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The Pro12Ala polymorphism of the PPARγ2 gene is associated with hepatic glucose uptake during hyperinsulinemia in subjects with type 2 diabetes mellitus

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Abstract

The Ala12 allele of the peroxisome proliferator–activated receptor γ gene (*PPARG2*) has been associated with reduced risk of type 2 diabetes mellitus (T2DM) and increased whole-body and skeletal muscle insulin sensitivity in nondiabetic subjects. The effect of the Pro12Ala polymorphism on tissue specific insulin sensitivity in subjects with T2DM has not been previously investigated. We studied the effect of the Pro12Ala polymorphism on the rates of whole-body, skeletal muscle, and subcutaneous adipose tissue glucose uptake (GU) in T2DM subjects, and the rates of hepatic GU in nondiabetic and T2DM subjects during hyperinsulinemia. Our study included 105 T2DM subjects whose whole-body, skeletal muscle, subcutaneous adipose tissue, and hepatic GUs were measured using ^{18}F-fluorodeoxyglucose and positron emission tomography during the hyperinsulinemic euglycemic clamp. Hepatic GU was also measured in 68 nondiabetic subjects. In obese (body mass index ≥27 kg/m^2) subjects with T2DM, the rate of hepatic GU was 28% lower in subjects with the Pro12Pro genotype than in carriers of the Ala12 allele (P = .001); and a similar trend was observed in nondiabetic obese subjects (P = .137). No effect of the Pro12Ala polymorphism on the rates of whole-body, skeletal muscle, or subcutaneous adipose tissue GU was observed in T2DM subjects. We conclude that the Ala12 allele of *PPARG2* is associated with higher hepatic GU in obese subjects with T2DM.

1. Introduction

Three isoforms of the peroxisome proliferator–activated receptor (PPAR) have been identified as major players in transcriptional control of glucose and fatty acid metabolism [1]. The PPARγ gene (*PPARG2*) produces 2 different proteins: PPARγ1 and PPARγ2. PPARγ2 is expressed almost exclusively in adipose tissue; however, small amounts of PPARγ2 can be found in other tissues such as skeletal muscle, liver, and pancreatic β-cells [1].

The Pro12Ala polymorphism of *PPARG2* has been associated with reduced receptor activity and better insulin sensitivity [2], whereas the more common Pro12Pro genotype has been associated with increased risk of type 2 diabetes mellitus (T2DM) [3]. Meta-analyses have shown about 1.25-fold increase in the risk of T2DM in subjects carrying the Pro12Pro genotype [3]. Increase in the relative risk of T2DM associated with the Pro12Pro genotype is considerably higher in nonobese subjects than in obese subjects. However, the mechanisms behind the insulin-sensitizing effect of the Pro12Ala polymorphism in different insulin-sensitive tissues have remained unclear. Possible mechanisms could include direct actions of PPARγ2 on the transcription of genes encoding proteins of the insulin

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signaling pathway. Other possible mechanisms involve indirect effects of free fatty acids (FFAs) and adipokines that are associated with insulin resistance and whose secretion from adipose tissue is controlled by PPARγ [4].

Liver regulates glucose homeostasis through a balance between hepatic glucose uptake (GU) and hepatic glucose production. Both hepatic GU and insulin suppression of hepatic glucose production are decreased in T2DM [5]. Free fatty acids and several adipokines are implicated in the regulation of hepatic insulin sensitivity. Because PPARγ regulates FFA and adipokine levels, it could potentially modulate hepatic insulin sensitivity as well [6,7].

We have previously reported that the Ala12 allele of the Pro12Ala polymorphism of *PPARG2* is associated with increased skeletal muscle and whole-body insulin sensitivity in nondiabetic nonobese, but not in obese, subjects [8]. The effect of the Pro12Ala polymorphism on skeletal muscle and subcutaneous adipose tissue glucose GU has not been previously studied in subjects with T2DM. Therefore, we also investigated the effect of the Pro12Ala polymorphism of *PPARG2* on the rates of whole-body, skeletal muscle, subcutaneous adipose tissue, and hepatic GU in obese and nonobese subjects with T2DM. In addition, the effect of the Pro12Ala polymorphism on hepatic GU in nondiabetic subjects was determined.

2. Materials and methods

2.1. Subjects

A total of 173 volunteers (68 nondiabetic and 105 subjects with T2DM), who had previously participated in positron emission tomography (PET) studies at Turku PET Center (Turku, Finland), were recruited for this study [9-10]. Fifty-one subjects in the nondiabetic study group were the same as in our previous study [8]. *Type 2 diabetes mellitus* was defined by the World Health Organization criteria [11]. Forty-six subjects with T2DM were receiving metformin, sulfonylurea, or both of these drugs. None of the subjects with T2DM was on insulin or thiazolidinedione treatment. Subjects were divided into 2 groups (nonobese and obese) using a cutoff point of 27 kg/m^2 for body mass index (BMI) as in our previous study [8]. All subjects gave a written informed consent. The study protocol was approved by the Ethics Committee of the Hospital District of South-West Finland (Turku, Finland).

2.2. Study design

Studies were performed after an overnight fast. The consumption of alcohol and caffeine was prohibited 12 hours before the study, and subjects were instructed to avoid strenuous physical activity 1 day before the study. The subjects were lying in a supine position throughout the PET scanning. Two cannulas were inserted: one in an antecubital vein for the infusion of glucose and insulin and the injection of ^{18}F-fluorodeoxyglucose ([^{18}F]FDG), and another in either the radial artery or the antecubital vein of the opposite upper extremity that was warmed with a heating pillow to arterialize venous blood. At 0 minute, an intravenous infusion of insulin (a priming dose for 7 minutes, followed by an insulin infusion of 1 mU·kg^{-1}·min^{-1}) was started for 140 ± 20 minutes. To determine the input function, arterial or arterialized blood samples were drawn for the measurement of plasma radioactivity. ^{18}F-fluorodeoxyglucose was injected at 90 ± 30 minutes, and dynamic scans were performed to obtain images of the liver (18-50 minutes) and the femoral region (20-30 minutes) as previously described [8,10]. Fifty-seven subjects performed intermittent isometric exercise with 1 leg during the scan; but only the measurements of the noncontracting, that is, control, leg were used in this report. The whole-body insulin sensitivity was assessed using the hyperinsulinemic euglycemic clamp technique [12]. The levels of insulin and FFAs were determined every 30 or 60 minutes, respectively. Blood samples for DNA analyses were drawn, and DNA analyses were performed at the University of Kuopio.

2.3. Production of PET tracers

^{18}F-fluorodeoxyglucose was synthesized with a computer-controlled apparatus according to a modified method of Hamacher et al [13].

2.4. Image acquisition and processing

Eight-ring ECAT 931/08 (Siemens/CTI, Knoxville, TN) and GE Advance (General Electric Medical Systems, Milwaukee, WI) tomographs were used. Photon attenuation was corrected by transmission scans on the femoral and abdominal region with a removable ring source containing ^{68}Ge. All data obtained were corrected for dead time, decay, and measured photon attenuation and were reconstructed into a 128 × 128 matrix. The Bayesian iterative reconstruction algorithm, using median root prior with iterations and a Bayesian coefficient of 0.3, was used for image processing when possible. A region of interest was drawn in the anteromedial muscular compartment of the femoral region in 4 planes in both legs as previously described [8]. Large blood vessels were avoided when outlining the regions. Regions of interest in femoral subcutaneous adipose tissue were drawn as previously described [14]. Free-shaped regions of interest were drawn on multiple planes in the right lobe of the liver avoiding large blood vessels as previously described [15].

2.5. Measurements of hepatic, skeletal muscle, and adipose tissue GU

Rates of hepatic, femoral muscle, and adipose tissue GU were calculated using the 3-compartment model of [^{18}F]FDG kinetics [16]; and plasma and tissue time-activity curves were graphically analyzed to quantitate the fractional phosphorylation rate (K_i) for the tracer [17]. The GU rates were obtained by multiplying K_i by the plasma glucose concentration divided by a lumped constant, which accounts

for the differences in transportation and phosphorylation of [^{18}F]FDG and glucose. A lumped constant value of 1.0 for liver, 1.2 for skeletal muscle, and 1.14 for adipose tissue was used, as previously described [18-20].

2.6. Measurement of whole-body GU

The rates of whole-body GU were measured independently of the PET measurements with the hyperinsulinemic euglycemic clamp technique [12]. In nondiabetic and diabetic subjects, euglycemia (plasma glucose, ~5 mmol/L) was maintained using a variable rate of 20% glucose infusion based on arterial plasma glucose measurements taken every 10 minutes. The rates of whole-body GU (*M* value; micromoles per kilogram per minute) were calculated between 60 and 120 minutes of hyperinsulinemia.

2.7. Biochemical analyses

Plasma glucose was determined in duplicate by the glucose oxidase method. Serum insulin concentration, determined every 30 minutes during the clamp, was measured by immunoassay; and serum FFA concentration was measured by a fluorometric method, as previously reported [8].

2.8. Genotyping of the Pro12Ala polymorphism of PPARG2

DNA samples were available from all subjects. Genotyping was performed using TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, CA). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 2700 (50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute), and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems). Genotyping success rate was 100% and error rate was 0% in 7.3% of samples regenotyped.

2.9. Statistical methods

All calculations were performed with the SPSS/Win statistical program (version 13.0 for Windows; SPSS, Chicago, IL). All data are represented as the mean ± SD. Weight, BMI, insulin and glucose concentrations, and hepatic GU were log transformed before statistical analysis to achieve normal distribution. Sex distributions were compared with either Pearson χ^2 test or Fisher exact test, when appropriate. Adjustment for sex, age, the use of oral antidiabetic drug treatment (none coded as 0, metformin coded as 1, sulfonylurea coded as 2, metformin and sulfonylurea coded as 3), and exercise during the clamp (no exercise coded as 0, exercise coded as 1) was performed with the analysis of covariance when comparing the genotype groups. *P* less than .05 was considered statistically significant.

3. Results

3.1. Clinical and laboratory characteristics

Frequency of the Ala12 allele was 0.13 in the diabetic subjects group and 0.19 in the nondiabetic subjects group

Table 1
Clinical and laboratory characteristics according to the Pro12Ala polymorphism of *PPARG2* in nonobese and obese nondiabetic and diabetic subjects

Nondiabetic subjects	BMI <27 kg/m^2		P^a	BMI ≥27 kg/m^2		P^a
	Pro12Pro	Ala12 allele		Pro12Pro	Ala12 allele	
n (men/women)	27 (21/6)	11 (9/2)	1.000	18 (10/8)	12 (5/7)	.456
Age (y)	34.3 ± 10.6	33.4 ± 10.2	.811	41.2 ± 11.4	45.4 ± 9.2	.292
BMI (kg/m^2)	23.2 ± 1.9	24.4 ± 1.8	.057	31.8 ± 3.9	31.8 ± 4.0	.893
Fasting						
Glucose (mmol/L)	5.2 ± 0.3	5.3 ± 0.4	.541	5.3 ± 0.4	5.6 ± 0.4	.043
Insulin (pmol/L)	31.9 ± 14.6	34.6 ± 15.3	.755	58.3 ± 30.1	52.5 ± 17.3	.652
FFA						
Fasting FFA (mmol/L)	0.52 ± 0.20	0.59 ± 0.15	.289	0.82 ± 0.25	0.70 ± 0.24	.091
During clamp FFA (mmol/L)	0.09 ± 0.06	0.12 ± 0.07	.345	0.11 ± 0.07	0.05 ± 0.02	.171
Diabetic subjects	**BMI <27 kg/m^2**		**P^b**	**BMI ≥27 kg/m^2**		**P^b**
	Pro12Pro	Ala12 allele		Pro12Pro	Ala12 allele	
n (men/women)	19 (15/4)	8 (7/1)	1.000	60 (38/22)	18 (13/5)	.487
Age (y)	63.5 ± 7.0	65.5 ± 11.5	.575	60.4 ± 7.5	60.4 ± 7.4	.969
BMI (kg/m^2)	25.1 ± 1.6	24.4 ± 1.3	.335	31.4 ± 3.4	31.6 ± 2.9	.691
Fasting						
Glucose (mmol/L)	7.6 ± 1.6	6.7 ± 1.3	.247	7.8 ± 1.8	8.1 ± 2.1	.495
Insulin (pmol/L)	36.1 ± 11.0	31.4 ± 12.3	.157	57.9 ± 30.8	72.1 ± 50.2	.136
FFA						
Fasting FFA (mmol/L)	0.70 ± 0.30	0.52 ± 0.30	.567	0.69 ± 0.26	0.69 ± 0.19	.944
During clamp FFA (mmol/L)	0.11 ± 0.04	0.11 ± 0.05	.825	0.16 ± 0.08	0.17 ± 0.09	.601

Data are presented as mean ± standard deviation.

[a] *P* values are adjusted for age, sex, and exercise status during clamp where appropriate.

[b] *P* values are adjusted for age, sex, medication class, and exercise status during clamp where appropriate.

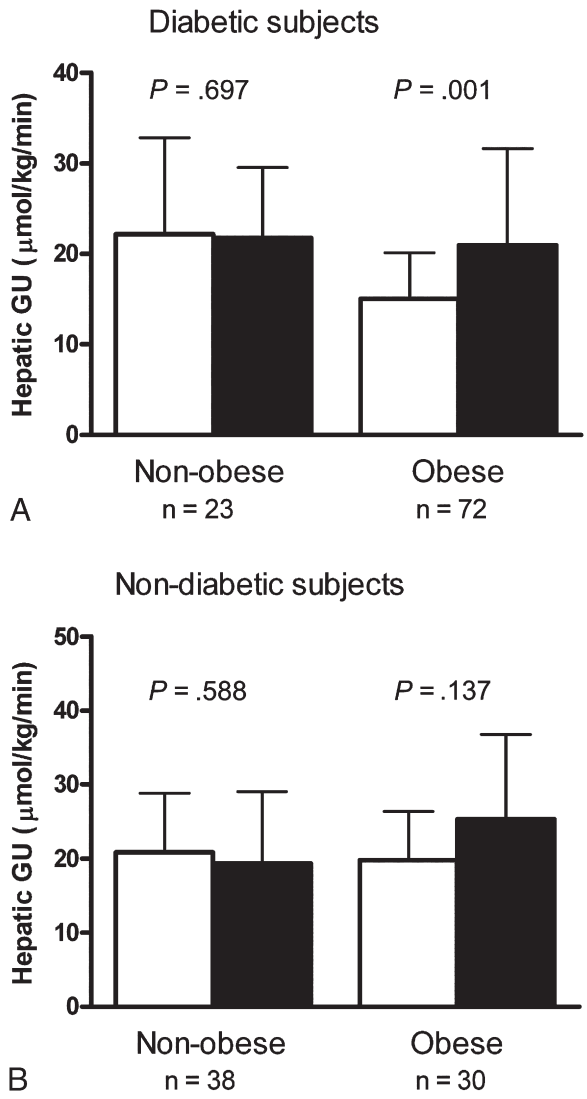

Fig. 1. The effect of the Pro12Ala polymorphism of *PPARG2* on the rates of hepatic GU in nonobese and obese diabetic (A) and nondiabetic (B) subjects (analysis of covariance; adjusted for sex, age, the use of oral antidiabetic drug treatment, and exercise during the clamp in diabetic subjects, and adjusted for sex, age, and exercise during the clamp in nondiabetic subjects). White bars indicate the Pro12Pro genotype carriers; black bars indicate carriers of the Ala12 allele. Error bars indicate SD.

(*P* = not significant). The genotype distribution was in the Hardy-Weinberg equilibrium. Because there was a significant interaction between BMI as a continuous variable and the effects of the Pro12Ala polymorphism on hepatic GU (*P* = .001), the results are presented separately for obese (BMI ≥27 kg/m^2) and nonobese (BMI <27.0 kg/m^2) subjects (*P* = .010 for interaction using 27.0 kg/m^2 as a cutoff point). Fasting glucose level was higher in carriers of the Ala12 allele compared with carriers of the Pro12Pro genotype among obese nondiabetic subjects (*P* = .043) (Table 1). There were no other statistically significant differences between the genotype groups among diabetic or nondiabetic subjects. Glucose level during the euglycemic

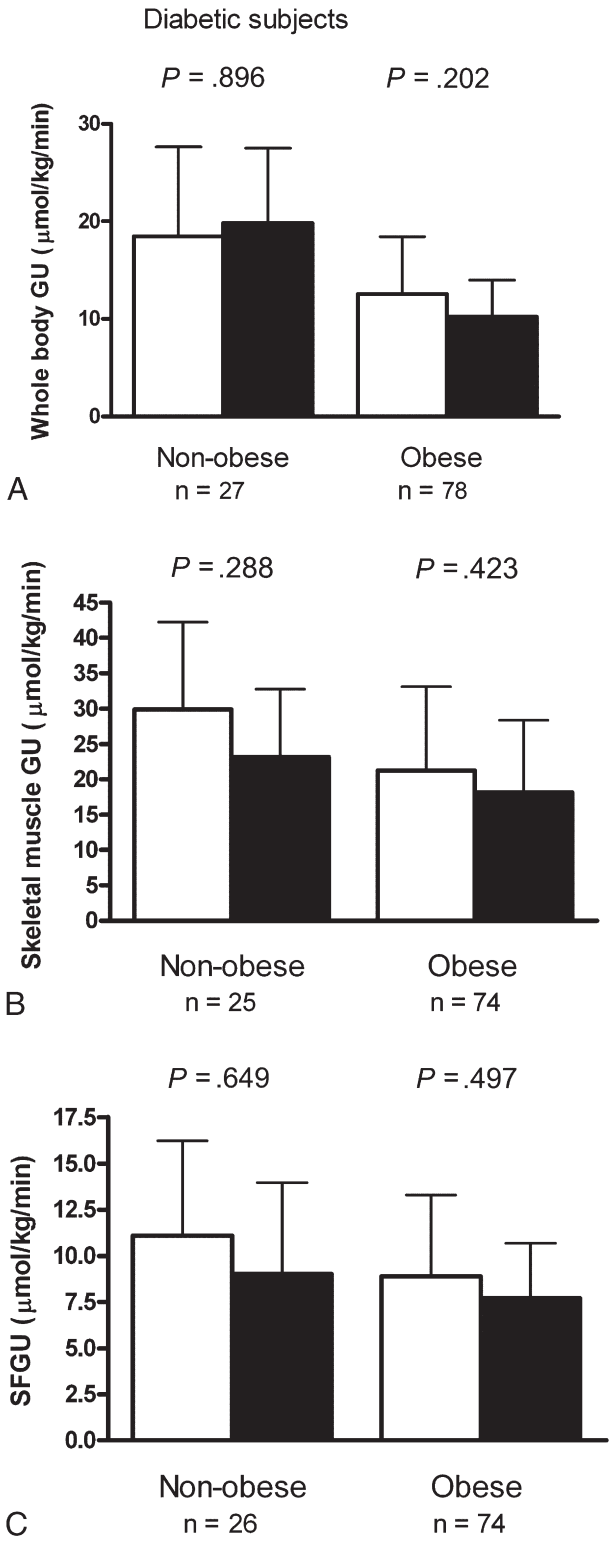

Fig. 2. The effect of the Pro12Ala polymorphism of *PPARG2* on the rates of whole-body (A), skeletal muscle (B), and subcutaneous adipose tissue (C) GU in nonobese and obese diabetic subjects (analysis of covariance; adjusted for sex, age, the use of oral antidiabetic drug treatment, and exercise during the clamp). White bars indicate the Pro12Pro genotype carriers; black bars indicate carriers of the Ala12 allele. Error bars indicate SD.

hyperinsulinemic clamp was 5.1 ± 0.4 mmol/L, and insulin level was 436 ± 97 pmol/L.

3.2. Hepatic GU

In obese subjects with T2DM, hepatic GU was 28% lower in carriers of the Pro12Pro genotype compared with carriers of the Ala12 allele (15.1 ± 5.1 vs 21.0 ± 10.6 μmol per kilogram of liver tissue per minute, $P = .001$, Fig. 1A), whereas in obese nondiabetic subjects, the 22% difference in hepatic GU did not reach statistical significance (19.8 ± 6.6 vs 25.3 ± 11.5 μmol per kilogram of liver tissue per minute, $P = .137$, Fig. 1B). There were no differences in hepatic GU between carriers of the Ala12 allele and subjects with the Pro12Pro genotype in nonobese subjects with T2DM (21.8 ± 7.8 vs 22.2 ± 10.6 μmol per kilogram of liver tissue per minute, Fig. 1A) or in nonobese nondiabetic subjects (19.4 ± 9.7 vs 20.8 ± 8.0 μmol per kilogram of liver tissue per minute, Fig. 1B).

3.3. Skeletal muscle, subcutaneous adipose tissue, and whole-body GU

There were no statistically significant differences in skeletal muscle GU between carriers of the Ala12 allele and subjects with the Pro12Pro genotype in nonobese (23.2 ± 9.5 vs 29.9 ± 12.4 μmol per kilogram of muscle tissue per minute) or in obese diabetic subjects (18.1 ± 10.3 vs 21.2 ± 11.9 μmol per kilogram of muscle tissue per minute) (Fig. 2B). There were no differences in subcutaneous adipose tissue GU between subjects with the Ala12 allele and subjects with the Pro12Pro genotype in nonobese (9.0 ± 5.0 vs 11.1 ± 5.2 μmol per kilogram of adipose tissue per minute) or in obese diabetic subjects (7.7 ± 3.0 vs 8.9 ± 4.4 μmol per kilogram of adipose tissue per minute) (Fig. 2C). In nonobese and obese diabetic subjects, the rates of whole-body GU did not differ between carriers of the Ala12 allele and subjects with the Pro12Pro genotype (19.8 ± 7.6 vs 18.5 ± 9.2 μmol per kilogram of body weight per minute and 10.2 ± 3.8 vs 12.6 ± 5.9 μmol per kilogram of body weight per minute, respectively) (Fig. 2A). The results remained practically unchanged if the rates of whole-body GU were expressed per body surface area instead of body weight.

4. Discussion

In this study, we investigated the effect of the Pro12Ala polymorphism of *PPARG2* on the rates of hepatic, skeletal muscle, subcutaneous adipose tissue, and whole-body GU in subjects with T2DM. We found a significant interaction between the effects of the Pro12Ala polymorphism and BMI on tissue and whole-body level of insulin sensitivity, as has been previously reported [8,21]; and therefore, the results were given separately for obese (BMI ≥27 kg/m^2) and nonobese (BMI <27.0 kg/m^2) subjects. The novel finding of our study was that carriers of the Ala12 allele had higher hepatic GU compared with carriers of the Pro12Pro genotype in obese subjects with T2DM, even after the adjustment for the use of oral drug treatment of diabetes (including the use of metformin that can modify both hepatic GU and hepatic glucose production). In contrast, we did not find differences in the rates of whole-body, skeletal muscle, or subcutaneous adipose tissue GU between carriers of the Ala12 allele and carriers of the Pro12Pro genotype in diabetic subjects. Therefore, the insulin-sensitizing effect of the Ala12 allele of *PPARG2* on skeletal muscle GU is either lost or masked by insulin resistance in T2DM.

Insulin is effective at stimulating hepatic GU under hyperglycemia and, to a lesser extent, during euglycemia. During euglycemic hyperinsulinemic clamp, skeletal muscle accounts for about 80% of the whole-body GU, whereas the contribution of hepatic GU is less than 10% [22]. Obesity per se is a major determinant of hepatic glucose output [23], whereas increased hepatic GU lowers glucose levels. Therefore, an increase in hepatic GU in obese carriers of the Ala12 allele in our study could be a compensatory mechanism to achieve better glycemic control because the liver is a major contributor to postprandial glucose disposal. In agreement with our findings, increased hepatic insulin sensitivity has been previously reported in carriers of the Ala12 allele [24]. We did not measure hepatic glucose production; but in this previous study [24], endogenous glucose production was 40% lower in carriers of the Ala12 allele compared with carriers of the Pro12Pro genotype during euglycemic hyperinsulinemic clamp.

Adipose tissue is a metabolically active organ that releases FFAs and adipose tissue–derived cytokines and adipokines into the blood circulation. Adiponectin and leptin have been associated with increased insulin sensitivity, whereas FFAs and tumor necrosis factor–α, interleukin-6, and resistin have been constantly associated with decreased insulin sensitivity; and their release is thought to be controlled by PPARγ [4]. A previous study reported higher suppression of FFA levels during the euglycemic hyperinsulinemic clamp among carriers of the Ala12 allele, suggesting better insulin suppression of lipolysis in adipose tissue [25]. However, we did not observe significant differences in FFA levels among obese subjects with T2DM according to the Pro12Ala polymorphism. Furthermore, in subjects without T2DM, the differences in FFA levels between the genotypes were lost after the adjustment for confounding factors. Therefore, differences in circulating FFAs are unlikely to explain the differences in hepatic GU between carriers of the Ala12 allele and the Pro12Pro genotype in our study, although it has been proposed that peripheral FFA concentrations may not reflect hepatic FFA delivery in the context of visceral obesity [26].

Our study has limitations. The number of subjects was quite small because of complicated protocol. This decreases the likelihood of finding statistically significant results. The lack of the association of the Pro12Pro genotype and a decrease in hepatic GU rate in obese nondiabetic subjects is

likely to be due to a small sample size. We investigated the association of the Pro12Ala polymorphism with GU in several tissues, which could lead to false-positive associations because of multiple testing. Finally, drug treatment of hyperglycemia could be a confounding factor. However, adjustment for medication class did not change our results. Because of these limitations, our findings need to be replicated in other studies.

In conclusion, we have reported for the first time that the 12Ala allele of *PPARG2* is associated with higher hepatic GU in obese subjects with T2DM.

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