

The minor allele of the PPAR γ 2 Pro12Ala polymorphism is associated with lower postprandial TAG and insulin levels in non-obese healthy men

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The PPAR γ Pro12Ala polymorphism has been associated in several studies with a decreased risk of obesity, type 2 diabetes and insulin resistance. Weak hints are available about the influence of PPAR γ Pro12Ala on postprandial metabolism. In 708 men, aged 45 to 65 years the PPAR γ 2 Pro12Ala genotypes were determined and postprandial TAG, insulin, glucose and NEFA after a standardized mixed fat meal and insulin and glucose after a glucose load (oral glucose tolerance test; OGTT) were assessed. Using the total sample, we did not find a significant impact of the genotype on the postprandial metabolism. In the subgroup with BMI < 30 kg/m², fasting and postprandial TAG and insulin levels as well as homeostasis model assessment of insulin resistance (HOMA) were significantly lower in the Ala12Ala group than in the Pro12Pro group after the mixed meal. In contrast, the groups did not differ in insulin levels and HOMA after the OGTT. To investigate if differences between a fat-containing meal and OGTT are caused by adiponectin, we examined a BMI- and age-matched subgroup. No differences were found between the genotypic groups. The effects of the PPAR γ 2 polymorphism on insulin sensitivity are mediated by affluent dietary fat. We did not find evidence that adiponectin as a fatty-acid-dependent adipocyte factor is a causative factor for this phenomenon.

PPAR γ 2 Pro12Ala: Insulin resistance: Type 2 diabetes: Single nucleotide polymorphism: Adiponectin

PPAR- γ is a transcriptional factor that belongs to the family of nuclear receptors such as steroid and thyroid hormone receptors (Desvergne & Wahli, 1999). PPAR γ forms heterodimers with the retinoid-X-receptor and binds to specific PPAR-response elements of DNA (Spiegelman, 1998). Through this mechanism, PPAR γ regulates the transcription of different target genes (Spiegelman, 1998). PPAR γ -regulated genes play a role in fatty acid transport, binding, oxidation and regulation (Desvergne & Wahli, 1999) and also in inflammatory response (Jiang *et al.* 1998). Ligands for PPAR γ , such as troglitazone, are used in clinical practice as insulin sensitizing drugs (Sewter & Vidal-Puig, 2002). Two main isoforms of PPAR γ are known: PPAR γ 1; PPAR γ 2. PAPR γ 1 is present in most tissues, whereas PPAR γ 2 is predominantly expressed in adipose tissue (Vidal-Puig *et al.* 1997). A single nucleotide polymorphism, at codon 12 of the exon 1 of PPAR γ 2, which leads to a switch of the amino acid proline to alanine, has recently been detected (PPAR γ 2 Pro12Ala) (Yen *et al.* 1997). In several studies, the PPAR γ 2 alanine coding genotype was associated with a diminished risk of the development of type 2 diabetes (Koch *et al.* 1999; Altshuler *et al.* 2000; Jacob *et al.* 2000; Ek *et al.* 2001; Gonzalez-Sanchez *et al.* 2002). In accordance with this, several studies have shown

an association with fasting glucose and TAG and insulin levels; however, an absent association has also been reported (Poirier *et al.* 2000; Vaccaro *et al.* 2002). At present, the reasons why diabetes, TAG and insulin resistance are associated with the PPAR γ 2 Pro12Ala polymorphism remain to be elucidated.

The induction of PPAR γ -regulated gene products in adipocytes such as lipoprotein-lipase (Schoonjans *et al.* 1996), apo CIII (Hertz *et al.* 1995), adipocyte lipid-binding protein (aP2) (Tontonoz *et al.* 1994; Li & Lazar, 2002), fatty acid binding proteins (Issemann *et al.* 1992; Darimont *et al.* 1998), acyl-CoA binding protein (Nitz *et al.* 2005), acyl-CoA synthetase (Schoonjans *et al.* 1995), glycerophosphat dehydrogenase (Li & Lazar, 2002), diacylglycerol acyltransferase (Li & Lazar, 2002) and carnitine-palmitoyl transferase (Brandt *et al.* 1998; Mascaro *et al.* 1998; Yu *et al.* 1998; Li & Lazar, 2002) may result in increased lipid uptake, storage and metabolism in these cells. As a result of this, NEFA release may be reduced. Lower NEFA result in higher insulin sensitivity in muscle tissue (Roden *et al.* 1996). Indeed, lower fasting (Temelkova-Kurktschiev *et al.* 2004; Tan *et al.* 2006) and postprandial NEFA concentrations (Tan *et al.* 2006) were found after a mixed meal in carriers of the

Abbreviations: AUC, area under the curve; HOMA, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test.

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alanine coding genotype, compared with Pro12Pro homozygotes. However, other studies showed no differences in the NEFA fasting concentrations of proline *v.* alanine coding genotypes (Stumvoll & Haring, 2002; Vaccaro *et al.* 2002; Vänttinen *et al.* 2005). In another study, the postprandial TAG, glucose and insulin after a fat ingestion and, in comparison, after a glucose load, were investigated in a young European cohort (Poirier *et al.* 2000). Under both meal conditions, no difference was found between the fasting and postprandial metabolic parameters of the different genotypes. The frequency of this single nucleotide polymorphism, however, considerably varied between cohorts from different European countries. Moreover, only young subjects with a low BMI were analysed. Recently published data from the Bogalusa Heart Study suggests that the association between insulin resistance and the PPAR γ 2 Pro12Ala polymorphism depends on the individual's age (Li *et al.* 2003). Insulin resistance was associated with the PPAR γ 2 Pro12Ala polymorphism in adults, whereas differences in young people were less pronounced. We therefore evaluated the role of the PPAR γ 2 Pro12Ala polymorphism in the postprandial metabolism of insulin and TAG in a regional cohort with people aged 45–65 years.

Research design and methods

Subjects

The Metabolic Intervention Cohort Kiel is dedicated to the study of gene–nutrient interaction with a particular emphasis on dietary fat, postprandial lipid metabolism and the metabolic syndrome (Syndrome X). A total of 722 men, aged 45 to 65 years, were recruited with the aid of the registrar of the city and surrounding area of Kiel, Germany. Patients with known diabetes, intestinal absorption disorders, liver diseases, renal diseases and intestinal surgical interventions within the last 3 months, thyroid disorders or hormone therapy were excluded. The volunteers underwent a clinical examination including a measurement of pulse, blood pressure, weight, height, waist circumference and hip circumference (World Health Organization, 1995; Lahti-Koski *et al.* 2000). Fasting plasma levels of TAG, cholesterol, LDL, HDL, insulin, glucose, glutamate oxalacetate transaminase, glutamate pyruvate transaminase, cholinesterase, alkaline phosphatase, C-reactive protein and blood cell count were assessed. Subjects underwent an oral glucose tolerance test and an oral metabolic tolerance test on different days with a minimum of 2 d in between tests. The local ethics committee approved the study design (AZ: A106/03 from 16 April 2003). The present study was performed according to the Declaration of Helsinki and informed consent was obtained from all subjects.

In the present study, data were only used from those subjects (n 708) for whom a complete dataset was available.

Oral metabolic tolerance test

To investigate the influence of a mixed meal on postprandial metabolism, we performed an oral metabolic tolerance test as described elsewhere (Schrezenmeir *et al.* 1992). This metabolic tolerance test is similar to a normal

Western-style fat-containing diet and allows the investigation of the physiological metabolic response. In brief, after a 12-h fasting period and after taking a fasting blood sample, a standardized liquid mixed meal containing 51.6 kJ% fat, 29.6 kJ% carbohydrates, 11.9 kJ% protein, with a total of 4406 kJ, was ingested within 5 min. At time points 0.5, 1, 2, 3, 4 and 5 h after ingestion had started, blood samples were taken for the analysis of serum insulin, glucose and TAG, NEFA and adiponectin, and 6, 7, 8 and 9 h after ingestion, for the analysis of serum TAG, NEFA and adiponectin.

Oral glucose tolerance test

To investigate the influence of the singular ingestion of carbohydrates on postprandial metabolism for detection of insulin resistance, we performed an oral glucose tolerance test according to the guidelines of the American Diabetes Association (2004). The blood sampling was extended up to 4 h. In short, the volunteers were examined after a 12-h fasting period. Fasting blood samples were collected and 75 g glucose, dissolved in 250 ml water, was ingested within 5 min. At time-points 0.5, 1, 2, 3 and 4 h after ingestion had started, blood samples were taken.

Plasma and serum samples

Blood was taken on ice and centrifuged, and plasma and serum were deep frozen for later analysis. Serum was used for insulin, TAG and NEFA determination, fluoride-plasma for glucose determination and EDTA plasma with aprotinin was used for adiponectin determination. Leukocytes and haemoglobin were assessed in EDTA blood. Other parameters were assessed in lithium-heparin plasma. Insulin levels were determined by RIA, according to the instruction manual of the producer (Adaltis, Bologna, Italy). Adiponectin levels were determined by ELISA, according to the instruction manual of the producer (R&D Systems, Minneapolis, MN, USA). Other parameters were measured enzymatically using a clinical laboratory analyser (Konelab, Espoo, Finland), according to the producer's manual.

Genotyping

Genomic DNA was isolated from 10 ml frozen blood samples using the Gigikit DNA extraction kit (Invitec, Berlin, Germany). Taqman probes and primers (hcv 1129864) were designed by the supplier (Applied Biosystems, Foster City, CA, USA). Taqman analysis was performed as described elsewhere (Hampe *et al.* 1999). In brief, genomic DNA was arrayed and dried on 96-well plates. Taqman PCR was set up with Genesis pipetting robots (Tecan, Männedorf, Switzerland). We amplified samples with ABI 9700 PCR machines (Applied Biosystems) and measured fluorescence with ABI 7700 and ABI 7900 fluorometers (Applied Biosystems).

Data analysis

SPSS (SPSS for windows, version 14.0.0; LEAD Technologies Inc., Charlotte, NC, USA) was used for the statistical analysis. Allele frequencies were estimated by the

maximum-likelihood approach. Deviation from the Hardy–Weinberg equilibrium was tested and rejected by using Pearson's χ^2 test. Deviations from normal distribution were tested using the Shapiro–Wilk test. Differences between the three genotypic groups were tested by the Kruskal–Wallis test. The Mann–Whitney U test was used to test for significant association under a recessive model for the Ala allele. Fasting insulin sensitivity was expressed according to the homeostasis model assessment of insulin resistance (HOMA) using the following formula: HOMA = insulin ($\mu\text{U}/\text{ml}$) * glucose (mmol/l) / 22.5 (Haffner *et al.* 1997). The area under the postprandial curves (AUC) was calculated using the following formula:

$$\frac{yt_0+yt_1}{2} \times (t_1-t_0) + \frac{yt_1+yt_2}{2} \times (t_2-t_1) + \dots + \frac{yt_{n-1}+yt_n}{2} \times (t_n-t_{n-1})$$

Results

The allele frequency was 0.85 for the proline allele and 0.15 for the alanine allele (Pro12Pro, n 515; Pro12Ala, n 176; Ala12Ala, n 17). The non-obese subgroup with BMI $<30\text{ kg/m}^2$ was made up of 555 subjects (Pro12Pro, n 403; Pro12Ala, n 141; Ala12Ala, n 11).

Table 1. Anthropometric, fasting and postprandial blood parameters of different genotypes of the PPAR γ 2 Pro12Ala polymorphism
(Mean values with their standard errors)

Parameter	n.d.	Pro12Pro (n 515)		Pro12Ala (n 176)		Ala12Ala (n 17)		Global test	Recessive model
		Mean	SEM	Mean	SEM	Mean	SEM		
Anthropometric parameters									
Age (years)	–	59.09	0.24	58.81	0.42	57.47	1.39	0.477	0.243
Body weight (kg)	–	86.15	0.62	86.12	1.121	86.7	3.35	0.961	0.930
BMI (kg/m^2)	–	27.36	0.18	27.54	0.33	27.53	0.94	0.935	0.988
Waist circumference (cm)	–	99.95	0.54	100.55	0.94	99.18	3.36	0.800	0.505
Waist:Hip ratio (cm/cm)	–	0.989	0.003	0.993	0.005	0.983	0.02	0.676	0.511
Systolic blood pressure (mmHg)	–	128.68	0.75	131.48	1.57	130.29	4.08	0.567	0.719
Diastolic blood pressure (mmHg)	–	80.16	0.45	81.59	0.91	80.00	3.00	0.512	0.670
Pulse (beats/min)	–	68.06	0.48	69.61	0.83	67.41	2.56	0.226	0.831
Fasting parameters									
Cholesterol (mmol/dl)	–	5.84	0.046	5.94	0.08	5.92	0.44	0.621	0.730
HDL (mmol/dl)	–	1.38	0.017	1.40	0.03	1.40	0.09	0.740	0.672
LDL (mmol/l)	+	3.73	0.04	3.71	0.06	3.81	0.28	0.967	0.833
TAG (mmol/l)	–	1.58	0.04	1.65	0.11	1.24	0.16	0.200	0.082
NEFA (mmol/l)	–	0.426	0.009	0.442	0.017	0.418	0.037	0.868	0.945
Glucose (mmol/l)	–	5.82	0.04	5.82	0.08	5.65	0.17	0.903	0.658
Insulin (pmol/l)	–	103.2	3.83	103.34	6.18	77.30	11.39	0.090	0.039
HOMA (mmol/l * $\mu\text{U}/\text{ml}/22.5$)	–	4.05	0.19	4.07	0.32	2.91	0.51	0.144	0.064
OMTT									
TAG (AUC mmol/l * h)	–	19.51	0.43	19.83	1.09	15.99	1.80	0.293	0.155
NEFA (AUC mmol/l * h)	–	4.403	0.056	4.333	0.098	3.99	0.239	0.290	0.159
Glucose AUC (mmol/l * h)	–	29.07	0.26	28.92	0.36	28.81	1.01	0.923	0.874
Insulin (AUC pmol/l * h)	–	1419.90	51.94	1441.99	93.02	1102.38	228.6	0.168	0.063
HOMA (mmol/l * $\mu\text{U}/\text{ml}/22.5 \cdot \text{h}$)	–	60.25	2.93	59.88	4.79	47.29	12.87	0.274	0.119
OGTT									
Glucose (AUC mmol/l * h)	–	25.93	0.31	25.72	0.48	26.81	1.38	0.685	0.386
Insulin (AUC pmol/l * h)	–	1218.33	42.83	1199.77	69.84	915.64	137.6	0.224	0.110
HOMA (mmol/l * $\mu\text{U}/\text{ml}/22.5 \cdot \text{h}$)	–	65.10	2.84	63.60	4.52	52.38	11.5	0.545	0.354

Homozygote carriers of the wild-type genotype coding for proline (P12P), heterozygote carriers (P12A) and homozygote carriers of the alanine coding genotype (A12A). n.d., Normal distribution was tested using Shapiro–Wilk test; (+) designates the presence of a normal distribution; (–) not normal distribution. HOMA, homeostasis model assessment of insulin resistance; OMTT, oral metabolic tolerance test; OGTT, oral glucose tolerance test. AUC, area under the curve. Differences between the three genotypic groups were tested using Kruskal–Wallis test (global test). The Mann–Whitney U test (recessive model) was used to test for significant association under a recessive model for the Ala allele. For details of subjects and procedures, see Research design and methods.

Total cohort

Only LDL-cholesterol showed a normal distribution. All other anthropometric and metabolic parameters were not normally distributed (Table 1). PPAR genotypic groups did not significantly differ in weight, length, BMI, waist circumference, waist:hip ratio, blood pressure, pulse, cholesterol, HDL-cholesterol, LDL-cholesterol, fasting glucose, fasting insulin, fasting HOMA, fasting TAG and fasting NEFA when compared with all genotypes (Kruskal–Wallis test) (Table 1). Although postprandial parameters after an oral metabolic tolerance test tended to be lower in the PPAR γ 2 Ala12Ala homozygotes than in carriers of the PPAR γ Pro12 genotype, no significance was attained (Table 1). When tested under a recessive model for the Ala allele (Pro12Pro + Pro12Ala v. Ala12Ala) fasting insulin levels were significantly lower in the Ala12Ala group (Mann–Whitney U test).

Non-obese sub-cohort

Obesity is associated with insulin resistance and the development of type 2 diabetes. In order to evaluate the association of the PPAR γ 2 Pro12Ala polymorphism, irrespective of obesity, we carried out an association study in a non-obese sub-cohort (BMI $<30\text{ kg/m}^2$). Because of the deviations from normal

distribution of anthropometric and most metabolic parameters, we could employ an adjustment for confounders possible for parametric methods.

Anthropometric and fasting laboratory parameters

Only body weight and LDL-cholesterol levels showed a normal distribution (Table 2). PPAR-genotypic groups did not significantly differ in weight, length, BMI, blood pressure, pulse, fasting cholesterol, fasting HDL-cholesterol, fasting LDL-cholesterol and fasting glucose (Table 2). The waist circumference, fasting TAG, fasting insulin and fasting HOMA were significantly lower in the Ala12Ala group than in the Pro12Pro group (Table 2).

Oral metabolic tolerance test

Postprandial TAG levels differed significantly between genotypes (Fig. 1; Table 2). The individual maximum levels of postprandial TAG after the intake of mixed meals were significantly higher in the Pro12Pro group than in the Ala12Ala group.

Neither fasting NEFA nor postprandial NEFA differed significantly between genotypes. However, NEFA tended to be lower in the postprandial phase (Fig. 1; Table 2).

Table 2. Subgroup BMI <30 kg/m². Anthropometric and fasting and postprandial blood parameters of different genotypes of the PPAR γ 2 Pro12Ala polymorphism

(Mean values with their standard errors)

Parameter	n.d.	Pro12Pro (n 403)		Pro12Ala (n 141)		Ala12Ala (n 11)		Global test	Recessive model
		Mean	SEM	Mean	SEM	Mean	SEM		
Anthropometric parameters									
Age (years)	–	59.39	0.28	58.90	0.49	58.27	1.83	0.588	0.65
Body weight(kg)	+	80.57	0.47	80.69	0.81	78.9	2.46	0.535	0.264
BMI (kg/m ²)	–	25.56	0.11	25.74	0.19	24.96	0.46	0.194	0.115
Waist circumference (cm)	–	95.61	0.40	95.71	0.68	90.55	1.82	0.052	0.015
Waist:Hip ratio (cm/cm)	–	0.974	0.003	0.977	0.004	0.950	0.01	0.138	0.053
Systolic blood pressure (mmHg)	–	125.43	0.81	128.42	1.61	128.64	5.27	0.363	0.712
Diastolic blood pressure (mmHg)	–	78.28	0.5	80.07	0.98	79.55	2.9	0.318	0.945
Pulse (beats/min)	–	67.32	0.55	68.90	0.96	66.55	3.5	0.164	0.807
Fasting parameters									
Cholesterol (mmol/dl)	–	5.85	0.005	6.00	0.09	6.04	0.50	0.426	0.898
HDL (mmol/dl)	–	1.42	0.02	1.47	0.033	1.54	0.10	0.179	0.221
LDL (mmol/l)	+	3.70	0.042	3.72	0.07	3.82	0.29	0.937	0.891
TAG (mmol/l)	–	1.49	0.04	1.59	0.12	0.99	0.12	0.06	0.02
NEFA (mmol/l)	–	0.412	0.009	0.427	0.02	0.403	0.04	0.883	0.906
Glucose (mmol/l)	–	5.68	0.04	5.68	0.07	5.44	0.21	0.568	0.294
Insulin (pmol/l)	–	87.58	2.85	88.41	5.21	55.21	5.21	0.008	0.003
HOMA (mmol/l * μ U/ml/22.5)	–	3.26	0.12	3.34	0.26	1.96	0.24	0.016	0.005
OMTT									
TAG (AUC mmol/l*h)	–	18.78	0.48	19.44	1.29	13.30	1.66	0.131	0.050
NEFA (AUC mmol/l*h)	–	4.27	0.06	4.19	0.10	3.76	0.18	0.268	0.142
Glucose AUC (mmol/l*h)	–	28.05	0.26	28.15	0.31	27.13	0.64	0.509	0.454
Insulin (AUC pmol/l*h)	–	1189.98	36.17	1215.17	81.28	735.40	108.6	0.028	0.009
HOMA (mmol/l * μ U/ml/22.5*h)	–	46.63	1.69	48.40	4.35	27.40	4.55	0.054	0.019
OGTT									
Glucose (AUC mmol/l*h)	–	25.06	0.32	24.85	0.43	25.53	1.07	0.678	0.382
Insulin (AUC pmol/l*h)	–	1033.43	30.70	1046.51	61.53	802.29	90.47	0.996	0.690
HOMA (mmol/l * μ U/ml/22.5*h)	–	52.40	1.96	54.08	4.25	42.53	5.77	0.729	0.691

Homozygote carriers of the wild-type genotype coding for proline (P12P), heterozygote carriers (P12A) and homozygote carriers of the alanine coding genotype (A12A). n.d., Normal distribution was tested using Shapiro-Wilk test; (+) designates the presence of a normal distribution, (–) not normal distribution. HOMA, homeostasis model assessment of insulin resistance; OMTT, oral metabolic tolerance test; OGTT, oral glucose tolerance test. AUC, area under the curve. Differences between the three genotypic groups were tested using Kruskal-Wallis test (global test). The Mann-Whitney *U* test (recessive model) was used to test for significant association under a recessive model for the Ala allele. For details of subjects and procedures, see Research design and methods.

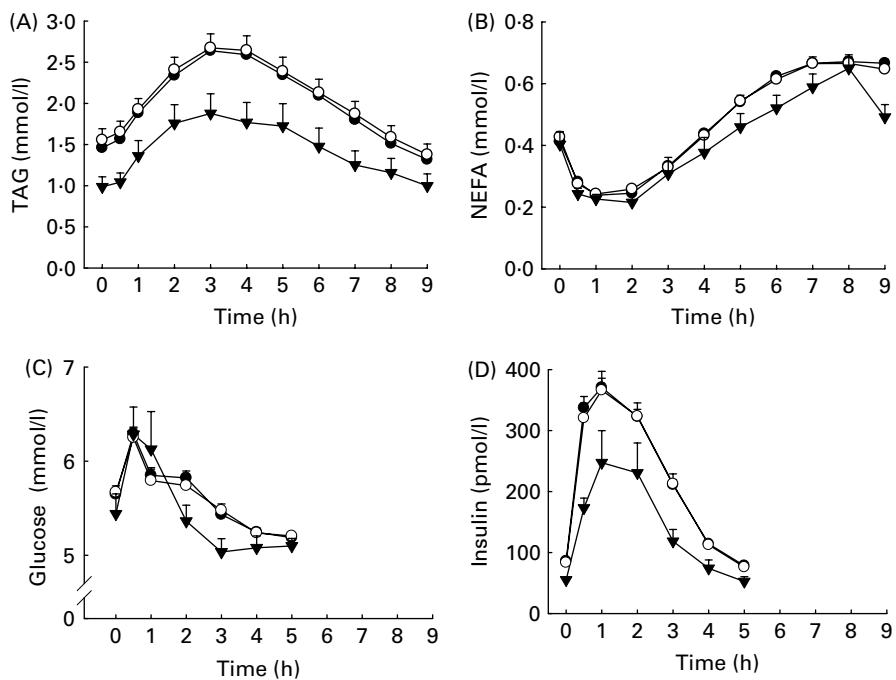


Fig. 1. Postprandial blood levels of the non-obese subgroup ($BMI < 30 \text{ kg/m}^2$) after a mixed meal (oral metabolic tolerance test, OMTT). Data are presented as means with their standard errors. PPAR γ 2 Pro12Pro, —●—; PPAR γ 2 Pro12Ala, —○—; PPAR γ 2 Ala12Ala, —▼—. (A) Postprandial TAG-curve after ingestion of a mixed meal; (B) postprandial serum levels of NEFA after OMTT; (C) postprandial glucose levels after OMTT; (D) postprandial insulin levels after OMTT.

Discussion

In non-obese males, we were able to show an association between PPAR γ 2 Ala12Ala homozygosity and lower fasting and lower postprandial TAG levels (AUC) after a mixed

meal (Fig. 1). Furthermore, PPAR γ 2 Ala12Ala was associated with lower fasting insulin levels, lower postprandial insulin levels (AUC) and higher postprandial insulin sensitivity (HOMA AUC) after the intake of a fat-containing meal

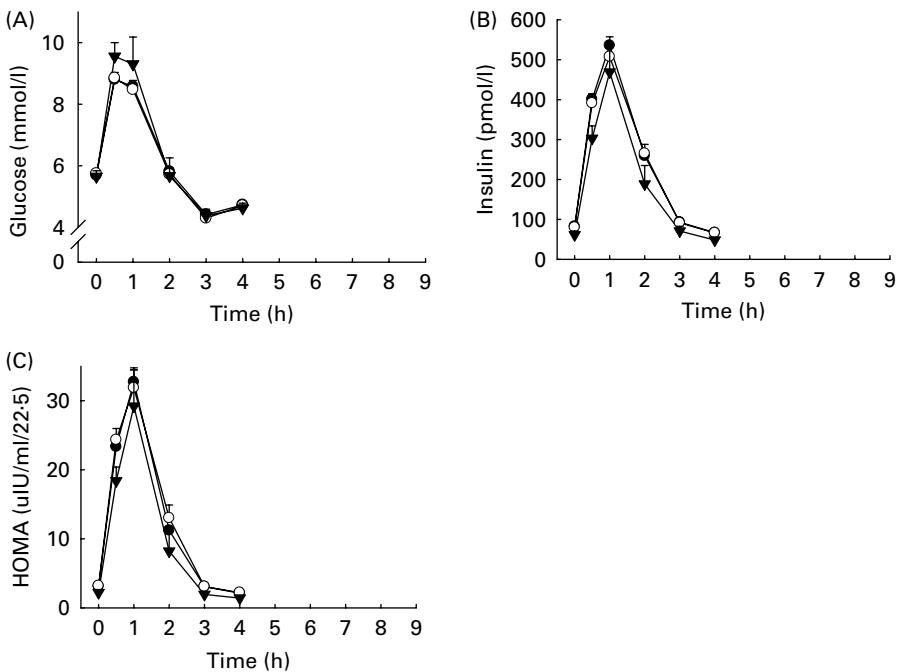


Fig. 2. Postprandial blood levels of the non-obese subgroup ($BMI < 30 \text{ kg/m}^2$) after a glucose load (oral glucose tolerance test, OGTT). Data are presented as means with their standard errors. PPAR γ 2 Pro12Pro, —●—; PPAR γ 2 Pro12Ala, —○—; PPAR γ 2 Ala12Ala, —▼—. (A) Postprandial glucose levels after OMTT; (B) Postprandial insulin levels after OMTT; (C) Postprandial homeostasis model assessment of insulin resistance levels (HOMA) after OGTT. For details of subjects and procedures, see Research design and methods.

(Fig. 1). This, however, was not observed after an oral glucose load (Fig. 2). In non-obese males, a lower waist circumference was found in the Ala12Ala group compared with the Pro12Pro group, despite equal BMI values (Table 2).

Subjects with the Pro12Ala genotype had values similar to those with the Pro12Pro genotype (Tables 1 and 2; Fig. 1). Up until now, only three studies have calculated the Ala12Ala genotype separately, probably because of the low frequency of this genotype (Valve *et al.* 1999; Frederiksen *et al.* 2002; Temelkova-Kurttschiew *et al.* 2004). As in the present study, in these studies, the Ala12Ala genotype clearly differed from the Pro12Ala and Pro12Pro genotype with regard to BMI (Valve *et al.* 1999), blood pressure and TAG (Frederiksen *et al.* 2002), and intima thickness (Temelkova-Kurttschiew *et al.* 2004), whereas the difference between Ala12 carriers and Pro12Pro homozygotes was less pronounced than as described by other studies (Altshuler *et al.* 2000).

In concordance with the present study, Poirier *et al.* (2000) did not find a different glucose and insulin response to a glucose load between the different genotypes. However, whereas they also did not find a different response to a lipid load, we did find a different response to a mixed meal containing fat. This difference was only detected by comparing the homozygous carriers of the rare Ala12Ala genotype with Pro12 carriers. Poirier and co-workers examined men aged 18 to 28 years from eleven different European countries, whereas the present study focused on men aged 45 to 65 years from one region. In the Bogalusa Heart study, Li *et al.* (2003) showed that differences in the association to insulin resistance in Ala12 carriers depended on the individual's age. While significant differences were found between homozygous wild-type carriers and carriers of the alanine coding allele (homozygote and heterozygote) for insulin resistance in adults, only insignificant trends were seen in young people (Li *et al.* 2003). Furthermore, the heterogeneous origin of the participants and their dietary habits (Mediterranean diet in the south and more saturated fat in the north) may obscure phenotypic differences between genotypes. The association between the Pro12Ala polymorphism and BMI and insulin levels seems to depend on the ingestion of unsaturated fatty acids (Luan *et al.* 2001).

Recently, Vänttinen *et al.* (2005) showed that the glucose uptake of the whole body and the skeletal muscle is higher in Ala12 carriers than in the Pro12Pro group after a euglycaemic clamp. However, this higher insulin sensitivity was found

only in non-obese and not in obese subjects (Vänttinen *et al.* 2005). We were able to confirm these findings in the cohort presented here after a mixed meal, where we only found significant differences between Ala12Ala genotypes and Pro12 carriers when obese subjects were excluded.

In the present study, weight and BMI were not affected by the polymorphism. However, waist circumference was lower in the Ala12Ala group. This is in agreement with the findings of Gonzalez-Sanchez *et al.* (2002), who showed that Pro12Pro homozygotes have centrally distributed fat and that Ala12 carriers have more peripheral fat distribution. Since visceral fat is assumed to be the driving force behind the development of insulin resistance (Giorgino *et al.* 2005), one might argue that the higher insulin sensitivity of the Ala12Ala group could be caused by lower abdominal fat. Visceral adipose tissue releases NEFA, which first pass the liver where they may be incorporated into VLDL more easily. Fasting NEFA, however, did not differ either in the Ala12Ala or in the Pro12-carriers of the total or the non-obese group (Fig. 1). In contrast with this, postprandial NEFA tended to be lower (Fig. 1). This may suggest that NEFA were released more slowly by lipoprotein lipase from postprandial-affluent TAG-rich lipoproteins. Indeed, the expression of lipoprotein lipase, and in particular adipocyte LDL, is regulated by PPAR γ (Schoonjans *et al.* 1996). This may explain why the PPAR γ 2 Pro12Ala polymorphism may have an impact on postprandial NEFA release from TAG-rich lipoproteins, although it is quite specifically expressed in adipose tissue (Desvergne & Wahli, 1999). The data obtained by Tan *et al.* (2006), however, do not seem to support the theory that dietary fat causes a different release of NEFA. Interestingly, they found a faster NEFA clearance by muscle tissue (Tan *et al.* 2006). Accordingly, Vänttinen *et al.* (2005) found that the glucose uptake of the whole body and the skeletal muscle is increased in Ala12 carriers, compared with Pro12Pro homozygotes in a euglycaemic clamp. Stefan *et al.* (2001) showed that the intravenous infusion of fatty acids leads to an increase in the insulin resistance of the Pro12Pro group and a decrease in the insulin resistance of the Ala12 carriers. This explains why we found no differences in insulin sensitivity between Ala12Ala and Pro12-carrier homozygotes after a pure carbohydrate load (oral glucose tolerance test) (Fig. 2), whereas such a difference became overt after a fat-containing mixed meal (Fig. 1). Since PPAR γ 2 is quite specifically expressed by adipose tissue (Desvergne & Wahli, 1999), we postulate that a factor

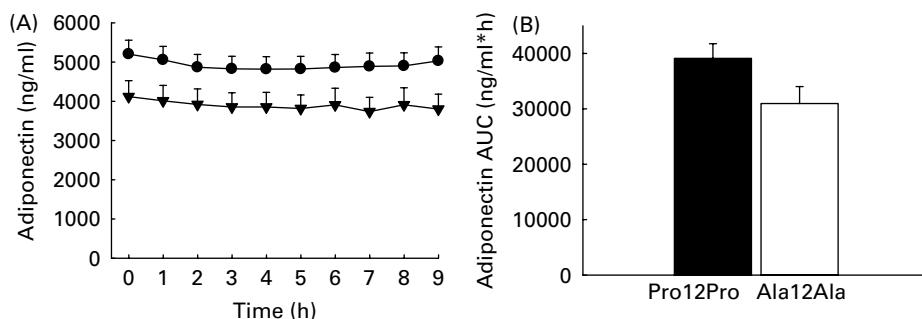


Fig. 3. Postprandial blood levels of the non-obese BMI-matched subgroup after a mixed meal (oral metabolic tolerance test, OMTT). Data are presented as means with their standard errors. PPAR γ 2 Pro12Pro, –●–; PPAR γ 2 Ala12Ala, –▼–. (A) Postprandial adiponectin levels after OMTT; (B) postprandial adiponectin levels area under the curve (AUC) of homozygote carriers. For details of subjects and procedures, see Research design and methods.

released by adipocytes causes this PPAR γ 2 genotypic dependence of the fatty acid-mediated effect on insulin sensitivity. Therefore, we investigated the peripheral blood levels of an adipocyte factor, which is under regulatory control of PPAR γ and has an influence on insulin sensitivity. The adipokine adiponectin – which increases insulin sensitivity – met this claim (Yamauchi *et al.* 2001). Fasting and postprandial adiponectin levels in the BMI-matched sub-cohort did not significantly differ between the Pro12Pro and the Ala12Ala group (Fig. 3). They even tended towards lower levels in the Ala12Ala group (Fig. 3). Yamamoto *et al.* (2002) recently also found reduced fasting adiponectin levels in Ala12 carriers, compared with Pro12Pro carriers. They subsumed that this finding provides evidence that the PPAR γ 2 Pro12Ala polymorphism has a functional impact on adipocyte tissue because this single nucleotide polymorphism is associated with decreased activity and results, therefore, in decreased PPAR γ -dependent adiponectin levels (Yamamoto *et al.* 2002).

The causative PPAR γ -dependent adipocyte-derived factor, which regulates insulin metabolism depending on the ingested meal, remains to be discovered from approximately fifty adipokines known at present (Trayhurn & Wood, 2004).

In conclusion, PPAR γ 2 Ala12Ala is associated with lower fasting and postprandial TAG levels, lower insulin levels and higher postprandial insulin sensitivity after a mixed meal containing fat. In contrast with this, postprandial insulin levels and insulin sensitivity were not affected after an oral glucose load. This may indicate that the effects of PPAR γ 2 on insulin sensitivity are mediated by postprandial-affluent dietary fat and that the PPAR γ 2 polymorphism has some functional impact on postprandial metabolism. Since PPAR γ 2 is predominantly expressed in adipocytes, we postulate that a PPAR γ 2 and fatty acid-dependent adipocyte factor affects insulin sensitivity. NEFA and adiponectin were excluded as causative adipokines.

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