, Dolan CV, Boomsma DI. Statistical power to detect genetic and environmental influences in the presence of data missing at random. Twin Res Hum Genet 2007;10:159–167
37. Vollmer K, Holst JJ, Baller B, et al. Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. Diabetes 2008;57:678–687
38. Martinez-Hervas S, Argente C, Garcia-Jodar J, et al. Misclassification of subjects with insulin resistance and associated cardiovascular risk factors by homeostasis model assessment index. Utility of a postprandial method based on oral glucose tolerance test. Metabolism 2011;60:740–746