Original Article

Role of the *PPARα* Leu162Val and *PPARγ2* Pro12Ala Gene Polymorphisms in Weight Change after 2.5-Year Follow-up in Czech Obese Women

(PPARγ2 / Pro12Ala polymorphism / obesity / PPARα / Leu162Val polymorphism / body mass index changes / psycho-behavioural factors and genetics)

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Abstract. The aim of this study was to investigate the possible effect of *PPARα* and *PPARγ2* variants on weight and eating attitudes as well as on their changes after 2.5-year follow-up. The study was carried out in 246 Czech non-diabetic obese women (age 49.0 ± 11.9 years; BMI 38.1 ± 7.0 kg/m²). The comprehensive weight management programme included low-energy diet, increased physical activity and lifestyle modification. Anthropometric parameters (body weight and height, waist and hip circumferences) and body composition were measured. The Three-Factor Eating Questionnaire and Beck Depression Inventory were evaluated. At baseline and after the follow-up period, fasting levels of serum glucose, plasma adiponectin, ghrelin, leptin, and lipid profile were determined. The dependence of monitored parameters on the Pro12Ala in *PPARγ2* and Leu162Val in *PPARα* and stage of the treatment (baseline; 2.5-year follow-up) was evaluated using the repeated measures ANOVA model. The cohort was re-examined after 2.5 years, independent of regular check-ups and adherence to lifestyle recommendation. Significant favourable changes in anthropometric indexes, lipid profile, leptin, ghrelin and adiponectin levels as well as in dietary restraint and hunger scores were revealed at 2.5-year check-up. However, no changes in the scores of disinhibition and depression were demonstrated. Despite several observed significant differences between carriers and non-carriers of the minor alleles at baseline and at the follow-up, the repeated measures ANOVA did not reveal any significant effect of the *PPARα* and *PPARγ2* polymorphisms on anthropometric, biochemical, hormonal and psycho-behavioural characteristics, neither at baseline nor at the 2.5-year follow-up.

Introduction

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily. PPARs participate in many actions related to energy homeostasis and hence obesity development. Three types of PPARs have been identified: α, β (δ), and γ (Meirhaeghe and Amouyel, 2004).

PPARα

Peroxisome proliferator-activated receptor α (PPARα) regulates a variety of genes involved in lipid metabolism, including fatty acid oxidation (Desvergne and Wahli, 1999). A Leu162Val polymorphism (rs1800206) of the *PPARα* gene has been associated with lipid levels, but those results have been contradictory (Tai et al., 2002; Nielsen et al., 2003; Skogsberg et al., 2003; Robitaille et al., 2004; Uthurralt et al., 2007). Evans et al. (2001) observed similar allelic frequency of the

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Abbreviations: BDI – Beck Depression Inventory, BMI – body mass index, FM – fat mass, FFM – fat-free mass, HDL-cholesterol – high-density lipoprotein cholesterol, LDL-cholesterol – low-density lipoprotein cholesterol, PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism, PPAR – peroxisome proliferator-activated receptor, TFEQ – Three-Factor Eating Questionnaire.

Table 1. Genotype distribution of the PPARα Leu162Val and the PPARγ Pro12Ala polymorphisms in European studies

PPARα Populations	N	Sex (M/F)	Age (years)	BMI (kg/m²)	Genotype			Val allelic frequency	Reference
					Leu/Leu	Leu/Val	Val/Val	(%)	
Danish	1383	830/553	59.0 ± 11.0	29.4 ± 5.1	1228	148	7	5.8	Sparso et al., 2007
German	842	481/261	-	-	754	73	5	9.0	Gouni-Berthold et al., 2004
German	370	66/304	39.0 ± 9.0	51.0 ± 8.0	326	41	3	6.3	Evans et al., 2001
Czech	246	0/246	49.0 ± 11.9	38.1 ± 7.0	227	19	0	3.9	Present study
PPARγ2 Populations	N	Sex (M/F)	Age (years)	BMI (kg/m²)	Genotype			Val allelic frequency	Reference
					Pro/Pro	Pro/Ala	Ala/Ala	(%)	
Finnish	490	161/329	40.0–68.0	31.1 ± 4.6	337	140	13	16.9	Lindi et al., 2002
Polish	366	196/170	59.9 ± 9.3	30.9 ± 5.8	256	99	11	16.5	Malecki et al., 2003
Spanish	464	210/254	49.0 ± 8.0	28.0 ± 4.4	385	75	4	8.9	Gonzalez Sanchez et al., 2002
Italian	566	211/355	36.6 ± 12.0	25.4 ± 4.5	501	64	1	5.8	Morini et al., 2008
French	124	0/124	55.8	31.8 ± 5.9	93	26	2	12.0	Meirhaeghe et al., 2005
Swedish	284	284/0	58.0	-	186	92	6	18.3	Rosmond et al., 2003
Danish	752	184/180	31.0	43.1 ± 6.2	540	191	21	15.5	Ek et al., 1999
Czech	326	99/225	32.0 ± 11.0	23.9 ± 4.0	233	83	8	15.1	Bendlova et al., 2008
Czech	246	0/246	49.0 ± 11.9	38.1 ± 7.0	189	49	8	13.2	Present study

Leu162Val polymorphism in subjects with hyperlipidaemia and type 2 diabetes, in morbidly obese patiens as well as in healthy individuals. He therefore concluded that this polymorphism has no major role in the development of these conditions. Similar results were reported in another study (Sparso et al., 2007). Val allelic frequency in different European populations is presented in Table 1.

A study performed in healthy adults has shown that the Val carriers had significantly lower body mass index (BMI) and percentage of body fat in comparison to wild-type, but this association was completely abolished after adjustment for total body fat and gender (Bosse et al., 2003). This is in agreement with a study that showed no effect on the BMI level (Gouni-Berthold et al., 2004). So far, no data have been published on the effect of the Leu162Val polymorphism on psycho-behavioural indexes and long-term weight change.

PPARγ

Peroxisome proliferator-activated receptor γ (PPARγ) as a transcriptional factor regulates adipocyte differentiation, adipocyte-specific gene expression and insulin action (Deeb et al., 1998; Auwerx, 1999; Zietz et al., 2002). Its activation leads to an increased differentiation of preadipocytes to adipocytes. There exist four isoforms of *PPARγ*: *PPARγ1, PPARγ2, PPARγ3* and *PPARγ4.* The *PPARγ2* is considered a candidate gene for the regulation of body weight as it is almost exclusively expressed in adipose tissue (Auwerx, 1999). It is also known that the expression of *PPARγ2* mRNA in adipose tissue is increased in obese subjects (Vidal-Puig et al., 1997). A strong positive correlation was identified between the ratio of PPARγ2/γ1 and the BMI (Vidal-Puig et al., 1997).

The allelic frequency of the Pro12Ala polymorphism (rs1801282) ranges between 2 and 23 % in different ethnic groups (Deeb et al., 1998; Altshuler et al., 2000; Stumvoll and Haring, 2002). Among Caucasians this polymorphism presents with an allelic frequency as high as 20 % (Meirhaeghe and Amouyel, 2004). Table 1 shows the Ala allelic frequency in different European populations. Numerous studies have investigated the association of the common polymorphism Pro12Ala of *PPARγ2* with body weight. The results have been controversial, as the Ala12 allele has been associated with lower BMI (Deeb et al., 1998) in some studies and with higher BMI in others (Beamer et al., 1998; Valve et al., 1999; Meirhaeghe and Amouyel, 2004). Meirhaeghe and Amouyel (2004) could not find such an association at all. A meta-analysis comprising more than 19,000 subjects with BMI above 27 kg/m² revealed a positive correlation of the Pro12Ala polymorphism with BMI under a recessive model (Masud and Ye, 2003). Inconsistent results were reported with regard to the effect of this polymorphism on the development of type 2 diabetes (Altshuler et al., 2000; Meirhaeghe and Amouyel, 2004). Many published studies have reported an association between the Ala allele and improved insulin sensitivity estimated by HOMA IR (Meirhaeghe and Amouyel, 2004).

Both the magnitude of weight loss and weight maintenance may be partially influenced by genetic factors (Marti et al., 2004; Hainer et al., 2008). Prospective and intervention studies have shown that the Pro12Ala polymorphism is associated with weight gain over time or weight regain after diet-induced weight loss (Ek et al., 1999; Nicklas et al., 2001), in contrast to the results of the Finnish intervention study in which Ala12Ala carriers lost more weight than the Pro allele carriers (Lindi et al., 2002). Vogels et al. studied the relation of weight maintenance to biological, psychological determinants and *PPARγ2* genotype (Vogels et al., 2005). They showed that more subjects with successful weight maintenance exhibited the Pro/Pro genotype compared with the other two genotypes.

To date, not much is known about the effect of *PPARγ2* polymorphism on psycho-behavioural indexes

in Caucasians. It is known that eating behaviour has a major effect on body weight and obesity-related phenotypes (Steinle et al., 2002). The Three-Factor Eating Questionnaire (TFEQ) is widely used to measure components of eating attitudes by scoring restriction, disinhibition, and hunger (Stunkard and Messick, 1985). It has been demonstrated that the components of TFEQ are influenced by genetic background (Neale et al., 2003). Disinhibition is as high as 45 % determined by heritability (Bryant et al., 2008). Lower effect of genetics was demonstrated on restraint and hunger score (Bryant et al., 2008). A decrease of disinhibition score is related not only to weight reduction, but also to weight maintenance (Hainer et al., 2005). In addition, the genotype of glucocorticoid receptor gene determines the decrease of disinhibition score and predicts weight maintenance after weight reduction (Vogels et al., 2005). However, no association between *PPARγ2* genotypes and factors of the TFEQ was shown (Vogels et al., 2005). Neuromedin β and glutamate decarboxylase 2 genes have also been identified as candidate genes influencing eating behaviour (Boutin et al., 2003; Bouchard et al., 2004). The linkage analysis of eating attitudes in Amish population showed a peak linkage to disinhibition at the region that encodes *PPARγ* (Steinle et al., 2002). An association between gene variants and depression was proved for several genes (Comings et al., 1996; Krishnamurthy et al., 2008; Fuemmeler et al., 2009; Kapoor et al., 2009). However, no data is available concerning the association of *PPARα* and *PPARγ* variants with depression.

Material and Methods

Subjects

The cohort of Czech obese Caucasian women comprised 246 unrelated non-diabetic subjects with mean age of 49.0 ± 11.9 years and mean BMI 38.1 ± 7.0 kg/m^2. Obesity was defined as a BMI ≥ 30.0 kg/m^2. Patients were followed in the Obesity Management Center of the Institute of Endocrinology in Prague and in the Obesity Management Unit of the Clinical Centre ISCARE I.V.F. in Prague and evaluated by a multidisciplinary team, which included an obesity specialist, a psychologist and a dietician. Our study was carried out over a period of 2.5 years. The comprehensive weight management programme included low-energy diet (recommended daily energy deficit of 2.5 MJ), increased physical activity (recommended 30 min of aerobic exercise per day) and behavioural lifestyle modification provided by a psychologist. All patients underwent a control examination 2.5 years after the enrollment, regardless of their adherence to the weight reduction programme or their participation in regular check-ups.

Anthropometric and clinical assessment methods

Anthropometric parameters (body weight, body height, waist and hip circumferences) were measured according to the WHO recommendations (WHO Expert Committee, 1995). Body composition (fat mass %, fat-free mass %) was assessed by using bipedal-bimanual bioimpedance analyser – TANITA BC-418MA. Daily energy and macronutrient intake was evaluated from one-week dietary record and analysed by a computerized version of the Czech Nutrition Programme "*Výživa*". Eating attitudes (dietary restraint, disinhibition and susceptibility to hunger) were assessed by TFEQ (Stunkard and Messick, 1985). The depression score was measured by the Beck Depression Inventory (BDI) (Beck et al., 1961). At baseline and after an average follow-up period of 2.5 years, fasting levels of serum glucose and lipid profile were determined and analysed by standard assays. Plasma adiponectin, ghrelin and leptin were measured by commercial RIA kits (Linco Research, Inc., St. Charles, MO).

Informed consent was obtained from all subjects. The study protocol was approved by the Ethics Committee of the Institute of Endocrinology in Prague and was in accordance with the principles of the Helsinki Declaration II.

Genetic analysis

Genomic DNA was extracted from peripheral leukocytes using a commercially available kit (QIAamp Blood Kit, Qiagen, Hilden, Germany). The Leu162Val polymorphism of the *PPARα* (rs1800206) was genotyped as described previously (Vohl et al., 2000). In the case of *PPARα*, genotyping was performed in 237 women only. The Pro12Ala polymorphism of the *PPARγ2* (rs1801282) was assessed by TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) with the LightCycler 480 Real-Time PCR System (ROCHE, Basel, Switzerland).

Statistical analyses

Due to the small number of Ala homozygotes and the same baseline characteristics with Pro12Ala heterozygotes in the *PPARγ2*, the Ala homozygotes were combined with Pro12Ala heterozygotes and compared to Pro12Pro homozygotes for all analyses. The difference in all parameters was compared with regard to the *PPARγ2* polymorphism (Ala allele carriers vs. Ala allele non-carriers) and with regard to the *PPARα* polymorphism (Val allele carriers vs. Val allele non-carriers). The dependence of all monitored parameters on the Pro12Ala polymorphism of the *PPARγ2* and the Leu162Val polymorphism of the *PPARα* and stage of the treatment (baseline; 2.5-year follow-up) was evaluated using the repeated measures ANOVA model consisting of subject factor, between-subject factor "polymorphism", within-subject factor "stage of the treatment" and interaction "polymorphism" × "stage of the treatment". Due to non-Gaussian data distribution in most of the dependent variables, this data underwent a power transformation to attain a distributional symmetry and a constant variance. The statistical software NCSS 2004 (NCSS, Kaysville, UT) was used for data analysis. A P value < 0.05 was considered significant.

Results

PPARα

The allelic frequency of the Leu162Val polymorphism in the *PPARα* gene was 3.9 % in the studied cohort. Within the cohort, 19 subjects (8.0 %) were identified as heterozygous for the variant. No homozygous subject (Val/Val genotype) was detected. At baseline, heterozygous carriers of the Val allele had significantly higher the following parameters: BMI, body weight, waist and hip circumferences ($P < 0.05$) (Table 2). In addition, the Val carriers had significantly lower levels of adiponectin, glucose, triglyceride and total cholesterol (Table 2). No baseline differences at eating attitude parameters or depression scores were observed.

Both groups significantly decreased energy and nutrient intake after the 2.5-year follow-up (Table 2). The trends of change in body weight, BMI, waist circumference, lipid profile, restraint and hunger scores were comparable in *PPARα* wild-type genotype and Val carriers. However, the change in these parameters was significant only in the wild-type carriers ($P < 0.05$) (Table 2). Leptin and ghrelin levels declined in both groups, whereas adiponectin concentration increased. All these changes in hormonal levels were found significant except for a decrease in the leptin level in Val carriers (Table 2). At follow-up, the restraint score increased and hunger score decreased, but significantly only in non-carriers of the Val allele. Neither the score of disinhibition nor the depression score were significantly affected. The body weight change and changes of other screened parameters after the 2.5-year period did not differ between the two genotypes (data not shown).

PPARγ2

The allelic frequency of the Pro12Ala polymorphism in the *PPARγ2* gene was 13.2 % among 246 Czech obese women. Within the cohort of obese women, 189 subjects (76.8 %) had the Pro/Pro genotype, 49 subjects (19.9 %) were identified as Pro/Ala heterozygotes and 8 subjects (3.3 %) had the Ala/Ala genotype. At baseline, no significant differences in any of the studied parameter between the two genotypes were observed (Table 3).

During the follow-up period both groups demonstrated a significant reduction in hip circumference, ghrelin level, in energy and macronutrient intake, as well as an increase in dietary restraint and decrease in hunger score (Table 3). Both groups exhibited a reduction of body weight, BMI and plasma leptin level and an increase of the adiponectin level; the significance was reached in Ala non-carriers (Table 3). Both genotype groups showed a similar trend of lipid profile improvement (except for HDL-cholesterol), although with variable significance (Table 3). Significant reduction in the triglyceride level was demonstrated in Ala carriers only. However, the differences in changes of all parameters before and after the follow-up were not significant between the two genotype groups. There was no effect of the particular genotype on any of the studied parameters when the three genotype groups (Pro/Pro, Pro/Ala, Ala/Ala) of the *PPARγ2* gene were evaluated (data not shown).

Table 2. The Leu162Val polymorphism of the PPARα – baseline anthropometric, biochemical, hormonal, nutritional and psycho-behavioural characteristics of non-diabetic obese women and their changes at follow-up expressed as a median (lower confidence limit; upper confidence limit)

	The Leu162Val polymorphism of *PPARα*					
	Val allele non-carriers (N = 218)			**Val allele carriers (N = 19)**		
	At baseline	**After 2.5-year follow-up**	**P value**	**At baseline**	**After 2.5-year follow-up**	**P value**
BMI (kg/m^2)	36.1 (35.1; 37.3)*	35.7 (35.0; 36.6)	NS	40.5 (36.0; 42.7)*	40.0 (36.7; 42.3)	NS
Body weight (kg)	99.5 (97.7; 102.0)*	97.2 (93.6; 100.0)	P < 0.05	106.6 (96.6; 110.5)*	104.5 (96.9; 112.5)	NS
Waist circumference (cm)	107.5 (105.0; 110.0)*	105.5 (103.0; 108.0)	NS	114.5 (99.0; 118.0)*	111.5 (108.0; 119.5)	NS
Hip circumference (cm)	123.0 (122.0; 125.0)*	122.0 (120.0; 124.0)	P < 0.05	131.5 (127.0; 137.0)*	133.0 (125.0; 138)	NS
Energy intake (kJ/day)	7153.0 (6894.0; 7489.0)	6148.5 (5848.0; 6413.0)	P < 0.05	7203.0 (6015.0; 8936.0)	6096.0 (6551.0; 7116.0)	P < 0.05
Protein intake (g/day)	64.9 (63.4; 68.0)	63.6 (58.5; 66.9)	P < 0.05	75.0 (53.8; 83.1)	61.0 (54.1; 64.9)	P < 0.05
Carbohydrate intake (g/day)	208.0 (198.7; 225.5)	181.7 (171.8; 197.2)	P < 0.05	226.6 (184.7; 241.5)	179.8 (165.9; 228.7)	P < 0.05
Fat intake (g/day)	62.6 (58.4; 66.1)	50.4 (47.7; 53.7)	P < 0.05	65.2 (48.9; 84.7)	55.6 (42.7; 62.7)	P < 0.05
TFEQ – restraint score	10.0 (9.0; 11.0)	14.0 (13.0; 15.0)	P < 0.05	12.0 (8.0; 15.0)	14.0 (13.0; 16.0)	NS
TFEQ – disinhibition score	6.0 (5.0; 7.0)	6.0 (5.0; 7.0)	NS	6.0 (4.0; 9.0)	5.0 (4.0; 6.0)	NS
TFEQ – hunger score	4.0 (3.0; 4.0)	3.0 (2.0; 3.0)	P < 0.05	4.0 (2.0; 6.0)	2.0 (1.0; 7.0)	NS
BDI – depression score	10.0 (9.0; 12.0)	9.0 (7.0; 11.0)	NS	11.0 (6.0; 23.0)	9.0 (6.0; 20.0)	NS
Glucose (mmol/l)	5.1 (4.9; 5.3)*	5.1 (4.9; 5.2)	NS	4.7 (4.5; 5.1)*	5.5 (4.4; 5.7)	NS
Total cholesterol (mmol/l)	5.2 (5.1; 5.4)*	4.9 (4.7; 5.2)	NS	5.0 (4.5; 5.6)*	5.0 (3.8; 5.5)	NS
HDL-cholesterol (mmol/l)	1.5 (1.4; 1.6)	1.4 (1.3; 1.5)	P < 0.05	1.6 (1.5; 1.8)	1.5 (1.1; 1.8)	NS
LDL-cholesterol (mmol/l)	3.1 (2.8; 3.2)	2.9 (2.5; 3.0)	P < 0.05	3.0 (2.4; 3.6)	2.8 (2.1; 3.6)	NS
Triglyceride (mmol/)	1.4 (1.3; 1.5)*	1.2 (1.1; 1.3)	P < 0.05	1.3 (1.2; 1.5)*	1.2 (1.0; 1.6)	NS
Leptin (ng/ml)	45.7 (40.9; 57.3)	40.9 (32.1; 53.5)	P < 0.05	52.1 (25.9; 117.9)	40.7 (20.2; 98.5)	NS
Ghrelin (pg/ml)	893.9 (791.9; 1008.4)	805.8 (697.4; 919.4)	P < 0.05	893.5 (594.5; 1270.1)	557.0 (401.2; 1020.1)	P < 0.05
Adiponectin (mg/l)	9.6 (7.8; 10.3)*	12.2 (10.7; 13.3)	P < 0.05	6.9 (4.1; 16.0)*	10.2 (4.1; 14.4)	P < 0.05

NS – not significant

* Significant differences in baseline characteristics between Val allele non-carriers vs. Val-allele carriers ($P < 0.05$)

Table 3. The Pro12Ala polymorphism of the PPARγ2 – baseline anthropometric, biochemical, hormonal, nutritional and psycho-behavioural characteristics of non-diabetic obese women and their changes at follow-up expressed as a median (lower confidence limit; upper confidence limit)

	The Pro12Ala polymorphism of PPARγ2					
	Ala allele non-carriers (N = 189)			Ala allele carriers (N = 57)		
	At baseline	After 2.5-year follow-up	P value	At baseline	After 2.5-year follow-up	P value
BMI (kg/m^2)	36.6 (35.4; 37.7)	36.3 (35.1; 37.1)	P < 0.05	36.5 (34.5; 39.3)	35.4 (33.2; 38.5)	NS
Body weight (kg)	100.0 (97.7; 102.6)	97.2 (94.0; 101.0)	P < 0.05	101.8 (95; 109.8)	97.8 (92.1; 106.2)	NS
Waist circumference (cm)	108.0 (104.0; 111.0)	106.0 (103.0; 110.0)	NS	110.0 (105.0; 114.0)	106.0 (101.0; 111.0)	P < 0.05
Hip circumference (cm)	124.0 (123.0; 126.0)	123.0 (120.0; 126.0)	P < 0.05	125.0 (120.0; 131.0)	122.0 (119.0; 127.0)	P < 0.05
Energy intake (kJ/day)	7262.5 (6894.0; 7561.0)	6096.0 (5921.0; 6426.0)	P < 0.05	7213.5 (6227.0; 7894.0)	6242.0 (5629.0; 6931.0)	P < 0.05
Protein intake (g/day)	66.1 (63.5; 69.0)	63.2 (58.7; 67.0)	P < 0.05	64.7 (57.1; 71.3)	61.5 (57.0; 64.9)	P < 0.05
Carbohydrate intake (g/day)	208.1 (197.0; 220.6)	181.2 (171.8; 193.8)	P < 0.05	223.6 (189.1; 237.7)	186.7 (169.7; 209.7)	P < 0.05
Fat intake (g/day)	62.5 (58.2; 66.8)	50.7 (48.2; 55.1)	P < 0.05	64.6 (50.7; 75.3)	53.3 (43.5; 60.3)	P < 0.05
TFEQ – restraint score	9.0 (8.0; 11.0)	13.0 (13.0; 14.0)	P < 0.05	11.0 (8.0; 12.0)	15.0 (12.0; 16.0)	P < 0.05
TFEQ – disinhibition score	7.0 (6.0; 8.0)	6.0 (5.0; 7.0)	NS	5.0 (4.0; 8.0)	5.0 (3.0; 7.0)	NS
TFEQ – hunger score	4.0 (3.0; 5.0)	3.0 (2.0; 4.0)	P < 0.05	3.0 (3.0; 4.0)	2.0 (1.0; 3.0)	P < 0.05
BDI – depression score	10.0 (9.0; 12.0)	9.0 (7.0; 11.0)	NS	11.0 (10.0; 13.0)	9.0 (7.0; 14.0)	NS
Glucose (mmol/l)	5.1 (4.9; 5.3)	5.1 (5.0; 5.3)	NS	5.1 (4.7; 5.3)	5.1 (4.8; 5.5)	NS
Total cholesterol (mmol/l)	5.2 (5.1; 5.4)	4.9 (4.7; 5.2)	P < 0.05	5.2 (4.8; 5.7)	4.8 (4.3; 5.3)	P < 0.05
HDL-cholesterol (mmol/l)	1.5 (1.4; 1.6)	1.4 (1.3; 1.6)	P < 0.05	1.6 (1.4; 1.6)	1.4 (1.2; 1.7)	NS
LDL-cholesterol (mmol/l)	3.0 (2.8; 3.2)	2.8 (2.5; 3.0)	P < 0.05	3.1 (2.6; 3.4)	3.0 (3.3; 3.4)	NS
Triglyceride (mmol/l)	1.4 (1.2; 1.5)	1.2 (1.1; 1.4)	NS	1.5 (1.3; 1.8)	1.1 (0.9; 1.4)	P < 0.05
Leptin (ng/ml)	48.7 (42.5; 57.6)	36.8 (31.9; 52.8)	P < 0.05	44.7 (34.6; 90.4)	41.2 (32.1; 62.4)	NS
Ghrelin (pg/ml)	891.7 (791.9; 983.7)	752.2 (627.7; 874.9)	P < 0.05	973.3 (761.6; 1406.3)	900.4 (688.7; 1000.0)	P < 0.05
Adiponectin (mg/l)	9.27 (7.11; 9.9)	12.0 (9.2; 12.8)	P < 0.05	10.5 (6.5; 16.0)	12.2 (7.3; 18.5)	NS

NS – not significant

In both gene analyses, no significant changes of the glucose profile, HOMA indexes, fat mass and fat-free mass at baseline and after 2.5 years were demonstrated (data not shown). Different genotype combinations of both genes (*PPARα*, *PPARγ2*) did not have any significant impact on the studied parameters (data not shown). Only seven subjects carrying both polymorphisms of *PPARα* and *PPARγ2* genes were identified. Due to the low statistical power, analyses are not presented.

Discussion

PPARα

No homozygous subjects of the Leu162Val polymorphism of the *PPARα* were detected. The genotype frequency of *PPARα* in the present study is similar to previous published studies of European populations (Table 1). We found that Val-allele carriers had lower lipid levels than wild-type carriers, which is consistent with previous findings (Nielsen et al., 2003; Robitaille et al., 2004; Uthurralt et al., 2007). Interestingly, these subjects exhibited lower lipid levels in spite of their higher baseline adiposity. We might suggest that the Val allele has a protective effect on serum lipids and an unfavourable effect on body adiposity (Table 2). On the other hand, Sparso et al. (2007) found increased fasting serum triglycerides and total cholesterol concentrations in Val homozygotes vs. non-homozygotes.

According to our results, the Leu162Val polymorphism of *PPARα* does not influence the level of weight reduction after 2.5-year follow-up, as no differences between the genotype groups were observed. The favourable changes in energy and nutrient intake were not due to the gene effect, as no differences between genotypes were detected. We assume that this improvement is entirely due to the intervention.

We found that this polymorphism had no effect on baseline scores of TFEQ and BDI, even though the restraint score of Val carriers had a tendency to be higher than in the wild-type carriers. After a 2.5-year follow-up, only wild-type carriers significantly improved their eating attitudes. We assume that the intervention might have led to a higher increase of restraint score in the wild-type group as its baseline level was lower. Further, we may argue that this improvement (higher dietary restraint and lower hunger score) might explain the significant body weight loss in the wild-type group after the 2.5-year follow-up. Similar changes in body weight, BMI, waist circumference, lipid profile, restraint and hunger scores were found in both genotype groups. The significance observed in wild-type carriers only can either be due to the diminished size of the Val-carrier cohort, or to the baseline differences. As demonstrated in previously published studies, these differences were not influenced by the gene effect itself (Bosse et al., 2003; Gouni-Berthold et al., 2004).

PPARγ2

The allelic frequency of the Pro12Ala polymorphism of the *PPARγ2* in Czech obese women was similar to previous studies in obese women (Beamer et al., 1998; Nicklas et al., 2001; Goyenechea et al., 2006) and in Czech adult subjects (Sramkova et al., 2002; Pinterova et al., 2004; Bendlova et al., 2008). Genotype frequencies of the Pro12Ala polymorphism in European popu-

lations are compared in Table 1. We did not find any differences in any of the studied parameters at baseline between the genotypes, which is in accordance with previously published studies performed on either population-based cohorts (Lindi et al., 2001; Luan et al., 2001) or obese subjects (Nicklas et al., 2001; Goyenechea et al., 2006). The lacking association of baseline hormonal and biochemical characteristics in our study is consistent with a study on metabolic syndrome conducted in obese subjects which did not support the impact of *PPARγ2* polymorphism on the components of metabolic syndrome except for minor effect (P = 0.04) on the glucose level (Montagnana et al., 2008).

On the other hand, a study performed on 112 obese American Caucasian women reported a higher body weight (P = 0.001), BMI (P = 0.004) and waist circumference (P = 0.001) in subjects with Ala12Ala and Pro12Ala genotypes when compared to wild-type carriers (Beamer et al., 1998). However, the lipid profile, glucose and insulin levels were not different between the two genotypes (Beamer et al., 1998). Exactly the opposite results were reported by Vogels et al. (2005), who found that wild-type obese subjects had significantly higher baseline body weight (P = 0.02), BMI (P = 0.05) and waist circumference (P = 0.02) than obese subjects with the Pro12Ala genotype. We suppose that these conflicting results might be due to the fact that cohorts differ in the characterization (obese vs. general population), their size, ethnicity, profile of consumed nutrients, degree of physical activity, allele frequency (especially low Ala homozygotes) and degree of obesity in obese subjects.

In the present study, wild-type carriers after the follow-up period of 2.5 years exhibited a significant weight loss. This finding is in agreement with studies performed in the general population (Lindi et al., 2001) and in subjects with juvenile obesity (Ek et al., 1999). The body weight and significant waist and hip circumference reductions were also apparent in our Ala carriers. No effect of the Pro12Ala polymorphism on weight loss and weight maintenance was demonstrated in previously published studies (Nicklas et al., 2001; Sesti et al., 2005; Goyenechea et al., 2006).

Obese women were able to maintain dietary recommendations throughout 2.5 years. The Pro12Ala polymorphism did not affect the scores of the TFEQ factors. We assume that the increase in dietary restraint and the decrease in hunger score were entirely influenced by the lifestyle intervention. We were not able to prove that *PPARγ2* can modify the eating attitudes as suggested by Steinle et al. (2002). The adiponectin and leptin change in Ala carriers did not reach a significant level, probably due to the lower number of Ala carriers in comparison to Ala non-carriers. The different impact of the three genotype groups on the weight change after 2.5-year follow-up reported in general population was not confirmed in our study (Lindi et al., 2001). These conflicting results might be due to different characteristics in the studied cohorts (obese vs. general population), duration of follow-up (2.5 years vs. 10 years) and to the very low Ala/Ala frequency in the study of Lindi et al. (2001).

In conclusion, follow-up of obese women over a 2.5-year period revealed some favourable changes not only in the anthropometric characteristics, but also in the lipid profile, leptin, ghrelin and adiponectin levels, as well as in dietary restraint and hunger scores. This improvement was demonstrated although the investigated cohort also included the patients who failed to adhere to lifestyle recommendations and participate in regular check-ups. Despite some observed significant differences between the carriers and non-carriers of the risk alleles both at baseline and at 2.5-year follow-up, the repeated measures ANOVA did not reveal any significant effect of the *PPARα* and *PPARγ2* polymorphisms on anthropometric, biochemical, hormonal and psychobehavioural characteristics, neither at baseline nor after the 2.5-year follow-up, and on their changes over the follow-up period. The Val allele of *PPARα* might have a favourable impact on the lipid profile and an unfavourable effect on adiposity in obese Caucasian women.

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