The Effects of the Pro12Ala Polymorphism of the Peroxisome Proliferator–Activated Receptor-γ2 Gene on Glucose/Insulin Metabolism Interact With Prenatal Exposure to Famine

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OBJECTIVE — An adverse fetal environment may permanently modify the effects of specific genes on glucose tolerance, insulin secretion, and insulin sensitivity. In the present study, we assessed a possible interaction of the peroxisome proliferator–activated receptor (PPAR)-γ2 Pro12Ala polymorphism with prenatal exposure to famine on glucose and insulin metabolism.

RESEARCH DESIGN AND METHODS — We measured plasma glucose and insulin concentrations after an oral glucose tolerance test and determined the *PPAR-γ2* genotype among 675 term singletons born around the time of the 1944–1945 Dutch famine.

RESULTS — A significant interaction effect between exposure to famine during midgestation and the *PPAR-γ2* Pro12Ala polymorphism was found on the prevalence of impaired glucose tolerance and type 2 diabetes. The Ala allele of the *PPAR-γ2* gene was associated with a higher prevalence of impaired glucose tolerance and type 2 diabetes but only in participants who had been prenatally exposed to famine during midgestation. Similar interactions were found for area under the curve for insulin and insulin increment ratio, which were lower for Ala carriers exposed to famine during midgestation.

CONCLUSIONS — The effects of the *PPAR-γ2* Pro12Ala polymorphism on glucose and insulin metabolism may be modified by prenatal exposure to famine during midgestation. This is possibly due to a combined deficit in insulin secretion, as conferred by pancreatic β-cell maldevelopment and carrier type of the Ala allele in the *PPAR-γ2* gene.

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The fetal origins hypothesis proposes that metabolic and cardiovascular disease originates through adaptations made by the fetus in response to an adverse fetal environment (1). An adverse fetal environment may alter gene expression and lead to physiological or morphological phenotypes associated with disease (2). Based on this hypothesis, one can expect the effects of polymorphisms associated with specific diseases to depend on the type of fetal environment. Size at birth, a marker of the fetal environment, has been shown to modulate the effects of a number of genetic polymorphisms (3–6). In particular, there is accumulating evidence for an interaction between size at birth and the effects of the Pro12Ala polymorphism of the peroxisome proliferator–activated receptor (PPAR)-γ2 gene.

PPAR-γ2 is one of three *PPAR-γ* isoforms and is a member of the nuclear hormone receptor subfamily of transcription factors, which regulate transcription of various genes (7). *PPAR-γ* is implicated in adipocyte differentiation, regulating glucose, and lipid homeostasis (7). The *PPAR-γ* gene contains nine exons and spans >100 kb of genomic DNA on chromosome 3p25 (8,9). Of several mutations identified on the *PPAR-γ2* gene, the proline-to-alanine change in codon 12 of exon B is one (10). The Ala allele reduces the transcriptional activity of *PPAR-γ* and may protect against type 2 diabetes compared with the more common Pro/Pro genotype (11,12). In people with low birth weight, the Pro/Pro genotype has been shown to be associated with raised systolic blood pressure, increased insulin resistance, and elevated plasma insulin concentrations (13,14). Because this association was not observed in people with normal birth weights, it may be concluded that the fetal environment interacts with the effects of the *PPAR-γ2* Pro12Ala polymorphism. Birth weight is, however, a summary measure of the fetal environment. The Dutch famine birth cohort study provides the opportunity to study the direct effects of a specific adverse fetal environment, namely maternal undernutrition. The Dutch famine birth cohort consists of people born as term singletons in the Wilhelmina Gasthuis in Amsterdam around the time of the Dutch famine. People who were exposed to fam-

ine during gestation had higher 120-min plasma glucose and insulin concentrations at age 50 years compared with people prenatally unexposed to famine (15). The purpose of the current study was to determine whether famine exposure during gestation interacts with the effects of the *PPAR-γ*2 Pro12Ala polymorphism on glucose and insulin metabolism.

RESEARCH DESIGN AND METHODS — Selection procedures

The Dutch famine birth cohort consists of 2,414 men and women born as term singletons in the Wilhelmina Gasthuis in Amsterdam between 1 November 1943 and 28 February 1947 (15). All 1,423 members of the cohort who lived in the Netherlands on 1 September 2002 and whose current address was available were invited to the hospital. Of the cohort of 1,423 eligible people, a total of 810 people agreed to participate in the study. The local medical ethics committee had approved the study, which was also carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Exposure to famine

Exposure to famine was defined according to the official daily food rations for the general population aged ≥21 years (16). An individual was considered to be prenatally exposed to famine if the average daily food ration of the mother during any 13-week period of gestation contained <1,000 calories. Based on this definition, babies born between 7 January 1945 and 8 December 1945 were exposed in utero. We delineated periods of 16 weeks each to differentiate between those who were exposed in late gestation (born between 7 January and 28 April 1945), in midgestation (born between 29 April and 18 August 1945), and in early gestation (born between 19 August and 8 December 1945). People born before 7 January 1945 and conceived and born after 8 December 1945 were considered as unexposed to famine in utero.

Study parameters

The medical birth records provided information about the mother, the course of the pregnancy, and the size of the baby at birth (15). Trained nurses took all measurements and conducted a standardized interview. We measured height using a fixed or portable stadiometer and weight with Seca and portable Tefal scales. Information about socioeconomic status, medical history, lifestyle, and use of medication was obtained in a standardized interview. We defined current socioeconomic status according to International Socioeconomic Index of Occupational Status 92, which is based on the participant's, or their partner's, occupation, whichever status was highest (17). After an overnight fast, we performed an oral glucose tolerance test (OGTT) with a standard load of 75 g. Venepuncture was performed at 0, 30, 60, and 120 min after the administration of the oral glucose load to assess plasma glucose and insulin concentrations. DNA material was extracted from the fasting blood sample. Participants with preexistent diabetes, defined as taking oral or injected antidiabetic medication, were excluded from the OGTT. Plasma glucose concentrations were measured by standardized enzymatic photometric assay on a Modular P analyzer (Roche, Basel, Switzerland) and plasma insulin concentrations by an immunoluminometric assay on an Immulite 2000 analyzer (Diagnostic Product, Los Angeles, CA). The insulin assay sensitivity was 15 pmol/l. The Pro12Ala polymorphism of the *PPAR-γ*2 gene was determined by the PCR restriction fragment–length polymorphism method. The following primers were used: forward primer 5′-CAAGC CCAGTCCTTTCTGTG-3′ and reverse primer 5′-AGTGAAGGAATCGCTT TCCG-3′.

Statistical methods

Impaired glucose tolerance was defined as a 120-min glucose level between 7.8 and 11.0 mmol/l. Type 2 diabetes was defined as a 120-min glucose level of >11.0 mmol/l (18). The ratio of the 30-min increment in insulin to 30-min increment in glucose concentrations was used as an index of insulin secretion (19). Insulin resistance was estimated by homeostasis model assessment (HOMA-IR) (20). The area under the curve (AUC) was calculated for insulin according to the following formula: $[15*\log(\mathrm{ins0min})] + [30*\log(\mathrm{ins30min})] + [45*\log(\mathrm{ins60min})] + [30*\log(\mathrm{ins120min})]/120$. Logarithmic transformations were applied to glucose, insulin, insulin increment ratio, HOMA-IR, and BMI values because they had skewed distributions. Allele frequencies were estimated by gene counting, and departure from Hardy-Weinberg equilibrium was tested using a χ^2 test with one degree of freedom. Genotypes were treated as categorical variables.

We used linear regression analysis to compare glucose and insulin concentrations and logistic regression to compare the prevalence of impaired glucose tolerance and type 2 diabetes between genotype groups and exposure groups. Possible interactions between the effects of prenatal famine exposure and the Pro12Ala polymorphism of the *PPAR-γ*2 gene on glucose/insulin metabolism were assessed by adding an interaction term (genotype*exposure) to the regression equation. We adjusted for sex and BMI in all analyses. Additional adjustment was done for maternal and birth characteristics, smoking, and current socioeconomic status. We considered differences to be statistically significant if *P* values were ≤0.05.

RESULTS — Genotypes of the *PPAR-γ*2 Pro12Ala polymorphism were available for 772 of 810 participants. Sixty-two of 772 participants had to be excluded from participating in the OGTT because they had preexisting diabetes. The test was not performed on another 35 participants due to the fact that they had not adhered to fasting instructions (*n* = 7) or due to difficulties in venepuncture (*n* = 28). Of 675 participants who underwent an OGTT and for whom a genotype was available, 280 (42%) had been exposed to famine in utero (Table 1). People with type 2 diabetes and people with impaired glucose tolerance were grouped together for analysis because of the relatively small number of people with type 2 diabetes based on the OGTT (31 cases).

The prevalences of the Pro/Ala and the Ala/Ala genotypes were 25 and 2%, respectively (Table 1). The frequency of the Ala allele was 0.148. The observed genotype frequencies were in Hardy-Weinberg equilibrium for all study groups. The Ala carriers were grouped together for analysis because of the low frequency of the Ala/Ala genotype. Excluding participants with the Ala/Ala genotype from analyses did not change the results (data not shown). The proportions of Ala carriers did not significantly differ between the unexposed group (27%) and the groups exposed in late (29%), mid (28%)-, and early gestation (23%). Table 2 shows that there were no significant differences between participants carrying the Pro/Pro genotype and those carrying the Ala allele in terms of BMI, plasma glucose and insulin concentrations, the prevalence of impaired glucose tolerance and

Table 1—*Maternal and birth and adult characteristics according to timing of prenatal exposure to the Dutch famine*

		Exposure to famine					
	Born before	In late gestation	In midgestation	In early gestation	Conceived after	All	n
General							
n	208	116	103	61	187	675	—
Proportion of men (%)	47	41	40	40	52	46	675
Age (years)	59	59	58	58	57	58 ± 1	675
Number of participants with Pro/Ala genotype	57	33	25	12	43	170	—
Number of participants with Ala/Ala genotype	3	1	4	2	5	15	—
Maternal characteristics							
Age at delivery (years)	29	31*	29	27	29	29 ± 6	675
Primiparous (%)	36	22*	21	43	35	33	675
Manual labor (%)	82	73	71	59*	69	73	549
Weight gain (third trimester) (kg)	2.8	0.1*	4.4*	4.9*	3.5	2.9 ± 2.9	470
Weight at last antenatal visit (kg)	66.4	63.1*	63.6*	69.3	69.4	66.5 ± 8.6	593
Birth outcomes							
Gestational age (days)	285	284	286	288*	286	285 ± 11	580
Birth weight (g)	3,393	3,208*	3,189*	3,486	3,494	3,366 ± 474	675
Adult characteristics at age 58 years							
BMI (kg/m²)†	27.7	27.7	27.4	27.4	28.6	27.9 ± 1.2	675
Current smoking (%)	22	26	25	33*	23	24	673
Current socioeconomic status (International Socioeconomic Index of Occupational Status)	48	52*	52*	47	49	50 ± 14	675

Data are means ± SD, except where given as numbers and percentages. *Statistically significant difference ($P \leq 0.05$) compared with participants unexposed to famine in utero. †Geometric means ± SD.

type 2 diabetes, AUC for insulin, insulin increment ratio, and insulin resistance.

Participants who were prenatally exposed to famine had 120-min glucose concentrations that were 0.4 mmol/l (95% CI 0.1–0.7) higher and 120-min insulin concentrations that were 27 pmol/l (0–57) higher than the glucose and insulin concentrations of unexposed participants. The prevalence of impaired glucose tolerance and type 2 diabetes, AUC for insulin, insulin increment ratio, and HOMA-IR did not differ between participants exposed and participants unexposed to famine in utero.

Table 3 shows that carriers of the Ala allele in the group exposed to famine during midgestation had a higher prevalence of impaired glucose tolerance and type 2 diabetes than carriers of the Pro/Pro genotype (odds ratio adjusted for sex and BMI 2.7 [95% CI 0.9–7.8]). Conversely, in the group unexposed to famine during gestation, carriers of the Ala allele had a lower prevalence of impaired glucose tolerance and type 2 diabetes compared with carriers of the Pro/Pro genotype (0.6 [0.3–1.2]). The interaction between famine exposure in midgestation and carrying the Ala allele on the prevalence of impaired glucose tolerance and type 2 dia-

betes was statistically significant ($P = 0.03$, Fig. 1). Inclusion of the 62 participants with known type 2 diabetes who were excluded from the OGTT yielded the same results ($P = 0.02$ for interaction, data not shown).

Carriers of the Ala allele exposed in midgestation had a lower AUC for insulin (−54 pmol/l [95% CI −117 to −2]) and a lower insulin increment ratio (−22 [−42 to 7]) than midexposed participants with the Pro/Pro genotype. Carriers of the

Table 2—*Geometric means ± SD for BMI, plasma glucose and insulin concentrations, and prevalence of impaired glucose tolerance and type 2 diabetes according to* **PPAR-γ2** *gene polymorphism*

	Pro/Pro	Ala	P
n	490	185	
BMI (kg/m²)	27.9 ± 1.2	28.0 ± 1.2	0.64
OGTT			
Glucose 0 min (mmol/l)	5.6 ± 1.7	5.5 ± 1.7	0.19
Glucose 30 min (mmol/l)	8.6 ± 2.2	8.6 ± 2.2	0.74
Glucose 60 min (mmol/l)	8.2 ± 2.1	8.1 ± 2.1	0.43
Glucose 120 min (mmol/l)	6.0 ± 1.8	5.8 ± 1.8	0.11
Insulin 0 min (pmol/l)	57 ± 4.0	55 ± 4.0	0.29
Insulin 30 min (pmol/l)	297 ± 1.8	299 ± 1.9	0.95
Insulin 60 min (pmol/l)	386 ± 1.8	394 ± 1.8	0.74
Insulin 120 min (pmol/l)	254 ± 2.1	232 ± 2.2	0.12
Prevalence of impaired glucose tolerance and diabetes (%)	20.6	18.6	0.54
AUC of insulin (pmol/l)	252 ± 1.7	252 ± 1.7	0.85
Insulin increment ratio	80 ± 2.4	84 ± 2.4	0.55
HOMA-IR index (mmol × pmol/l²)	14.2 ± 2.7	13.5 ± 2.6	0.21

P values for differences adjusted for sex, BMI, and prenatal exposure to famine during late, mid-, and early gestation.

Table 3—*Prevalence of impaired glucose tolerance and type 2 diabetes and geometric means for insulin-associated variables according to timing of prenatal exposure to the Dutch famine and Pro12Ala polymorphism*
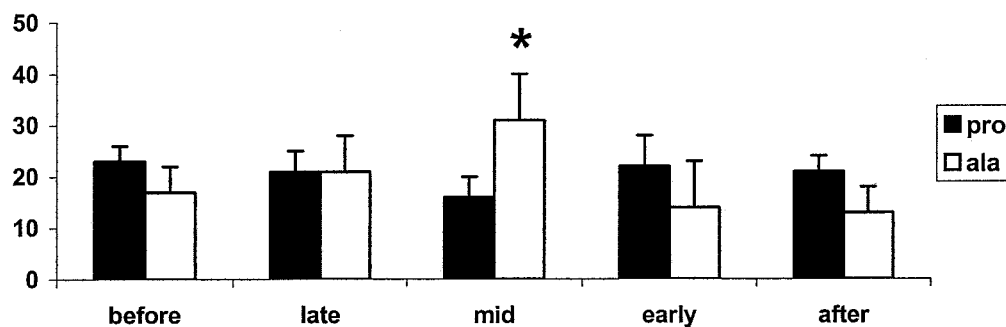
	Exposure to famine				
	Born before (148/60)	In late gestation (82/34)	In midgestation (74/29)	In early gestation (47/14)	Conceived after (139/48)
Prevalence of impaired glucose tolerance/diabetes (%)					
Pro/Pro	22.5	20.5	15.9	21.7	20.6
Ala	16.9	20.6	31.0	14.3	12.8
All	20.4	20.5	20.4	20.0	18.6
P value*	—	0.46	0.03	0.84	—
AUC of insulin (pmol/l)					
Pro/Pro	248	245	268	274	247
Ala	270	270	221	218	249
All	255	252	253	260	247
P value*	—	0.80	0.03	0.09	—
Insulin increment ratio					
Pro/Pro	81	79	91	78	73
Ala	87	86	69	60	95
All	82	81	84	74	79
P value*	—	0.65	0.05	0.17	—
HOMA-IR (mmol × pmol/l^2)					
Pro/Pro	14.1	13.4	13.9	15.0	14.6
Ala	14.1	15.1	12.1	12.7	12.6
All	14.1	13.9	13.4	14.4	14.0
P value*	—	0.19	0.56	0.49	—

Frequencies of Pro/Pro/Ala carriers in parentheses. *P values for interaction genotype times famine exposure during late, mid-, and early gestation, adjusted for sex and BMI.

Ala allele unexposed to famine, on the other hand, had a higher AUC for insulin (12 pmol/l [−15 to 44]) and a higher insulin increment ratio (14 [−3 to 35]) than unexposed participants with the Pro/Pro genotype. The interactions between famine exposure in midgestation and carriership of the Ala allele were both significant on AUC for insulin and insulin increment ratio (P = 0.03 and 0.05, respectively). The participants exposed to famine in midgestation who carried the Ala allele also had a lower HOMA-IR compared with carriers of the Pro/Pro genotype, but

this interaction was not statistically significant. Additional adjustment for confounding variables other than sex and BMI (including maternal characteristics, birth weight, gestational age, current smoking, and current socioeconomic status) did not significantly alter the results. There were no significant interactions between the Pro12Ala polymorphism and birth weight in terms of plasma glucose and insulin concentrations, prevalence of impaired glucose tolerance and type 2 diabetes, AUC for insulin, insulin increment ratio, and insulin resistance.

CONCLUSIONS — Our findings show that prenatal exposure to famine during midgestation interacts with the Pro12Ala polymorphism of the *PPAR-γ2* gene in influencing the prevalence of impaired glucose tolerance and type 2 diabetes. The Ala allele of the *PPAR-γ2* gene was associated with a higher prevalence of impaired glucose tolerance and type 2 diabetes but only in participants who had been prenatally exposed to famine during midgestation. The Ala allele was also associated with lower insulin concentrations but, again, only in participants who

Figure 1—*Prevalence of impaired glucose tolerance and type 2 diabetes (±SE) for participants carrying either the Pro/Pro genotype or the Ala allele according to timing of prenatal exposure to the Dutch famine. *Indicates a statistically significant interaction between genotype and famine exposure during midgestation.*

had been exposed to famine during midgestation.

Although this study is the first to demonstrate that genetic influences can be modified by prenatal nutrition, gene-nutrient interaction regarding the Pro12Ala polymorphism has been shown before. Women who carried the Ala allele and who were given a hypocaloric diet for 6 months had a greater increase in insulin sensitivity and fasting carbohydrate oxidation and a greater decrease in fasting lipid oxidation compared with women on the diet who carried the Pro/Pro genotype (21).

Our findings differ from the study of Eriksson et al. (13), who reported that the effects of low birth weight on raised fasting insulin concentrations and insulin resistance were confined to carriers of the Pro/Pro genotype. We did not find evidence that the effect of the Pro/Pro genotype on fasting insulin concentrations or insulin resistance is modified by famine exposure or size at birth. Rather, we found the highest prevalence of impaired glucose tolerance and type 2 diabetes in carriers of the Ala allele who were exposed to famine during midgestation. The fact that our results diverge from the results of the study of Eriksson et al. may lie in the difference between the use of size at birth and maternal undernutrition as markers of fetal environment. Size at birth is a summary measure of fetal growth and a result of a wide range of maternal, placental, and fetal factors, of which maternal nutrition is just one. It has been shown in animals as well as in humans that restricted maternal nutrition can produce permanent effects on adult health without affecting size at birth (22–24). Our inability to show associations with birth weight may have resulted from differences in population characteristics such as the older age and the surplus of women in the Helsinki cohort used in Eriksson et al.'s study and the interference of famine exposure in our cohort. Alternatively, the fact that, compared with Eriksson et al.'s study, in our study maternal nutrition may have had a relatively large contribution to birth weight compared with placental, fetal, and other maternal characteristics may have contributed to the discrepancy.

The combination of carrying the Ala allele and prenatal exposure to famine during midgestation did not only affect the prevalence of impaired glucose tolerance and type 2 diabetes but also resulted in lower insulin concentrations and a lower insulin increment ratio. These findings suggest that this group of people is insulin deficient. There is evidence that carrying the Ala allele leads to impaired insulin secretion of the pancreatic β-cell (25). On the other hand, the Ala allele exhibits a reduced ability for transcriptional activity of PPAR-γ, leading to improved insulin sensitivity because lower levels of adipose tissue mass are accumulated (11). Fetal undernutrition has been shown to decrease the number and function of β-cells in rats as well as in humans (26,27). In humans, β-cells are found at ~10–11 weeks of gestation and develop for the most part during midgestation (28). It is possible that prenatal famine exposure in midgestation impairs the development of the β-cells, leading to impaired insulin secretion. Carrying the Ala allele may further reduce insulin secretion. We speculate that the combination of impaired β-cell development and Ala allele carriership produces a degree of insulin deficiency that can no longer be compensated for by improved insulin sensitivity related to carrying the Ala allele.

The interaction between effects of the Pro12Ala polymorphism and prenatal exposure to famine seemed to involve exposure to famine during midgestation only. Although participants who were exposed to famine during late and early gestation also had higher 120-min glucose concentrations predisposing to type 2 diabetes, we did not find interactions with the Pro12Ala polymorphism for these groups. This might indicate that depending on trimester of exposure to famine, different mechanisms relating to impaired glucose tolerance may be affected. As mentioned above, the Pro12Ala polymorphism may have interacted with famine during midgestation because midgestation is an important period for the development of the β-cells (28).

A limitation of the study is the relatively small sample size. In our cohort, only 29 participants who carried the Ala allele had been exposed to famine during midgestation. Our findings should therefore be confirmed by future studies. Another limitation is that our finding is a post hoc finding. We aimed to look for an interaction between prenatal exposure to famine and the Pro12Ala polymorphism on glucose/insulin metabolism but did not have clear hypotheses about the direction of the interaction and investigated several glucose/insulin-associated variables.

In conclusion, our study provides the first evidence that genetic influences can be modified by nutrition of the human fetus in utero. The effects of the PPAR-γ2 Pro12Ala polymorphism on the glucose/insulin metabolism are modified by prenatal exposure to famine during midgestation. This is possibly due to a combined deficit in insulin secretion, as conferred by pancreatic β-cell maldevelopment and carrier type of the Ala allele in the PPAR-γ2 gene.

References
1. Barker DJP: *Mothers, Babies and Health in Later Life.* Edinburgh, U.K., Churchill Livingstone, 1998
2. West-Eberhard MJ: Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst* 20:249–278, 1989
3. Cambien F, Leger J, Mallet C, Levy-Marchal C, Collin D, Czernichow P: Angiotensin I-converting enzyme gene polymorphism modulates the consequences of in utero growth retardation on plasma insulin in young adults. *Diabetes* 47:470–475, 1998
4. Dennison E, Arden N, Keen R, Syddall H, Day I, SPector T, Cooper C: Birth weight, vitamin D receptor genotype and the programming of osteoporosis. *Paediatr Perinat Epidemiol* 15:211–219, 2001
5. Kajantie E, Rautanen A, Kere J, Andersson S, Yliharsila H, Osmond C, Barker DJP, Forsen T, Eriksson J: The effects of the ACE gene insertion/deletion polymorphism on glucose tolerance and insulin secretion in elderly people are modified by birth weight. *J Clin Endocrinol Metab* 89:5738–5741, 2004
6. Kubaszek A, Markkanen A, Eriksson JG, Forsen T, Osmond C, Barker DJP, Laakso M: The association of the K121Q polymorphism of the plasma cell glycoprotein-1 gene with type 2 diabetes and hypertension depends on size at birth. *J Clin Endocrinol Metab* 89:2044–2047, 2004
7. Auwerx J: PPAR gamma, the ultimate thrifty gene. *Diabetologia* 42:1033–1049, 1999
8. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J: The organization, promoter

analysis, and expression of the human PPARgamma gene. *J Biol Chem* 272:18779–18789, 1997

9. Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh L, Greene G, Nimer SD: Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expr* 4:281–299, 1995

10. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J, Shuldiner AR: Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 241:270–274, 1997

11. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J: A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 20:284–287, 1998

12. Buzzetti R, Petrone A, Ribaudo MC, Alemanno I, Zavarella S, Mein CA, Maiani F, Tiberti C, Baroni MG, Vecci E, Arca M, Leonetti F, Di Mario U: The common PPAR-gamma2 Pro12Ala variant is associated with greater insulin sensitivity. *Eur J Hum Genet* 12:1050–1054, 2004

13. Eriksson JG, Lindi V, Uusitupa M, Forsen TJ, Laakso M, Osmond C, Barker DJP: The effects of the Pro12Ala polymorphism of the peroxisome proliferator–activated receptor-γ2 gene on insulin sensitivity and insulin metabolism interact with size at birth. *Diabetes* 51:2321–2324, 2002

14. Yliharsila H, Eriksson JG, Forsen T, Laakso M, Uusitupa M, Osmond C, Barker DJP: Interactions between peroxisome proliferator-activated receptor-gamma 2 gene polymorphisms and size at birth on blood pressure and the use of antihypertensive medication. *J Hypertens* 22:1283–1287, 2004

15. Ravelli ACJ, van der Meulen JHP, Michels RPJ, Osmond C, Barker DJP, Hales CN, Bleker OP: Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 351:173–177, 1998

16. Burger GCE, Sandstead HR, Drummond JC: *Malnutrition and Starvation in Western Netherlands, September 1944 to July 1945. Part I and II.* The Hague, Netherlands, General State Printing Office, 1948

17. Bakker B, Sieben I: Maten voor prestige, sociaal-economische status en sociale klasse voor de standaard beroepenclassificatie 1992. *Sociale Wetenschappen* 40:1–22, 1997

18. World Health Organization: *Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications: Report of a WHO Consultation. Part 1, Diagnosis and Classification.* Geneva, World Health Org., 1999

19. Phillips DIW, Clark PM, Hales CN, Osmond C: Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. *Diabet Med* 11:286–292, 1994

20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostatis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985

21. Nicklas BJ, van Rossum EFC, Berman DM, Ryan AS, Dennis KE, Shuldiner AR: Genetic variation in the peroxisome proliferator–activated receptor-γ2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain. *Diabetes* 50:2172–2176, 2001

22. Harding J: The nutritional basis of the fetal origins of adult disease. *Int J Epidemiol* 30:15–23, 2001

23. Langley-Evans SC, Welham SJM, Jackson AA: Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sci* 64:965–974, 1999

24. Roseboom TJ, van der Meulen JHP, Osmond C, Barker DJP, Ravelli ACJ, Bleker OP: Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *Am J Clin Nutr* 72:1101–1106, 2000

25. Stefan N, Fritsche A, Haring H, Stumvoll M: Effect of experimental elevation of free fatty acids on insulin secretion and insulin sensitivity in healthy carriers of the Pro12Ala polymorphism of the peroxisome proliferator–activated receptor-γ2 gene. *Diabetes* 50:1143–1148, 2001

26. Van Assche FA, De Prins F, Aerts L, Verjan M: The endocrine pancreas in small-for-dates infants. *Br J Obstet Gynaecol* 84:751–753, 1977

27. Dahri S, Snoeck A, Reusens-Billen B, Remacle C, Hoet JJ: Islet function in offspring of mothers on low-protein diet during gestation. *Diabetes* 40:115–120, 1991

28. Van Assche FA, Aerts L: The fetal endocrine pancreas. *Contrib Gynecol Obstet* 5:44–57, 1979