Pro12Ala Polymorphism in the Peroxisome Proliferator–Activated Receptor-γ_2 Gene Is Associated With Increased Antilipolytic Insulin Sensitivity

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The Pro12Ala polymorphism of the peroxisome proliferator–activated receptor (PPAR)-γ_2 is associated with reduced transcriptional activity in vitro and increased insulin sensitivity in humans in vivo. The mechanism by which this polymorphism influences insulin sensitivity in humans is unclear. PPAR-γ_2 is mainly expressed in adipocytes, and free fatty acids released from adipose tissue are key mediators of peripheral insulin resistance. Therefore, we examined insulin suppression of lipolysis in 51 subjects without (Pro/Pro) and 17 subjects with the polymorphism (X/Ala). Both groups were lean (BMI <27.0 kg/m^2) and matched for age, BMI, waist-to-hip ratio, and sex. The isotopically (infusion of d_5 glycerol) determined glycerol rate of appearance was used as an index of lipolysis. Insulin sensitivity of lipolysis was expressed as the insulin concentration resulting in half-maximal suppression (EC_{50}). This was directly determined during a three-step hyperinsulinemic-euglycemic clamp (n = 21) or estimated indirectly during a standard hyperinsulinemic-euglycemic clamp (n = 47). The insulin sensitivity index (ISI) of glucose disposal was 0.095 ± 0.006 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1} \cdot l^{-1}$ in the control group and 0.129 ± 0.008 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1} \cdot l^{-1}$ in the X/Ala group (P = 0.003). The EC_{50} was 56 ± 2 pmol/l in the control group and 44 ± 3 pmol/l in the X/Ala group (P = 0.001). The EC_{50} of lipolysis and ISI was significantly correlated (r = 0.42, P = 0.002). In conclusion, in lean subjects, the Pro12Ala polymorphism is associated with increased insulin sensitivity of glucose disposal and suppression of lipolysis. This result suggests that an altered transcriptional activity of PPAR-γ_2 in X/Ala subjects either causes a more efficient suppression of lipolysis in adipose tissue, which in turn results in improved insulin-stimulated glucose disposal in muscle, or, alternatively, beneficially affects insulin signaling in both tissues independently of one another. *Diabetes* 50: 876–881, 2001

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EC_{50}, insulin concentration resulting in half-maximal suppression; FFA, free fatty acid; GIR, mean infusion rate of exogenous glucose; ISI, insulin sensitivity index; MCR, metabolic clearance rate of glucose; PPAR, peroxisome proliferator–activated receptor; R_a, rate of appearance.

Insulin resistance is an important factor in the development of type 2 diabetes, and multiple mechanisms are thought to contribute to its pathogenesis (1,2). Among these, the role of adipose tissue and obesity are of paramount significance. Development and metabolism of adipocytes are complexly regulated, and the important role of the transcription factor peroxisome proliferator–activated receptor (PPAR) isoform γ has recently been recognized (3,4). PPAR-γ controls not only the expression of key enzymes of lipid metabolism (e.g., lipoprotein lipase, fatty acid binding and transfer proteins, and hormone-sensitive lipase) but also of secretory proteins such as leptin (5) and tumor necrosis factor-α (6), possibly influencing skeletal muscle insulin sensitivity.

A CCA → GCA mutation (codon 12 of exon 2) of the PPAR-γ_2 gene resulting in the substitution of proline by alanine was described in humans (allele frequency ~15%) (7–12). Recently, an association of this Pro12Ala polymorphism with decreased risk of type 2 diabetes was demonstrated (13). There is evidence that this polymorphism is associated with reduced transcriptional activity in vitro (8). In humans, the Pro12Ala polymorphism was found to be associated with a lower BMI and lower levels of fasting insulin, suggesting increased insulin sensitivity (8). In obese carriers of the Ala allele, increased insulin sensitivity based on euglycemic-hyperinsulinemic clamps was recently reported for Caucasian (10,14) and Japanese (15) subjects.

It is currently unclear by which mechanisms an altered transcriptional activity of PPAR-γ_2 could influence peripheral insulin sensitivity. Although the isoform PPAR-γ_1 is expressed in many tissues, PPAR-γ_2 is mainly expressed in adipocytes (3,16). Free fatty acids released from adipose tissue triglycerides via lipolysis have long been known to impair insulin-stimulated glucose disposal (17). Conceivably, the differences in peripheral insulin sensitivity observed in subjects with the PPAR-γ_2 Pro12Ala polymorphism may be secondary to altered regulation of lipolysis.

Therefore, we examined insulin suppression of lipolysis in 17 subjects with (X/Ala, 16 heterozygous and 1 homozygous) and 51 subjects without the polymorphism (Pro/Pro). Subjects were recruited from an ongoing family study in Tübingen. The selection was random because the PPAR-γ status was unknown at the time of recruitment. To

TABLE 1
Characteristics of subjects undergoing the two clamp protocols

	Standard hyperinsulinemic clamp		Three-step hyperinsulinemic clamp		All subjects		
	Pro/Pro	X/Ala	Pro/Pro	X/Ala	Pro/Pro	X/Ala	*P**
n (M/F)	34 (17/19)	11 (8/3)	14 (11/3)	7 (2/4)	51 (28/23)	17 (10/7)	
Age (years)	30 ± 1	32 ± 2	26 ± 1	30 ± 2	29 ± 1	31 ± 2	0.35
Weight (kg)	70.0 ± 1.7	76.3 ± 4.0	76 ± 4	65 ± 3	72 ± 2	72 ± 3	0.92
BMI (kg/m^2)	23.5 ± 0.5	24.1 ± 1.3	24.2 ± 1.1	22.7 ± 1.0	23.7 ± 0.5	23.7 ± 0.9	0.94
Waist-to-hip ratio	0.84 ± 0.01	0.85 ± 0.03	0.84 ± 0.02	0.79 ± 0.03	0.84 ± 0.01	0.83 ± 0.02	0.65
Fasting serum glucose (mg/dl)	89 ± 1	91 ± 2	86 ± 1	85 ± 3	88 ± 1	89 ± 2	0.51
Fasting serum insulin (pmol/l)	47 ± 6	40 ± 5	36 ± 4	30 ± 5	44 ± 4	36 ± 4	0.29

Data are *n* or means ± SE. **P* value denotes difference between all subjects with wild-type (Pro/Pro) and heterozygous plus homozygous (X/Ala) polymorphisms in the PPAR-γ_2 gene (codon 12).

minimize potentially overwhelming influences of obesity on the regulation of lipolysis (18), only nonobese subjects were included (BMI <27.0 kg/m^2). Lipolysis was determined using the isotopically determined rate of appearance of glycerol in plasma. Insulin sensitivity of lipolysis was expressed as the insulin concentration resulting in half-maximal suppression (EC_{50}). This was directly determined during a three-step hyperinsulinemic-euglycemic clamp ($n = 21$) and estimated from the standard hyperinsulinemic clamp ($n = 47$) using an index derived from the glycerol rate of appearance (R_a) at 60 min, which was recently validated for lean people (19).

RESEARCH DESIGN AND METHODS

Subjects. In the Tübingen Family Study for type 2 diabetes to date, >600 subjects of Caucasian origin have been metabolically characterized. As part of a more recent add-on protocol, lipolysis was determined during standard hyperinsulinemic and stepwise hyperinsulinemic-euglycemic clamps using stable isotope methodology. At the time of recruitment, the PPAR-γ status was unknown, and subjects were included in an unselected fashion. After excluding extremely lean (BMI <18 kg/m^2) and overweight (BMI >27 kg/m^2) individuals, 68 normal glucose-tolerant unrelated subjects were eligible for the analysis presented in this article. A total of 51 subjects were homozygous for the wild-type allele (Pro/Pro), 16 were heterozygous for the Pro12Ala polymorphism (Pro/Ala), and 1 was homozygous for the polymorphism (Ala/Ala). This allelic frequency is similar to that reported by other groups (8,9,11,12). The characteristics of all subjects and the groups undergoing the standard hyperinsulinemic ($n = 47$) or three-step hyperinsulinemic-euglycemic clamps ($n = 21$) are shown in detail in Table 1. The Pro/Pro group and the X/Ala group were reasonably well matched for BMI, waist-to-hip ratio, age, and sex.

All subjects underwent the standard preparatory procedures and investigations of the protocol of the Tübingen Family Study (medical history, physical examination, routine blood test, electrocardiogram, and oral glucose tolerance test). The protocols were approved by the local ethical committee, and after the nature of the study was explained, all subjects gave informed written consent.

Experimental protocol. Subjects were studied after an overnight fast. At ~6:00 A.M., an antecubital vein was cannulated for infusion of insulin, glucose, and isotopes. A dorsal hand vein on the contralateral arm was cannulated retrogradely and placed under a heating device to permit sampling of arterialized blood. A primed continuous infusion of [$^2H_5$]glycerol (Cambridge Isotope Laboratories, Andover, MA; 1 μmol/kg, 4 μmol/min) was immediately started. At 8:00 A.M., one of the following clamp protocols was started.

Three-step hyperinsulinemic-euglycemic clamp. After the baseline period, subjects received sequential insulin infusions at rates of 0.1, 0.25, and 1.0 mU · kg^{-1} · min^{-1} for 2 h each rate. Blood was drawn every 5–10 min for determination of blood glucose, and a glucose infusion was adjusted appropriately to maintain the baseline glucose level. Arterialized blood samples were obtained at −20, −10, and 0 min before the start of the insulin infusion and at 100, 110, and 120 min of each 2-h insulin infusion rate for determination of plasma [$^2H_5$]glycerol enrichments, plasma glycerol concentrations, and serum insulin and free fatty acid (FFA) concentrations.

Standard hyperinsulinemic-euglycemic clamp. After the baseline period, subjects received a primed insulin infusion at a rate of 1.0 mU · kg^{-1} · min^{-1} for 2 h (20). Blood was drawn every 5–10 min for determination of blood glucose, and a glucose infusion was adjusted appropriately to maintain the baseline glucose level. Arterialized blood samples were obtained at −20, −10, and 0 min before the start of the insulin infusion and at 30, 60, 90, 100, 110, and 120 min for determination of plasma [$^2H_5$]glycerol enrichments, plasma glycerol concentrations, and serum insulin and FFA concentrations.

Analytical procedures. Blood glucose was determined using a bedside glucose analyzer (glucose oxidase method; Yellow Springs Instruments, Yellow Springs, CO). Serum insulin was measured with a microparticle enzyme immunoassay (Abbott, Wiesbaden, Germany), serum FFA with an enzymatic method (Wako Chemicals, Neuss, Germany), and plasma glycerol with an enzymatic method (Sigma Diagnostics, Deisenhofen, Germany). Plasma [$^2H_5$]glycerol enrichment was determined by gas chromatography mass spectrometry using the trimethylsilyl derivative of glycerol. Electron impact ionization was applied, and the mass-to-charge ratios 205 and 208 were monitored (21). The Pro12Ala polymorphism in exon 2 of the PPAR-γ_2 gene was determined by polymerase chain reaction and subsequent restriction enzyme analysis with *Bst*XI as reported previously (7).

Calculations. The plasma R_a of glycerol was used as an index for systemic lipolysis. In the steady state, i.e., at baseline and during the last 20 min, glycerol R_a was assumed to equal the rate of disappearance (R_d) and was calculated according to the steady-state equation $R_a = (ENR_{inf}/ENR_{pl} - 1) \times F$, where ENR_{inf} is the isotopic enrichment of the infusate, ENR_{pl} is the isotopic enrichment of plasma (both in atom percent excess), and F is the rate of the isotope infusion (in micromoles per minute). The mean of the −20-, −10-, and 0-min and the 100-, 110-, and 120-min values of each step were used as steady-state values. During the standard hyperinsulinemic-euglycemic clamp at 30, 60, 90, 100, 110, and 120 min, DeBodo's modification of Steele's non–steady-state equations was used (22) to calculate R_a. A fractional pool size of 0.5 and a volume of distribution of 650 ml/kg were assumed (23).

Insulin sensitivity of systemic lipolysis was assessed as the serum insulin concentration that effectively suppressed plasma glycerol R_a by 50% of the maximal suppression (EC_{50}). The EC_{50} for suppression of glycerol R_a was estimated by fitting a monoexponential function (glycerol R_a = max + $a \times e^{-Ins \times k}$) to the mean basal and the three mean steady-state values, where max equals maximal suppression, a and k represent the fitted parameters, and Ins represents the serum insulin concentration. The EC_{50} was calculated as $EC_{50} = Ins_0 + \ln 2/k$, where Ins_0 represents the serum insulin concentration at baseline. In the subjects undergoing the standard hyperinsulinemic clamp, the insulin EC_{50} for the suppression of lipolysis was estimated using the following equation: EC_{50} = 90 – glycerol $R_{a(60\ min)}$ (% decrease from baseline) × 0.79 (19).

The insulin sensitivity index (ISI) (in μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1}) for systemic glucose uptake was calculated as the mean infusion rate of exogenous glucose (GIR) (in μmol · kg^{-1} · min^{-1}) necessary to maintain euglycemia during the last 60 min of the standard clamp (third step of the three-step clamp) divided by the steady-state serum insulin concentration during step 3. The metabolic clearance rate of glucose (MCR) was calculated as the GIR divided by the steady-state glucose concentration.

Statistical analysis. Data are given as mean ± SE unless otherwise stated. Differences between the two groups were analyzed using an unpaired Student's t test. The correlation between the EC_{50} and ISI was analyzed using least-square regression analysis. A P value <0.05 was considered to be statistically significant. The statistical software package JMP (SAS Institute, Cary, NC) was used for the statistical analyses.

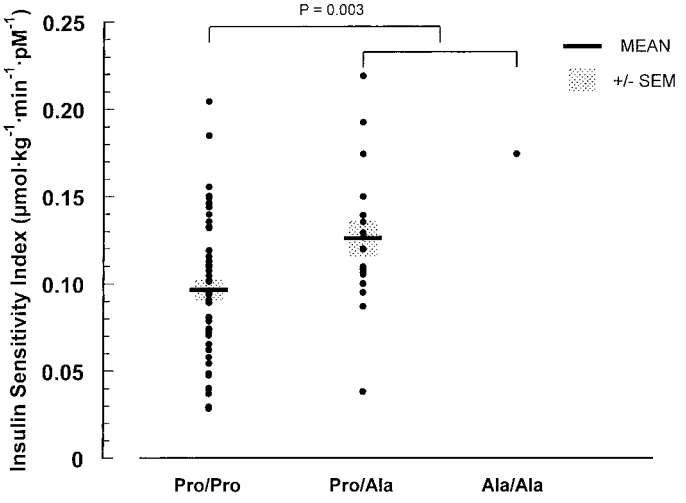

FIG. 1. ISI (glucose disposal per unit insulin) in subjects without the Pro12Ala polymorphism in the PPAR-γ gene (Pro/Pro, n = 51), heterozygous for the Pro12Ala polymorphism (Pro/Ala, n = 16), and homozygous for the Pro12Ala polymorphism gene (Ala/Ala, n = 1).

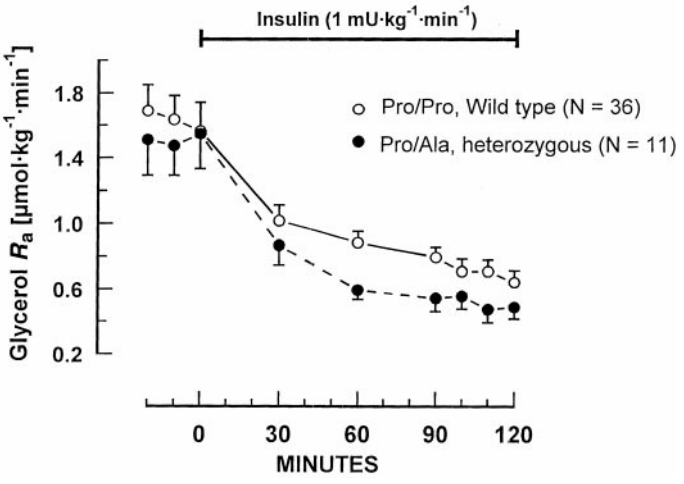

FIG. 2. Plasma R_a of glycerol during the standard hyperinsulinemic clamp in subjects without the Pro12Ala polymorphism in the PPAR-γ gene (Pro/Pro, n = 36), heterozygous or homozygous for the Pro12Ala polymorphism (Pro/Ala, n = 11).

RESULTS

Insulin sensitivity of glucose disposal. During the standard hyperinsulinemic-euglycemic clamp protocol, serum insulin increased to 385 ± 11 pmol/l in the control (Pro/Pro) group and 394 ± 19 pmol/l in the X/Ala group (P = 0.38). The ISI was 0.097 ± 0.007 μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1} in the control (Pro/Pro) group and 0.107 ± 0.008 μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1} in the X/Ala group (P = 0.44). The MCR was 7.2 ± 0.4 ml · kg^{-1} · min^{-1} in the control (Pro/Pro) group and 8.2 ± 0.5 ml · kg^{-1} · min^{-1} in the X/Ala group (P = 0.24).

During the three-step hyperinsulinemic clamp, serum insulin increased to 388 ± 17 pmol/l in the control (Pro/Pro) group and 319 ± 13 pmol/l in the X/Ala group (P = 0.03). The ISI in the third step was 0.096 ± 0.011 μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1} in the control (Pro/Pro) group and 0.160 ± 0.016 μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1} in the X/Ala group (P = 0.01). The MCR was 7.6 ± 0.9 ml · kg^{-1} · min^{-1} in the control (Pro/Pro) group and 10.2 ± 1.0 ml · kg^{-1} · min^{-1} in the X/Ala group (P = 0.11). The homozygous subject (Ala/Ala) had an ISI of 0.174 μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1} and an MCR of 9.4 ml · kg^{-1} · min^{-1}.

In both protocols combined, the ISI was 0.095 ± 0.006 μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1} in the control (Pro/Pro) group and 0.129 ± 0.008 μmol · kg^{-1} · min^{-1} · pmol^{-1} · l^{-1} in the X/Ala group (P = 0.003) (Fig. 1). The MCR was 7.3 ± 0.4 ml · kg^{-1} · min^{-1} in the control (Pro/Pro) group and 8.9 ± 0.5 ml · kg^{-1} · min^{-1} in the X/Ala group (P = 0.04).

Insulin sensitivity of lipolysis. In the standard hyperinsulinemic-euglycemic clamp protocol, the glycerol R_a in the control (Pro/Pro) group decreased from 1.63 ± 0.15 μmol · kg^{-1} · min^{-1} at baseline to 0.69 ± 0.07 μmol · kg^{-1} · min^{-1} at 120 min. In the Pro/Ala group, the glycerol R_a decreased from 1.51 ± 0.20 μmol · kg^{-1} · min^{-1} at baseline to 0.51 ± 0.07 μmol · kg^{-1} · min^{-1} at 120 min (Fig. 2). The relative decrease of the glycerol R_a from baseline at 60 min was 44 ± 2% in the control (Pro/Pro) group and 57 ± 4% in the X/Ala group (P = 0.007). The relative decrease of the glycerol R_a from baseline at 100–120 min was 50 ± 4% in the control (Pro/Pro) group and 64 ± 5% in the X/Ala group (P = 0.10). The estimated insulin EC_{50} for the suppression of lipolysis was 55 ± 2 pmol/l in the control (Pro/Pro) group and 40 ± 3 pmol/l in the X/Ala group ($P < 0.001$).

The mean glycerol R_a values at the end of each step in the stepwise clamp are shown in Fig. 3. In the subjects undergoing the three-step hyperinsulinemic-euglycemic clamp protocol, the directly determined insulin EC_{50} for the suppression of lipolysis was 61 ± 6 pmol/l in the control (Pro/Pro) group and 43 ± 4 pmol/l in the X/Ala group (P = 0.09). The homozygous subject (Ala/Ala) had an EC_{50} of 27 pmol/l. In both protocols combined, the EC_{50} was 56 ± 2 pmol/l in the control group and 44 ± 3 pmol/l in the X/Ala group (P = 0.001) (Fig. 4).

FFA concentrations during the clamps. In the standard hyperinsulinemic-euglycemic clamp protocol in the control (Pro/Pro) group, FFAs decreased from 460 ± 35 μmol/l at baseline to 53 ± 4 μmol/l at 120 min. In the Pro/Ala group, the glycerol R_a decreased from 333 ± 39 μmol/l at baseline to 37 ± 6 μmol/l at 120 min (P = 0.06). In the control (Pro/Pro) group undergoing the three-step

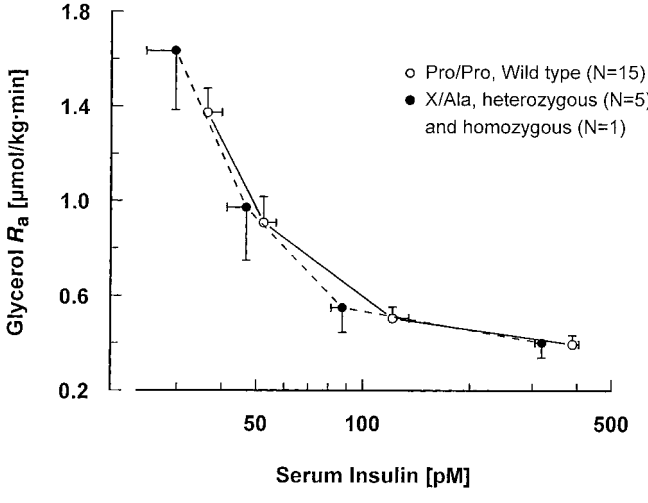

FIG. 3. Insulin dose-response curves of plasma glycerol R_a in subjects without the Pro12Ala polymorphism in the PPAR-γ gene (Pro/Pro, n = 15) and subjects heterozygous (n = 5) or homozygous (n = 1) for the Pro12Ala polymorphism (X/Ala).

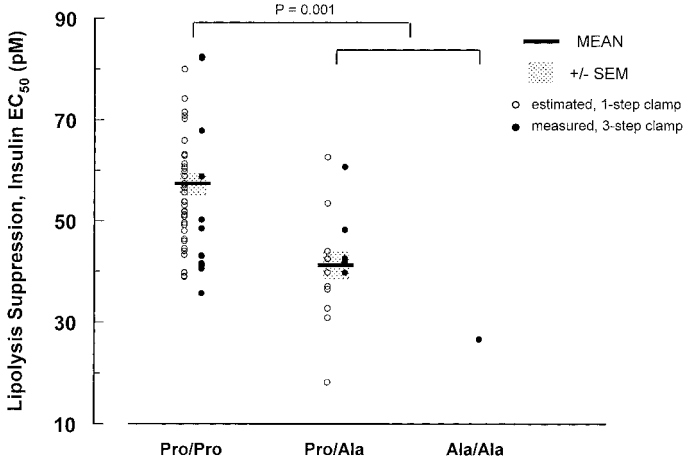

FIG. 4. Insulin sensitivity of suppression of lipolysis (insulin EC_{50}) in subjects without the Pro12Ala polymorphism in the PPAR-γ gene (Pro/Pro, n = 51), heterozygous for the Pro12Ala polymorphism (Pro/Ala, n = 16), and homozygous for the Pro12Ala polymorphism gene (Ala/Ala, n = 1).

hyperinsulinemic-euglycemic clamp protocol, FFAs decreased from 464 ± 36 μmol/l at baseline to 286 ± 37 μmol/l (step 1), 112 ± 15 μmol/l (step 2), and 39 ± 6 μmol/l (step 3). In the X/Ala group, FFAs decreased from 448 ± 55 μmol/l at baseline (P = 0.83) to 334 ± 73 μmol/l (step 1, P = 0.70), 97 ± 27 μmol/l (step 2, P = 0.63), and 27 ± 10 μmol/l (step 3, P = 0.51). In the homozygous subject (Ala/Ala), FFAs decreased from 480 to 7 μmol/l. In both protocols combined, FFAs decreased from 459 ± 27 μmol/l at baseline to 48 ± 3 μmol/l in the control (Pro/Pro) group and from 403 ± 37 to 33 ± 5 μmol/l in the X/Ala group (P = 0.03). The suppressed FFA concentrations at the end of the clamp are shown in Fig. 5.

Correlation between insulin sensitivity of glucose disposal and lipolysis. The EC_{50} of lipolysis and the insulin sensitivity of glucose disposal were significantly correlated (r = 0.42, P = 0.002) (Fig. 6). Of the subjects with the X/Ala polymorphism, 72% are located in the right lower quadrant of the distribution versus 21% of control subjects.

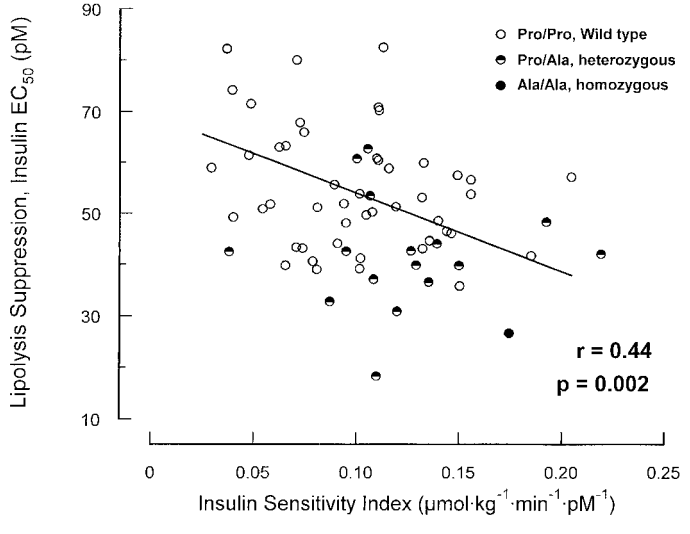

FIG. 5. FFA concentrations at the end of the euglycemic-hyperinsulinemic clamp in subjects without the Pro12Ala polymorphism in the PPAR-γ gene (Pro/Pro, n = 51), heterozygous for the Pro12Ala polymorphism (Pro/Ala, n = 16), and homozygous for the Pro12Ala polymorphism gene (Ala/Ala, n = 1).

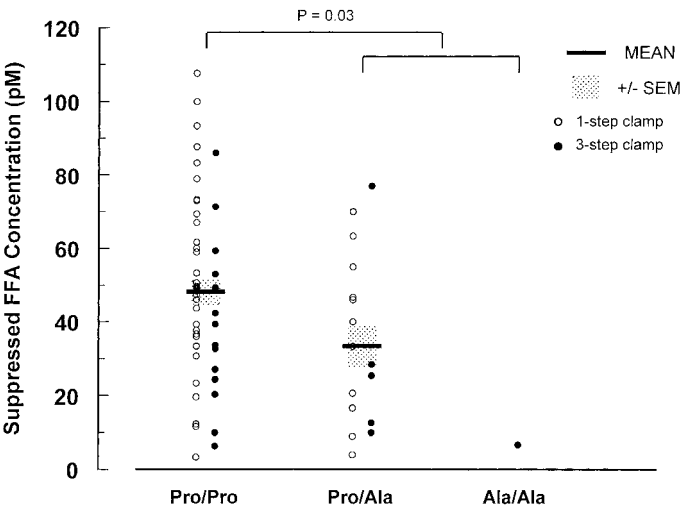

FIG. 6. Correlation between the ISI (glucose disposal per unit insulin) and suppression of lipolysis (insulin EC_{50}) in subjects without the Pro12Ala polymorphism in the PPAR-γ gene (Pro/Pro, n = 51), heterozygous for the Pro12Ala polymorphism (Pro/Ala, n = 16), and homozygous for the Pro12Ala polymorphism gene (Ala/Ala, n = 1).

DISCUSSION

The aim of the present study was to assess the functional impact of a single nucleotide polymorphism on metabolic processes. We specifically attempted to elucidate the mechanisms by which the Pro12Ala polymorphism in the PPAR-γ_2 gene influences insulin sensitivity. We found that both insulin sensitivity of glucose disposal and insulin sensitivity of lipolysis were greater in the subjects carrying the Ala allele. These observations were made in the absence of any differences in age, sex, adiposity (BMI), or fat distribution (waist-to-hip ratio). Moreover, insulin sensitivity of glucose disposal and lipolysis were significantly correlated across a narrow BMI range.

There are two possible mechanisms by which the presence of the Ala allele could influence insulin sensitivity. Theoretically, there may be a primary mechanism by which an altered transcriptional activity of the Ala variant somehow independently improves insulin signaling in both muscle and adipose tissue. The insulin signaling chain to lipolysis in adipocytes and to glucose transport and glycogen storage in muscle shares a number of proteins, such as the insulin receptor, the insulin receptor substrate family, and the phosphatidylinositol 3-kinase complex (24,25). However, because the PPAR-γ_2 protein has been shown to be virtually absent in skeletal muscle (16), this scenario appears to be unlikely.

Alternatively, alterations in the transcriptional activity of the Ala variant in adipocytes could somehow enhance insulin's action on suppression of lipolysis, resulting in a decreased release of FFAs or other factors yet to be identified in the circulation. Reduced availability of FFAs would thus permit muscle, the primary site of insulin-stimulated glucose disposal, to use more glucose (17). Our finding lower circulating FFAs at the end of the hyperinsulinemic clamp in the Pro12Ala group is consistent with this hypothesis.

The more efficient suppression of lipolysis by insulin in the Pro12Ala group may also provide an explanation for the significantly greater BMI recently observed in two cohorts of subjects heterozygous and homozygous for the Pro12 Ala polymorphism (11,12). The greater suppression of lipolysis should tilt the lipolysis-lipogenesis balance slightly toward lipogenesis. This would result in preservation of triglycerides in the adipocyte, at least during insulin stimulation, and in the long run, favor weight gain. Because we not only excluded overweight and obese subjects but also made an effort to match the two groups for BMI, no inferences on the relationship between BMI and the Pro12Ala polymorphism can be made in the present study.

The cellular mechanisms mediating the effect of altered transcriptional activity on enhanced insulin suppression of lipolysis in adipocytes are unclear. Recently, the heterozygous PPAR-γ knockout mouse has been shown to be more insulin sensitive than its wild-type littermates with respect to glucose uptake and suppression of glucose production (26). In this study, insulin suppression of FFA release was not determined but represents a prime candidate for mediating the other insulin effects. The greater insulin sensitivity in animals with 50% reduction of PPAR-γ activity is in apparent contradiction with the notion that thiazolidinediones (synthetic activators of PPAR-γ) improve insulin sensitivity in humans in vivo. The following largely speculative explanations, which would also apply to our data, have been put forward (26).

Endogenous PPAR-γ along with its natural ligand might primarily serve to dampen insulin action. Thiazolidinediones, most of which being both partial agonists and partial antagonists, could displace putative endogenous full agonists and eliminate their dampening effect on insulin sensitivity. A genetically reduced transcriptional activity, such as that shown for the Pro12Ala polymorphism, would also result in improved insulin sensitivity. Alternatively, PPAR-γ might act as a transcriptional repressor in the ligand-free state, thus inducing insulin resistance. Insulin sensitivity would be restored either by reversing this repression or by genetically reducing the activity of the repressor, i.e., by replacing proline by alanine.

A conceivable mechanism by which both thiazolidinediones and the Pro12Ala polymorphism improve insulin suppression of lipolysis could involve the proportion of large versus small adipocytes. Thiazolidinediones have been shown to promote the differentiation of preadipocytes to small adipocytes (27) and small adipocytes appear to be more insulin sensitive with respect to suppression of lipolysis than large adipocytes (28). Hence, by reasoning along the lines of the above paragraph, it is possible that both thiazolidinediones and the Pro12Ala polymorphism in PPAR-γ_2 lead to a higher proportion of small adipocytes for a given fat mass and consequently a more efficient insulin suppression of glycerol and FFA release. In support of this hypothesis, heterozygous PPAR-γ–deficient mice had smaller adipocytes and greater insulin sensitivity than wild-type mice (29).

In conclusion, in lean subjects, the Pro12Ala polymorphism is associated with increased insulin sensitivity of glucose disposal and suppression of lipolysis. Because the PPAR-γ_2 protein is virtually absent in skeletal muscle, it appears likely that an altered transcriptional activity of PPAR-γ_2 in subjects carrying the Ala allele causes a more efficient suppression of lipolysis in adipose tissue, which in turn results in improved insulin-stimulated glucose disposal in muscle via decreased availability of FFAs. The cellular mechanism mediating the effect of altered transcriptional activity of the Ala variant on enhanced insulin suppression of lipolysis in adipocytes may involve fat cell size.

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