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Endurance training–induced changes in the insulin response to oral glucose are associated with the peroxisome proliferator–activated receptor-γ2 Pro12Ala genotype in men but not in women

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Abstract

The present study sought to investigate, in sedentary men and women, (*a*) whether a common functional gene variant (peroxisome proliferator–activated receptor-γ2 [PPARγ2] Pro12Ala) predicts insulin action and (*b*) whether improvements in insulin action in response to endurance exercise training are associated with PPARγ2 Pro12Ala. Sedentary, 50- to 75-year-old men and women (N = 73) were genotyped and underwent oral glucose tolerance tests (OGTTs) before and after 6 months of endurance training. At baseline, men heterozygous for the Pro12Ala variant had a greater OGTT insulin area under the curve (AUC) as compared with Pro12 homozygous men ($P = .009$). Endurance training resulted in a significantly greater improvement in insulin AUC in Pro12Ala heterozygous men as compared with Pro12 homozygous men ($P = .003$) despite no genotype-specific differences with respect to training-induced changes in body weight, body mass index, and percent body fat. No differences between genotype groups were present at baseline or in response to training in women. Training did not alter the OGTT glucose AUC for the group as a whole, and the baseline, final, and change in glucose AUC were not dependent on PPARγ2 genotype and/or sex. In conclusion, these findings suggest that sedentary men with the PPARγ2 Pro12Ala variant have lower insulin action on glucose disposal as compared with their counterparts. However, these men are particularly responsive with respect to the magnitude of endurance training–induced improvement in insulin action.

1. Introduction

The common, missense variation (Pro12Ala) in the peroxisome proliferator–activated receptor-γ2 (PPARγ2) gene has been extensively studied for associations with insulin action and type 2 diabetes risk. The PPARγ2 gene codes for a ligand-activated transcription factor that is primarily expressed in adipocytes but is also expressed at lower levels in muscle [1]. As a transcription factor, the PPARγ2 protein regulates genes involved in lipid and carbohydrate metabolism [2], and it is involved in the regulation of adipogenesis [3]. The Pro12Ala substitution decreases the affinity of the PPARγ2 protein for its response elements in target genes and decreases its transcriptional activity by 50% [4,5]. From a physiological perspective, it might be predicted that the lower transcriptional activity of the Ala12 isoform of PPARγ2 would decrease insulin action because dominant negative mutations in human beings result in severe insulin resistance [6] and because pharmaceutical activation of PPARγ with thiazolidinediones increases insulin action in human beings [7]. Furthermore, it has recently been shown that insulin action is impaired in homozygous PPARγ2 knockout mice [3].

Studies on the association between PPARγ2 Pro12Ala genotype and insulin action in human beings have produced equivocal results. Some reports indicate that the Ala12 allele may be associated with greater insulin action and/or a lower risk for type 2 diabetes [4,8-10], while others indicate either

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the opposite [11,12] or no effect at all [13-16]. A potential explanation for these discrepant findings is that most of these studies did not account for the powerful and potentially confounding effect of physical activity levels on insulin action and type 2 diabetes risk [17]. Therefore, the purpose of the present study was to assess the relationship between the PPARγ2 Pro12Ala genotype and indices of insulin action in men and women before and after 6 months of supervised and standardized endurance exercise training. Because diet may also alter the relationship between the PPARγ2 genotype and insulin action [18], all subjects were trained by a registered dietician to consume a diet consistent with the American Heart Association dietary recommendations for the general population [19] and were required to be weight stable for 2 weeks before baseline testing. Lastly, because the relationship between the PPARγ2 Pro12Ala genotype and type 2 diabetes risk may be different for men and women [11], sex was included as a factor in our analyses.

2. Materials and methods

2.1. Subjects

Seventy-three sedentary, 50- to 75-year-old healthy men (n = 32) and women (n = 41) gave informed, written consent to participate in the present study, which was approved by the University of Maryland at the College Park Institutional Review Board. All subjects had normal glucose tolerance [20], and none was receiving medication known to affect glucose metabolism. Further details on subject screening and recruitment are published elsewhere [21].

2.2. Dietary control

For 6 weeks before baseline testing, all subjects attended twice weekly classes in which they were taught by a registered dietician to consume a diet consistent with the American Heart Association dietary recommendations for the general population [19]. Subjects were asked to maintain this diet throughout the duration of the study; compliance with the recommendations was monitored via 7-day diet records and food frequency questionnaires. Subjects who were noncompliant with the dietary recommendations during the study were counseled by a registered dietician until dietary compliance was again achieved.

2.3. Exercise intervention

The 6-month supervised endurance exercise training consisted of exercises such as treadmill walking and stationary cycling. Details of the training program have been published previously [21]. In brief, the exercise program gradually progressed over the first 10 weeks to 3 to 4 sessions of exercise per week for 40 minutes at 65% to 75% of heart rate reserve. Exercise heart rates were monitored with Polar heart rate monitors (Polar Beat model, Polar Electro Inc, Woodbury, NY).

2.4. Genotype

Subjects were genotyped by polymerase chain reaction/restriction fragment length polymorphism analysis of nuclear DNA from blood leukocytes according to procedures described by Nicklas et al [22] and Yen et al [23].

2.5. Dependent measures

Dependent data on subjects who attended at least 75% of the required exercise training sessions were collected. Training was continued until the last set of data was collected, and assessments at the end of the training intervention were made 24 to 36 hours after an exercise bout.

For the oral glucose tolerance test (OGTT), venous blood for glucose and insulin analyses was drawn into tubes containing 15% of potassium EDTA before and 30, 60, 90, 120, and 180 minutes after a 75-g oral glucose load. The samples were stored on ice for subsequent isolation of plasma via centrifugation (4°C and 1800*g* for 20 minutes), and the plasma was stored at −80°C. The OGTTs were started between 6:30 and 9:00 AM and after a 12- to 16-hour fast. Subjects consumed 250 g or more of carbohydrate per day for 3 days before each OGTT.

2.6. Body composition

Body fat mass, as a percentage of total body mass, was measured via whole-body dual-energy x-ray absorptiometry (model DPX-L, Lunar Corporation, Madison, Wis) as described elsewhere [24]. Body composition data were not available on 5 (2 homozygous Pro12 men, 2 homozygous Pro12 women, and 1 heterozygous man) of the 73 subjects.

2.7. Sample analysis

Glucose was analyzed using the glucose oxidase method, and insulin was determined by radioimmunoassay. All samples were run in duplicate, and when discrepancies between duplicate measures occurred (>0.1 mmol/L for glucose or a coefficient of variation of >0.10 for insulin), the sample was reassessed in a subsequent assay.

2.8. Calculations

Total areas under the curve (AUCs) were calculated for the OGTT plasma glucose and insulin responses using the trapezoidal rule. The product of the glucose AUC and insulin AUC ($AUC_{product}$) was calculated as an index of insulin resistance [25,26].

2.9. Maximum oxygen uptake

Maximum oxygen uptake (VO_2max) was determined by indirect calorimetry during an incremental treadmill exercise test to exhaustion as described elsewhere [27].

2.10. Statistics

Two-factor (genotype and sex) analyses of covariance were used for statistical analysis of outcome data. Data with

Table 1
Characteristics of subjects in the baseline, sedentary state

	Men		Women	
	Pro/Pro (n = 24)	Ala/Pro (n = 8)	Pro/Pro (n = 37)	Ala/Pro (n = 4)
Age (y)	59 ± 1	54 ± 2	59 ± 1	58 ± 3
Weight (kg)	84.6 ± 2.7*	89.3 ± 4.6*	76.8 ± 2.2	75.8 ± 6.6
Height (cm)	177 ± 1*	177 ± 2*	164 ± 1	164 ± 3
BMI (kg/m^2)	27 ± 1	29 ± 1	29 ± 1	28 ± 2
Percent body fat[a]	26.2 ± 1.4*	30.4 ± 2.6*	42.2 ± 1.1	41.3 ± 3.4
VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	29 ± 1*	28 ± 1*	23 ± 1	22 ± 1
Race				
White, n (%)	16 (67)	5 (62)	28 (76)	3 (75)
Black, n (%)	4 (17)	3 (38)	8 (22)	0 (0)
Other, n (%)	4 (17)	0 (0)	1 (3)	1 (25)

[a] Data are missing for 5 subjects. Data are least squares means ± SEM, except for race, where data are absolute and relative frequencies.
* $P \leq .05$ vs women of either genotype group.

nonnormally distributed residuals were log transformed for statistical analyses, and their resultant statistics (least squares means and SEMs) were reverse transformed for presentation. Age, body mass index (BMI), and their respective interactions with the PPARγ2 Pro12Ala genotype and sex were included in the initial model as covariates and were retained in the final model when their P values were $\geq .10$. Post hoc means comparisons were performed using protected least significant difference tests. A Fisher exact test was used to test for differences in the proportions of white and black subjects among the study groups. Spearman correlations were used for correlation analyses, and tests for homogeneity among correlation coefficients were performed using Fisher r-to-Z transformations as described elsewhere [28]. Analyses were performed at an α error rate of .05. Error terms are presented as SEM. SAS software (SAS version 8, SAS Institute, Inc, Cary, NC) was used for all analyses except for the tests for differences among correlation coefficients, which were done manually.

3. Results

Mean age for the 73 subjects at baseline was 58 ± 1 years. Baseline VO_2max was 25 ± 1 $mL \cdot kg^{-1} \cdot min^{-1}$, indicating that the subjects were sedentary, and baseline BMI was 28 ± 1, indicating that the subjects were overweight, on average. Fifty-two of the subjects classified themselves as whites, while the remainder classified themselves as blacks (n = 15) or members of other (n = 6) racial groups. Race was not used as a variable in the outcome analyses because none of the study outcomes was dependent on race (data not shown) and because the balance among the frequencies for whites, blacks, and "others" was similar among the study groups (Table 1; P = .24). Twenty-four men and 37 women were homozygous for the PPARγ2 Pro12 allele, 8 men and 4 women were heterozygous, and no subjects were homozygous for the Ala12 allele. These genotype frequencies were not different from the Hardy-Weinberg predicted frequencies for men, women, or for the group as a whole. At baseline, body weight, height, BMI, percent body fat, and VO_2max did not differ between genotype groups for either men or women (Table 1). Men were heavier and taller and had lower percent body fat and greater VO_2max values than women; however, these dimorphisms were not dependent on the PPARγ2 genotype.

At baseline, fasting insulin and insulin AUC from the OGTT were greater in PPARγ2 Pro12Ala heterozygous men than in Pro12 homozygous men or in women of either

Table 2
Results for subjects in the baseline, sedentary state

	Men		Women		P for interaction
	Pro/Pro	Ala/Pro	Pro/Pro	Ala/Pro	
Insulin AUC ($\times 10^3$ $pmol \cdot L^{-1} \cdot min^{-1}$)[a]	53 (4, 4)	98 (24, 19)*	50 (3, 3)	41 (9, 7)	.009
Glucose AUC ($\times 10^2$ $mmol \cdot L^{-1} \cdot min^{-1}$)	8.8 (0.4, 0.4)	8.8 (0.6, 0.6)	8.7 (0.3, 0.3)	8.0 (0.9, 0.9)	NS
$AUC_{product}$ ($\times 10^6$ units)[a]	45 (5, 5)	90 (30, 23)*	43 (8, 4)	33 (10, 8)	.02
Fasting insulin (pmol/L)[a]	79 (5, 5)	110 (21, 18)*	75 (4, 4)	54 (9, 8)	.01
Fasting glucose (mmol/L)	5.3 (0.1, 0.1)	5.7 (0.3, 0.3)	4.9 (0.3, 0.3)	5.0 (0.2, 0.2)	NS

[a] Data are least squares means (+ SEM, − SEM). NS indicates not significant at P = .05. Insulin AUC, $AUC_{product}$, fasting insulin, and fasting glucose data were compared after adjustment for age, baseline BMI, and an interaction between genotype and age. Glucose AUC data were compared after adjustment for baseline BMI. Residuals for insulin AUC, $AUC_{product}$, and fasting insulin were nonnormally distributed; data for these variables were therefore log transformed for statistical analyses, and their resultant statistics (least squares means and SEMs) were reverse transformed for presentation.
* $P < .01$ vs all other means.

Table 3
Endurance exercise training–induced changes

	Men		Women		*P* for interaction
	Pro/Pro	Ala/Pro	Pro/Pro	Ala/Pro	
Change in insulin AUC ($\times 10^3$ pmol·L^{-1}·min^{-1})	−10 ± 3	−39 ± 8*	−12 ± 2	−8 ± 7	.001
Change in glucose AUC ($\times 10^2$ mmol·L^{-1}·min^{-1})	0.0 ± 0.3	−0.5 ± 0.5	−0.1 ± 0.2	0.4 ± 0.7	NS
Change in $AUC_{product}$ ($\times 10^6$ units)	−9 ± 3	−38 ± 9*	−11 ± 3	−8 ± 8	.02
Change in fasting insulin (pmol/L)	−10 ± 4	−43 ± 10*	−10 ± 3	2 ± 9	.003
Change in fasting glucose (mmol/L)	0.1 ± 0.1	0.0 ± 0.2	0.1 ± 0.1	0.1 ± 0.3	NS
Change in weight (kg)	−1.9 ± 0.5	−1.5 ± 0.8	−1.0 ± 0.4	−0.1 ± 1.2	NS
Change in BMI (kg/m^2)	−0.6 ± 0.2	−0.5 ± 0.3	−0.4 ± 0.1	0.0 ± 0.4	NS
Change in percent body fat[a]	−1.4 ± 0.4	−0.3 ± 0.7	−1.5 ± 0.3	−0.2 ± 0.9	NS
Change in V_{O_2}max (% increase in mL·kg^{-1}·min^{-1})	19 ± 2	21 ± 4	13 ± 2	22 ± 5	NS

[a] Data are missing for 5 subjects. Data are least squares means (± SEM). Changes in insulin AUC and changes in $AUC_{product}$ were compared after adjustment for age, change in BMI, and an interaction between genotype and age. Changes in glucose AUC were compared after adjustment for changes in BMI. Changes in fasting insulin were compared after adjustment for age and an interaction between genotype and age.

* $P < .05$ vs all other means.

genotype group (Table 2). Because the means for OGTT glucose AUC were not different at baseline between genotypes in men, the differences in insulin AUC suggest greater insulin resistance in PPARγ2 Ala12 carriers. Furthermore, $AUC_{product}$ was 2-fold greater in Ala12 carrier men. None of the outcomes were different between genotypes in women at baseline.

Exercise training increased V_{O_2}max (initial = 26 ± 1 mL·kg^{-1}·min^{-1}; final = 30 ± 1 mL·kg^{-1}·min^{-1}; $P < .0001$) and decreased total body weight (initial = 82.3 ± 2.1 kg; final = 81.1 ± 2.1 kg; $P = .002$), BMI (initial = 28.2 ± 0.6 kg/m^2; final = 27.8 ± 0.6 kg/m^2; $P = .004$), and percent body fat (initial = 35.7 ± 1.2; final = 34.5 ± 1.2; $P \leq .0001$) for the group as a whole. These exercise training–induced changes were not different between PPARγ2 genotype groups, between men and women, or among genotype- and sex-specific groups (Table 3), although there was a tendency for greater decreases in percent body fat among homozygous Pro12 carriers (men and women combined) vs noncarriers (−1.4 ± 0.2 and − 0.2 ± 0.6, respectively; $P = .06$).

Decreases in fasting insulin and insulin AUC in response to endurance training were 4-fold greater in Pro12Ala heterozygous men as compared with Pro12 homozygous men; no such genotype-specific effects of exercise training were found in women (Table 3). Fasting glucose and glucose AUC remained unchanged in all study groups despite the sex- and genotype-specific changes in insulin. The decrease in $AUC_{product}$ was 4-fold greater in Pro12Ala men as compared with homozygous men and with women of either genotype.

For the group as a whole, the changes in body weight were correlated with changes in insulin AUC ($r = 0.42$; $P = .0002$) and glucose AUC ($r = 0.27$; $P = .02$). Likewise, the magnitude of change in BMI was correlated with changes in insulin AUC ($r = 0.43$; $P = .0001$) and glucose AUC ($r = 0.29$; $P = .01$). These correlations did not differ among the 4 study groups (all P values were ≥.34); however, it should be noted that tests for differences among correlation coefficients generally require sample sizes that are larger than those for 2 of the groups (Ala12 carrier men and women) in the present study [28]. After the 6-month exercise training program, none of the fasting or OGTT-based outcomes differed among the study groups, which indicates that all of the baseline differences were eliminated (Table 4).

Table 4
Results for subjects in the final, trained state

	Men		Women		*P* for interaction
	Pro/Pro	Ala/Pro	Pro/Pro	Ala/Pro	
Insulin AUC ($\times 10^3$ pmol·L^{-1}·min^{-1})	42 (4, 3)	51 (8, 7)	40 (3, 3)	35 (8, 6)	NS
Glucose AUC ($\times 10^2$ mmol·L^{-1}·min^{-1})	8.9 (0.4, 0.4)	8.5 (0.7, 0.7)	8.7 (0.3, 0.3)	7.8 (1.0, 1.0)	NS
$AUC_{product}$ ($\times 10^6$ units)	37 (4, 4)	41 (8, 7)	35 (3, 3)	28 (8, 6)	NS
Fasting insulin (pmol/L)	71 (4, 4)	64 (6, 6)	66 (3, 3)	57 (8, 7)	NS
Fasting glucose (mmol/L)	5.3 (0.1, 0.1)	5.7 (0.3, 0.3)	5.0 (0.1, 0.1)	5.1 (0.3, 0.3)	NS

Data are least squares means (+SEM, − SEM). Insulin AUC and fasting insulin data were compared after adjustment for final BMI. Glucose AUC data were compared after adjustment for age, final BMI, and an interaction between sex and final BMI. $AUC_{product}$ data were compared after adjustment for final BMI and an interaction between sex and final BMI. Fasting glucose data were compared after adjustment for age and an interaction between age and genotype. Residuals for insulin AUC, $AUC_{product}$, and fasting insulin were nonnormally distributed; data for these variables were therefore log transformed for statistical analyses, and their resultant statistics (least squares means and SEMs) were reverse transformed for presentation.

4. Discussion

Many studies have assessed the relationship between indices of insulin action or type 2 diabetes risk and the PPARγ2 Pro12Ala genotype; however, the results are equivocal. Some of the variability among previous reports may be because of a dependency of the association between the PPARγ2 genotype and insulin action on exercise training status [29], diet [18], and sex [11]. In the present study, subjects consumed a standardized diet, and sex- and genotype-specific associations with indices of insulin action were determined before and after supervised, standardized exercise training. The main finding from the present study is that sedentary PPARγ2 Pro12Ala heterozygous men have greater fasting and OGTT insulin concentrations than Pro12 homozygous men and women of either genotype. In response to exercise training, Pro12Ala heterozygous men experience greater decreases in fasting insulin and insulin AUC than Pro12 homozygous men despite similar changes in body weight, BMI, and percent body fat. Because these differences in the fasting and OGTT insulin among study groups occurred in the absence of differences in fasting and OGTT glucose, differences in insulin action are likely.

Kahara et al also assessed the association of the PPARγ2 Pro12Ala genotype with insulin action before and after endurance exercise training [29]. While no association between the PPARγ2 Pro12Ala genotype and insulin action was found in the initially sedentary Japanese men, the training-induced changes in insulin action were greater in heterozygous men as compared with Pro12 homozygous men, which is consistent with the findings of the present study. It is not clear why Kahara et al [29] did not find a PPARγ2 genotype dependency of insulin action among sedentary subjects while the present study did. One possibility is that the subjects in the present study were more overweight and older and, consequently, at greater risk for developing insulin resistance and type 2 diabetes than those studied by Kahara et al [29]. Alternatively, racial differences could explain the discrepancy between the studies because the cause of diabetes in Japanese Americans may be different from that found in other subjects [30]. Lastly, differences in dietary fat intake between the groups could account for the genotype-specific differences in baseline characteristics between studies [18].

Although no studies other than that of Kahara et al [29] and the present study specifically assessed the relationship between the PPARγ2 Pro12Ala genotype and endurance training–induced changes in insulin action, two other lifestyle modification studies warrant discussion. First, Nicklas et al [22] assessed the association between the PPARγ2 Pro12Ala genotype and insulin action in sedentary, overweight or obese, postmenopausal women. Consistent with our findings, genotype was not associated with insulin action at baseline in sedentary women. In response to diet-induced weight loss, however, Ala12 carrier women experienced 2-fold greater improvements in insulin action as compared with Pro12 homozygous women, despite similar changes in body weight and composition. Although it is not clear why restriction of energy intake in women resulted in genotype-specific improvements in insulin action while exercise training–induced did not, it is perhaps appropriate to consider diet- and exercise-induced improvements in insulin action as distinct phenotypes.

In a study from the Finnish Diabetes Prevention Study, Lindi et al [12] reported that in sedentary men and women with impaired glucose tolerance, PPARγ2 Ala12 allele carriers had a greater 3-year incidence of type 2 diabetes compared with noncarriers. Although an increased incidence of type 2 diabetes in Ala12 carriers seems contrary to the findings of others [10], an intervention consisting of exercise and diet modification fully abrogated the ill effect of the Ala12 allele on diabetes incidence. If poor insulin action is considered to be a risk factor for type 2 diabetes, our results are consistent with those of Lindi et al [12] in that lifestyle modifications such as exercise training may reduce the genetic predisposition to type 2 diabetes.

A limitation of the present study is the presence of only 4 women who were PPARγ2 Ala12 carriers. Conceivably, this could have led to a false-negative result (type II statistical error) for genotype-dependent differences in women. Power calculations are difficult in these circumstances because the genotype effect size for training-induced changes in insulin AUC for women is unknown. If the effect size seen in men in the present study is used as an estimate for the effect size in women (which assumes that men and women do not differ with respect to training-induced changes in insulin AUC), the power to detect a statistical difference between genotype groups in women is 0.98, which suggests that our findings are valid. Furthermore, if the 4 Ala12 carrier women are considered in the context of all women in the present study, it is noteworthy that 2 were above the 50th percentile and 2 were below the 50th percentile with respect to training-induced changes in insulin AUC. Taken together, although a type II statistical error cannot be ruled out, the findings in the present study appear to be valid.

In summary, sedentary men who were heterozygous for the PPARγ2 Pro12Ala genotype had greater fasting and OGTT insulin concentrations as compared with Pro12 homozygous men. Endurance training in men resulted in significantly greater improvement in insulin concentrations in Pro12Ala heterozygotes compared with Pro12 homozygotes, such that no differences in fasting and OGTT insulin concentrations existed between genotypes after endurance training. Lastly, the baseline, final, and training-induced changes in the plasma glucose response to glucose ingestion were not different among sex- and genotype-specific groups. Taken together, these findings suggest that sedentary men who are heterozygous for the PPARγ2 Pro12Ala genotype have lower insulin action relative to their counterparts but are more responsive to the insulin-sensitizing effect of endurance exercise training.

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References

[1] Vidal-Puig AJ, Considine RV, Jimenez-Linan M, et al. Peroxisome proliferator–activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. J Clin Invest 1997;99:2416-22.

[2] Tamori Y, Masugi J, Nishino N, et al. Role of peroxisome proliferator–activated receptor-gamma in maintenance of the characteristics of mature 3T3-L1 adipocytes. Diabetes 2002;51:2045-55.

[3] Zhang J, Fu M, Cui T, et al. Selective disruption of PPARgamma2 impairs the development of adipose tissue and insulin sensitivity. Proc Natl Acad Sci U S A 2004;101:10703-8.

[4] Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 1998; 20:284-7.

[5] Masugi J, Tamori Y, Mori H, et al. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator–activated receptor-gamma 2 on thiazolidinedione-induced adipogenesis. Biochem Biophys Res Commun 2000;268:178-82.

[6] Savage DB, Tan GD, Acerini CL, et al. Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator–activated receptor-gamma. Diabetes 2003;52: 910-7.

[7] Tonelli J, Li W, Kishore P, et al. Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. Diabetes 2004;53:1621-9.

[8] Ek J, Andersen G, Urhammer SA, et al. Studies of the Pro12Ala polymorphism of the peroxisome proliferator–activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant Caucasians. Diabetologia 2001;44:1170-6.

[9] Hara K, Okada T, Tobe K, et al. The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. Biochem Biophys Res Commun 2000;271:212-6.

[10] Altshuler D, Hirschhorn JN, Klannemark M, et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 2000;26:76-80.

[11] Hegele RA, Cao H, Harris SB, et al. Peroxisome proliferator–activated receptor-gamma2 P12A and type 2 diabetes in Canadian Oji-Cree. J Clin Endocrinol Metab 2000;85:2014-9.

[12] Lindi VI, Uusitupa MI, Lindstrom J, et al. Association of the Pro12Ala polymorphism in the PPAR-gamma2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. Diabetes 2002;51:2581-6.

[13] Ringel J, Engeli S, Distler A, et al. Pro12Ala missense mutation of the peroxisome proliferator activated receptor gamma and diabetes mellitus. Biochem Biophys Res Commun 1999;254:450-3.

[14] Mancini FP, Vaccaro O, Sabatino L, et al. Pro12Ala substitution in the peroxisome proliferator–activated receptor-gamma2 is not associated with type 2 diabetes. Diabetes 1999;48:1466-8.

[15] Oh EY, Min KM, Chung JH, et al. Significance of Pro12Ala mutation in peroxisome proliferator–activated receptor-gamma2 in Korean diabetic and obese subjects. J Clin Endocrinol Metab 2000;85:1801-4.

[16] Poirier O, Nicaud V, Cambien F, et al. The Pro12Ala polymorphism in the peroxisome proliferator–activated receptor gamma2 gene is not associated with postprandial responses to glucose or fat tolerance tests in young healthy subjects: the European Atherosclerosis Research Study II. J Mol Med 2000;78:346-51.

[17] Ivy JL, Zderic TW, Fogt DL. Prevention and treatment of non–insulin-dependent diabetes mellitus. Exerc Sport Sci Rev 1999;27:1-35.

[18] Luan J, Browne PO, Harding AH, et al. Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes 2001;50:686-9.

[19] Krauss RM, Eckel RH, Howard B, et al. AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000;102:2284-99.

[20] Anonymous. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2002;25: 5S-20S.

[21] Wilund KR, Ferrell RE, Phares DA, et al. Changes in high-density lipoprotein-cholesterol subfractions with exercise training may be dependent on cholesteryl ester transfer protein (CETP) genotype. Metabolism 2002;51:774-8.

[22] Nicklas BJ, van Rossum EF, Berman DM, et al. Genetic variation in the peroxisome proliferator–activated receptor-gamma2 gene (Pro12Ala) affects metabolic responses to weight loss and subsequent weight regain. Diabetes 2001;50:2172-6.

[23] Yen CJ, Beamer BA, Negri C, et al. 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 1997; 241:270-4.

[24] Mazess RB, Barden HS, Bisek JP, et al. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr 1990;51:1106-12.

[25] Levine R, Haft DE. Carbohydrate homeostasis (second of two parts). N Engl J Med 1970;283:237-46.

[26] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-70.

[27] Dengel DR, Hagberg JM, Coon PJ, et al. Effects of weight loss by diet alone or combined with aerobic exercise on body composition in older obese men. Metabolism 1994;43:867-71.

[28] Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research. 3rd ed. New York: W.H. Freeman and Company; 1995.

[29] Kahara T, Takamura T, Hayakawa T, et al. PPARgamma gene polymorphism is associated with exercise-mediated changes of insulin resistance in healthy men. Metabolism 2003;52:209-12.

[30] Chen KW, Boyko EJ, Bergstrom RW, et al. Earlier appearance of impaired insulin secretion than of visceral adiposity in the pathogenesis of NIDDM. 5-Year follow-up of initially nondiabetic Japanese-American men. Diabetes Care 1995;18:747-53.