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Short Communication

PPARγ2 Pro12Ala polymorphism is associated with improved lipoprotein lipase functioning in adipose tissue of insulin resistant obese women

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ABSTRACT

Lipoprotein lipase (LPL) plays a pivotal role in lipid metabolism, contributes to metabolic disorders related to insulin action and body weight regulation, and is influenced by inflammation. The Pro12Ala polymorphism of the peroxisome proliferator-activated receptor (*PPAR*)*γ2* gene seems to influence LPL functioning, but its role in obesity and insulin resistance status, which usually coexist in the clinical setting, has not been explored. Our aim was to analyze the association of obesity and insulin resistance with adipose LPL activity and expression, and the influence of the *PPARγ2* Pro12Ala polymorphism. A cross-sectional study was conducted in 58 reproductive-age women who underwent elective abdominal surgery. Free-fatty acids, glucose, insulin, and selected adipokines were measured in fasting blood samples. DNA was isolated and the polymorphism genotyped. Biopsies of abdominal subcutaneous adipose tissue obtained during surgery were used to determine enzymatic LPL activity and expression; and expression of selected cytokines. Overweight/obese women presented lower LPL activity ($P = 0.022$) and higher circulating TNF-α ($P = 0.020$) than controls. Insulin resistant women also showed borderline lower LPL activity than non-resistant ($P = 0.052$), but adiposity and inflammatory molecules were comparable. Nevertheless, LPL activity was higher in Pro12Ala carriers than in non-carriers after adjusting for obesity, insulin resistance and inflammation. Likewise, adipose LPL expression was increased in carriers while expression of cytokines was decreased. Our data suggest that insulin resistance is associated with low adipose LPL activity independently of obesity, but the *PPARγ2* Pro12Ala polymorphism seems to protect the LPL functioning of obese insulin resistant women, likely through regulating inflammation in adipose tissue.

1. Introduction

Lipoprotein lipase (LPL) is an enzyme that hydrolyzes circulating triglycerides (TG) either for oxidation or storage. It regulates tissue-specific substrate delivery and utilization and consequently may contribute to various aspects of metabolic disorders related to energy balance, insulin action, and body weight regulation, including obesity (Santamarina-Fojo and Dugi, 1994; Taskinen, 1987; Wang and Eckel, 2009). Adipose and blood LPL activity is influenced by nutritional factors such as circulating glucose and free fatty acids; hormonal environment, mainly concentrations of insulin, leptin, and adiponectin; and genetic factors as it is the presence of the Pro12Ala polymorphism of the transcription factor peroxisome proliferator-activated receptor γ2 (*PPARγ2*) gene among others. Circulating glucose, free fatty acids, insulin, leptin and adiponectin stimulate blood and adipose LPL activity (Kern, 1997; Von Eynatten et al., 2004; Wang and Eckel, 2009). In contrast, it has been reported that the *PPARγ2* Pro12Ala polymorphism decreases adipose LPL activity and expression (Schneider et al., 2002). However, other authors found such effect in women but not in men (Kolehmainen et al., 2003).

The relationship between obesity and LPL activity in adipose tissue is unclear. Some authors reported decreased enzymatic activity in obese, as compared to non-obese subjects (Dahms et al., 1981), but

Abbreviation: LPL, lipoprotein lipase; *PPARγ2*, peroxisome proliferator-activated receptor γ2; BMI, body mass index; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; TG, triglycerides; FFA, free fatty acids; OW, overweight/obese; IR, insulin resistant; OWIR, overweight/obese and insulin resistant; ARP, acidic ribosomal phosphoprotein.

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others have found it increased suggesting that such high activity is what impacts the fat storage of obese individuals (Benkalfat et al., 2011; Kern, 1997; Kern et al., 1990). Nevertheless, the interpretation of these studies is inconsistent. The study that compared LPL activity of obese and non-obese individual, informed that such activity was higher in the obese group (Kern et al., 1990), but the same author reported later that adipose LPL activity of obese individuals increased further after weight loss (Kern, 1997), suggesting that the enzyme activity was low when the subjects were obese. Likewise, in a more recent study conducted in experimentally obese rats fed a cafeteria-diet, it was found a high adipose LPL activity associated with high adiposity (Benkalfat et al., 2011), but the effect of adiposity was not separated from the effect of an inadequate diet. The association between LPL activity and insulin resistance is also unclear. Eckel (1989) found increased adipose LPL activity in insulin resistant individuals, but such increase was dependent on adiposity. In contrast, another study in which adiposity was controlled by pairing subjects according with their BMI, an inverse association between HOMA and adipose LPL activity was detected (Eriksson et al., 2003).

Scientific evidence also suggests that the obesity-related inflammatory status influences blood and adipose LPL activity independently of adiposity (Kern, 1997; Yudkin et al., 2000; Wu et al., 2004). Epidemiological studies have found positive associations among body mass index (BMI) and circulating C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)-α, and leptin (Aronson et al., 2004; Maachi et al., 2004); as well as positive correlations among these inflammatory molecules and fasting insulin (Giugliano et al., 2004; Wexler et al., 2005). Moreover, studies in animals suggest that elevated amounts of IL-6 and TNF-α hamper the insulin-receptor performance and decrease adipose LPL activity (Grupta and Khandelwal, 2004; Ruan and Lodish, 2003). In fact, experimental studies demonstrated that the inflammatory status interferes with LPL activity by infecting experimental animals, which resulted in depressed blood and tissue LPL activity, reduction in the clearance rate of TG, and hypertriglyceridemia (Bagby and Martinez, 1987; Lanza-Jacoby and Tabares, 1990; Picard et al., 2002).

The information above presented comes from studies that analyze separately the effect of obesity, insulin resistance, inflammation or the presence of the *PPARγ2* Pro12Ala polymorphism on LPL activity. Exploring associations among all these variables together in the clinical setting is complex because these conditions frequently coexist. Therefore, in this study we analyze the association of obesity and insulin resistance with adipose LPL activity and expression, as well as the influence of the *PPARγ2* Pro12Ala polymorphism, by comparing: circulating inflammatory markers and adipokines; adipose tissue LPL activity and expression; as well as the expression of selected cytokines in adipose tissue of reproductive-age women, across the following four categories that combine nutritional status and insulin resistance status: 1) Control: normal BMI, normal fasting insulin; 2) OW: high BMI, normal fasting insulin; 3) IR: normal BMI, high fasting insulin and; 4) OWIR: high BMI, high fasting insulin. Comparisons were conducted also between carriers and non-carriers of the *PPARγ2* Pro12Ala polymorphism. Variables known to influence LPL activity, such as fasting glucose, free fatty acids, leptin and adiponectin; as well as the inflammatory status, were taken into account.

2. Material and methods

2.1. Subjects and procedures

A clinical study using a cross-sectional design was conducted in the Unit of Research in Medical Nutrition (UIMN) of the Mexican Institute of Social Security (IMSS) in Mexico City. A sample of 58 women was recruited in the Reproductive Medicine Service of the Hospital de Gineco-Obstetricia "La Raza" of the IMSS. The study included reproductive-age women who underwent elective abdominal or laparoscopic surgery due to non-inflammatory conditions. Women with type 2 diabetes mellitus, polycystic ovary syndrome, and those with clinical evidence of infection, were excluded. Women who met the selection criteria and accepted to participate signed a written informed consent form. The study protocol was accepted by the Ethics Committee of the IMSS # CNIC 2005-785-138.

2.2. Anthropometry

Anthropometric measurements were obtained on the day of surgery before entering to the surgery room. Body weight and height were measured by trained field workers according to standard procedures. Body mass index was calculated dividing weight (kg) by height (m^2). The percentage body fat was obtained by assessing body composition with bioelectric impedance (Bodystat Model 1500 MDD. Tampa Fl, EEUU).

2.3. Blood samples

Once in surgery and before anesthesia, fasting blood samples were obtained from a peripheral vein, collected in EDTA tubes, and centrifuged at 4 °C for 10 min at 1500 *g*. Plasma and leukocytes were separated from blood samples within the first half hour. Plasma aliquots and leukocytes DNA were stored at −70 °C for future biochemical determination and genotyping of the *PPARγ2* Pro12Ala polymorphism.

2.3.1. Biochemical determinations

Plasma insulin, leptin, and adiponectin were assessed by RIA using commercial kits (Linco, USA). IL-6, TNF-α (Quantikine HS, Minneapolis, USA) and CRP (DSL, Active US, Inc. Texas, USA) were measured by ELISA methods also with commercial kits. Glucose, TG (Spinreact, S.A., Sant Esteve de Bas, Spain), and FFA (Roche Diagnostics GmbH, Penzberg, Germany) were determined by enzymatic assays.

2.3.2. Genotyping of the Pro12Ala PPARγ2 polymorphism

Genomic DNA leukocytes from peripheral blood were extracted according to standard techniques. The quality and quantity of DNA was ascertained by spectrophotometry and agarose gel electrophoresis criteria. *PPARγ2* Pro12Ala polymorphism was determined by PCR-SSCP (single-strand conformational polymorphism) analysis as described previously (Canizales-Quinteros et al., 2007). Quality control was evaluate in some randomly selected Ala12, Pro12 homozygotes and Pro12Ala heterozigotes samples by sequencing with asymmetric PCR using Big Dye Kit Terminators v3, and analyzed in an automated sequencer ABI 3100 according to the manufacturer's protocol (Applied Biosystems, Foster City, California).

2.4. Adipose tissue biopsies

After anesthesia, samples of subcutaneous adipose tissue were collected by the surgeon at the site of the surgical incision, in the periumbilical region. Adipose tissue biopsies were immediately washed with cold saline, and transported in liquid nitrogen to the laboratory of the UIMN for storage at −70 °C until LPL activity was determined and expression of LPL, IL-6 and TNF-α, were assayed.

2.4.1. Measurement of LPL activity

LPL activity was measured by the method of Nilsson-Ehle and Schotz (1976), with a [9,10-^{3}H] triolein substrate emulsified with L-α-phosphatidylcholine and glycerol. LPL activity was expressed as nanomols of fatty acid released per min per g tissue. Acetone powders of adipose tissue were extracted (10 mg of powder per ml of buffer) with 0.05 M Tris–HCl, pH 8.0, containing 1 M ethylene glycol. The supernatants obtained after centrifugation at 40,000 *g* were used as enzyme source in all experiments. The substrate was prepared by homogenization with a Polytron of 150 mg triolein 99% (Sigma-Aldrich) plus 0.25 ml of [9,10-^{3}H] triolein (5 mCi/10 ml) stored in toluene, using lecithin in chloroform as emulsifier. The

solvents were evaporated under a stream of nitrogen and the substrate was stored at room temperature for at least six weeks.

Enzymatic activity was measured after incubations at 37 °C for 60 min in a total volume of 0.2 ml with 0.1 ml of assay substrate and 0.1 ml enzyme and/or buffer. Fatty acids produced during the incubations were isolated using the liquid–liquid partition system. Reaction was stopped by adding 3.33 ml methanol–chloroform–heptane 1.41:1.25:1 (v/v/v) followed by 1.0 ml of 0.1 M potassium carbonate-borate buffer at pH 10.5. After vigorous mixing on a Vortex for 15 s, the tubes were centrifuged for 15 min at 3000 *g*. Aliquots of 0.5 ml of the methanol–water upper phase was counted in a Beckman LS 600 LL spectrometer using 5 ml Instage-toluene 1.1 as scintillator. An aliquot of 0.025 ml of assay substrate plus 5 ml of scintillator was assayed for radioactivity after the addition of 1 ml upper phase from control incubations without enzyme. Activity was calculated as follows: *Enzyme activity = net cpm × (1/incubation time) × (1/sp act) × 3 × 2.45 × (1.076)*, where enzymatic activity is expressed as mU in the sample assayed. One mU of lipolitic activity represents release of 1 nmol fatty acid per minute at 37 °C.

2.4.2. RNA isolation, cDNA synthesis and quantitative real-time (qRT)-PCR

Frozen adipose tissue was pulverized and total RNA was isolated using the RNeasy lipid tissue mini kit according to manufacturer instructions (QIAGEN Sciences. Maryland, USA). RNA concentration was determined by absorbance at 260 c 1 μg/μl in RNAse-free water, and its integrity was evaluated using electrophoresis with 1% agarose and ethidium bromide staining (1.25 ng/μl, Sigma, Mexico City). First-strand cDNA was synthesized from 1 μg of total RNA with oligo-dT oligonucleotide primer by using the TaqMan reverse transcription reagents kit (Applied Biosystems, Branchburg, New Jersey USA) according to manufacturer recommendations. Before qRT-PCR analysis, cDNA quality was tested by final-point PCR amplifying the reference acidic ribosomal phosphoprotein (ARP).

Relative mRNA levels of target genes and reference transcript ARP were determined by qRT-PCR using cDNA preparation for adipose tissue. Synthesized cDNA was mixed with Light Cycler Fast Start DNA Master^{PLUS} SYBR Green I (Roche, Indianapolis, IN) and with various sets of gene-specific forward and reverse primers as follows: IL-6 forward CCT TCC AAA gAT ggC TgA AA reverse gTA ggg gTg gTT ATT gCA T; TNF-α forward TCC TTC AgA CAC CCT CAA CC, reverse CAg ggA TCA AAg CTg TAg gC; LPL forward CCg CCg ACC AAA gAA gAg AT, T reverse TTC CTg TTA CCg TCC AgC CAT and ARP forward ggC ACC ATT gAA ATC CTg AgT gAT, reverse TTg Cgg ACA CCC TCC Agg AAg C. Then, it was subjected to RT-PCR quantification using the Light Cycler 3.5 detection System (Roche). qRT-PCR was performed in specially designed Light Cycler Capillaries in a total volume of 20 μl containing 2 μl of the cDNA sample, 40 pmol of each primer, and 4 μl of Master^{PLUS} SYBER Green. For each reaction, the polymerase was activated by preincubation at 95 °C for 10 min. Amplification was then performed with 35 cycles at 95 °C for 10 s and 60 °C or 62 °C for 7 s. Relative amounts of mRNA were calculated using the comparative threshold cycle method (Light Cycler software v.4.0, Roche). Expression values were normalized from tissue of control subjects. The dynamic range of amplification over which a reaction is linear (the highest to the lowest quantifiable copy number [threshold cycle (Ct)]) was determined by a calibration curve using serial dilutions (from 1:10 to 1:1000) for the genes analyzed. On average, there were ≤35 Ct cycles.

All reactions were performed in duplicate and fluorescent data were acquired during each extension phase. Each sample was normalized to ARP mRNA using the following equation: $\Delta Ct = Ct_{target\ gen} - Ct_{ARP.}$ Fold change in expression of the genes analyzed was calculated using the equation: $2^{(\Delta\Delta Ct\ gene)}$, where $\Delta\Delta Ct$ gene = mean $\Delta Ct_{target\ gene} - \Delta Ct_{target\ gene}$ of the controls subjects. Values represent mean fold changes ± SE.

2.5. Statistical analyses

The Minitab statistical software (Minitab 14, State College, PA) was used for statistical analyses. Differences were considered significant at $p \leq 0.050$. Unadjusted data are presented as median and range. Genotype distributions were tested for deviation from Hardy–Weinberg equilibrium in all groups. Allele and genotype frequencies were compared using the χ^2 test.

Non-parametric statistics was used for comparisons; Mann Whitney U-test and Kruskal Wallis test were used as appropriate. Nutritional status was analyzed using normal BMI (<25 kg/m^2) as control, and overweight and obese women were considered together as a group (BMI ≥ 25 kg/m^2). Insulin resistance status was evaluated by comparing non insulin resistant (fasting insulin ≤ 12 μU/ml) with insulin resistant (fasting insulin > 12 μU/ml) women (Carmina and Lobo, 2004). Comparisons were also conducted among the four groups that combined overweight/obesity with insulin resistance as follows: Control: BMI<25 k/m^2, insulin ≤ 12 μU/ml; OW: BMI ≥ 25 k/m^2, insulin ≤ 12 μU/ml; IR: BMI<25 k/m^2, insulin > 12 μU/ml; and OWIR: BMI ≥ 25 k/m^2, insulin > 12 μU/ml.

To identify variables associated with LPL activity, we used two regression models: a multiple regression analysis (model 1), and the general linear model approach (model 2). Regression models were tested for co-linearity and residuals. For model 1, LPL activity was the dependent variable and BMI, circulating glucose, insulin, FFA, adiponectin, leptin, IL-6, TNF-α and CRP were introduced as predictors; because co-linearity was found among IL-6, TNF-α, and CRP, CRP was selected to analyze the effect of inflammation. For model 2, LPL activity was considered the dependant variable and inflammation, nutritional status, insulin resistance status, and *PPARγ2* Pro12Ala polymorphism, were introduced as factors. Inflammation was coded as absent = 0, present = 1; nutritional status as: normal BMI = 0, overweight/obese = 1; insulin resistance as non-insulin resistant = 0, insulin resistant = 1; and the Ala12 allele as absent = 0, or present = 1.

3. Results and discussion

3.1. General characteristics

Fifty-eight women 20–50 year old were included. The surgical procedure was laparoscopic in 31 (61.4%) as part of a protocol for infertility, and abdominal in 23 (39.6%) mainly because of umbilical hernia. None of the women presented fever or leukocytosis at the moment of surgery. Statistical comparisons of anthropometric, biochemical, and adipose tissue characteristics between the two types of surgery showed no difference (P > 0.300) suggesting that the underlying pathological condition did not influence the results.

Anthropometric and biochemical parameters for the complete sample showed that median BMI and fasting insulin were above the cut-off points for overweight and insulin resistance, but 22% of women presented normal BMI and 33% were non-insulin resistant. Although median CRP was within the normal range, 12% of women presented a concentration above 10 μg/ml which is the cut-off point to identify systemic inflammation (Flores et al., 2007); the woman who presented the highest CRP value (147.99 μg/ml) presented also the highest concentration of IL-6 (128.12 pg/ml), but analysis without this woman did not modify the results (Table 1).

DNA specimens to genotype *PPARγ2* 12Ala allele were available for 48 women. This genetic variant was identified in 14 women, 4 homozygous (Ala12Ala) and 10 heterozygous (Pro12Ala).

3.2. Biochemical parameters and LPL activity stratified by nutritional and insulin resistance status

Comparisons between nutritional status groups demonstrated that overweight/obese women presented higher adiposity, plasma TNF-α

and TG, as well as borderline higher CRP, than normal BMI women (Table 2). Comparisons between insulin resistance status groups showed that concentrations of glucose, insulin, and TG were different between groups, but no differences in adiposity or inflammatory markers were detected (Table 3). LPL activity was lower in overweight/obese as compared to normal weight women; and borderline lower in insulin resistant than in non-resistant women (Table 4).

Regarding the combined groups, 9% of women were classified as Controls, 24% OW, 14% IR, and 53% as OWIR. Comparisons among these groups detected significant differences in CRP concentration and LPL activity. While CRP was significantly higher (P = 0.039) in those groups that included overweight/obese women (OW: median = 4.11 μg/ml; OWIR: median = 4.68 μg/ml) as compared to Control (median = 3.50 μg/ml) and IR groups (median = 2.69 μg/ml), LPL activity was lower only in women who presented both overweight/obesity and insulin resistance (OWIR) as compared to the other three groups (Table 4).

These analyses allowed us to observe separate effects of adiposity, insulin resistance and systemic inflammation on LPL activity. While inflammation was directly associated with adiposity, it was not related to LPL activity in adipose tissue. Our interpretation is that the main physiopathological mechanism involved in alterations of adipose LPL activity of obese individuals is the insulin resistance itself instead of inflammation. Insulin is a well known stimulator of LPL activity, but in the insulin resistance status the lack of entrance of glucose to the cell resembles the fasting state in which LPL activity is decreased in the adipose tissue to favor fuel utilization by muscle (Mead et al., 2002). This interpretation was supported by the results from the combined groups, which demonstrated that despite inflammation was present in the two groups that included overweight/obese women (OW and OWIR), LPL activity was decreased only in the OWIR group (Table 4).

3.3. PPARγ2 Pro12Ala polymorphism and LPL activity

The *PPARγ2* Pro12Ala polymorphism was genotyped in 48 women and identified in 29.2% (20.8% heterozygous, 8.3% homozygous), but because BMI and fasting insulin of these two groups were comparable, they were analyzed together as a group for comparisons with non-carriers. No differences in LPL activity, and circulating inflammatory molecules and hormones were detected between carriers and non-carriers of the polymorphism (Table 5). Although 77.5% of carriers were overweight or obese, and 71.4% were insulin resistant, this frequency of obesity and insulin resistance was similar to that in non-carriers (Fisher test, P > 0.500). None of the carriers presented systemic inflammation.

The prevalence of the Ala12 allele detected in our sample (29%) was greater than that reported for other populations; prevalence of 1% and 12% have been reported for Chinese and Caucasian populations respectively (Passaro et al., 2011; Stumvoll and Haring, 2002). Nevertheless, the distribution of Ala12 allele frequencies we found (Pro12Pro: 0.71, Ala12: 0.29) is comparable to that reported in other studies that included Mexican Mestizo individuals from central Mexico (Ramírez-Salazar et al., 2008; Canizales-Quinteros et al., 2007). The prevalence of the Ala12Ala genotype identified in our sample (8.3%) was higher than in those studies mentioned above, in which the homozygous variant was observed only in one subject (Ramírez-Salazar et al., 2008). Accordingly, our genotype frequencies were not in Hardy–Weinberg equilibrium, showing that the Ala12 homozygous allele is overrepresented likely due to the biased population and the small sample size. Thus, although our group of study is not representative of the Mexican population, it still allowed analyzing the effect of the polymorphism on LPL functioning of this sample of reproductive age women.

Our results are comparable with those of Ramírez-Salazar et al. (2008) regarding the lack of effect of the genetic variant on the metabolic and inflammatory parameters. These authors detected the polymorphism in 14.6% of non-obese and 23.5% of obese women, but no differences in fasting insulin, leptin and adiponectin between carriers and non-carriers were identified. Results of this study, and our results, suggest that alterations in insulin, leptin, and adiponectin that occur in obese women are not related to the genetic variant.

3.4. Expression of cytokines and LPL in adipose tissue

Samples of adipose tissue to determine IL-6, TNF-α and LPL mRNA were available in a subset of women. Comparisons among nutritional status groups showed that expression of IL-6 was higher in obese/overweight than in normal BMI women (P = 0.001), and expression of TNF-α was higher in obese, but not overweight, than in

Table 1
Anthropometric and biochemical parameters in the study sample, n = 58.

	Median	Minimum, maximum
Body mass index, kg/m^2	27.40	19.10, 44.10
Body fat, %	41.05	25.30, 61.40
Insulin, μU/ml	13.65	4.63, 29.52
Glucose, nmol/ml	4.66	3.02, 6.09
Free fatty acids, nmol/ml	0.288	0.075, 0.710
Interleukin-6, pg/ml	3.43	0.692, 128.12
Tumor necrosis factor-α, pg/ml	11.37	8.61, 55.17
C-reactive protein, μg/ml	3.57	0.40, 147.99
Leptin, ng/ml	18.05	5.20, 68.49
Adiponectin, μg/ml	8.82	0.14, 17.47
Triglycerides, nmol/ml	2.57	1.13, 5.51

Normal values for: BMI < 25 kg/m^2; fasting insulin ≤ 12 μU/ml; fasting glucose < 6.1 nmol/ml; fasting triglycerides < 1.7 nmol/ml; C-reactive protein ≤ 10 μg/ml.

Table 2
Anthropometric and biochemical parameters stratified by nutritional status[a].

	Normal	Overweight/obese	p-value[b]
Body mass index, kg/m^2	23.00 (19.10, 24.60)	28.00 (25.00, 44.10)	<0.001
Body fat, %	32.8 (25.8, 40.0)	43.20 (25.30, 61.40)	<0.001
Insulin, μU/ml	13.00 (6.29, 18.45)	13.99 (4.63, 29.52)	0.275
Glucose, nmol/ml	4.84 (3.73, 5.60)	4.63 (3.02, 6.09)	0.621
FFA, nmol/ml	0.296 (0.09, 0.66)	0.262 (0.08, 0.71)	0.447
IL-6, pg/ml	3.30 (2.49, 9.75)	3.49 (0.69, 128.12)	0.292
TNF-α, pg/ml	10.45 (8.61, 55.17)	11.72 (8.92, 34.38)	0.020
CRP, μg/ml	2.82 (0.99, 9.18)	4.38 (0.40, 147.99)	0.074
Leptin, ng/ml	13.72 (5.99, 35.97)	18.16 (5.20, 68.49)	0.159
Adiponectin, μg/ml	9.41(4.55, 17.47)	8.50 (0.14, 17.41)	0.288
TG, nmol/ml	1.00 (0.57, 1.91)	1.34 (0.68, 2.81)	0.039

FFA, free fatty acids; IL-6, interleukin-6; TNF, tumor necrosis factor; CRP, C-reactive protein; TG, triglycerides.
[a] Values are: median (minimum, maximum).
[b] Mann Whitney U-test.

Table 3
Anthropometric and biochemical parameters stratified by insulin resistance status[a].

	Insulin resistance	Non-insulin resistance	p-value[b]
BMI, kg/m^2	27.20 (20.58, 44.10)	27.70 (19.10, 36.60)	0.844
Body fat, %	41.25 (25.30, 61.40)	40.95 (25.80, 54.50)	0.851
Insulin, μU/ml	16.22 (12.13, 29.52)	8.56 (4.63, 11.80)	<0.001
Glucose, nmol/ml	4.80 (3.98, 6.09)	4.41 (3.02, 5.49)	0.001
FFA, nmol/ml	0.263 (0.075, 0.710)	0.309 (0.128, 0.661)	0.379
IL-6, pg/ml	3.39 (2.42, 128.12)	3.52 (0.69, 9.75)	0.750
TNF-α, pg/ml	11.14 (8.92, 34.38)	11.61 (8.61, 55.17)	0.883
CRP, μg/ml	3.57 (0.99, 147.99)	3.97 (0.40, 24.15)	0.872
Leptin, ng/ml	18.59 (9.16, 68.49)	15.24 (5.20, 38.82)	0.113
Adiponectin, μg/ml	8.46 (0.14, 17.41)	9.21 (2.03, 17.47)	0.457
TG, nmol/ml	1.43 (0.59, 2.81)	1.05 (0.57, 2.10)	0.002

FFA, free fatty acids; IL-6, interleukin-6; TNF, tumor necrosis factor; CRP, C-reactive protein; TG, triglycerides.
[a] Values are: median (minimum, maximum).
[b] Mann Whitney U-test.

Table 4
Activity of LPL in adipose tissue (nmol/g), stratified by nutritional status, insulin resistance status and a combination of nutritional and insulin resistance statuses.

	Median	Minimum, maximum	p-value[a]
Nutritional status			
Normal	49.20	5.85, 85.54	0.022
Overweight/obese	26.27	0.35, 105.95	
Insulin resistance			
No	42.11	0.35, 99.99	0.052
Yes	24.62	2.65, 105.95	
Combination			
Control	53.70	5.85, 70.40	0.013
OW	41.19	0.35, 99.99	
IR	46.43	18.07, 85.54	
OWIR	22.65	2.65, 105.95	

Control: BMI<25 kg/m^2, fasting insulin<12 μU/ml; OW: BMI>25 kg/m^2, fasting insulin<12 μU/ml; IR: BMI<25 kg/m^2, fasting insulin>12 μU/ml; OWIR: BMI>25 kg/m^2, fasting insulin>12 μU/ml.
[a] Kruskal Wallis or Mann Whitney U-test as appropriate.

normal weight women (P = 0.024). In contrast, the expression of LPL was not different among nutritional status groups (P = 0.511) (Fig. 1). Comparisons between insulin resistance groups demonstrated that expression of TNF-α was significantly higher in insulin resistant than in non-resistant women (1.24 vs. 1.00, P = 0.037), but expression of IL-6 or LPL was not different among groups (P > 0.550). These data demonstrate that although adipose LPL activity is decreased in obesity and insulin resistance status, the expression of the transcript is not affected, suggesting that posttranscriptional or posttranslational modifications are likely used as regulators of the enzymatic activity (Gouni et al., 1993).

On the other hand, comparisons between carriers and non-carriers of the *PPARγ2* Pro12Ala polymorphism demonstrated that expression of LPL was higher in carriers, but the expressions of IL-6 and TNF-α were lower, as compared to non-carriers of the genetic variant (Fig. 2). Implications of these results are that the Pro12Ala polymorphism acts against the deleterious effects of obesity by increasing the synthesis of LPL and decreasing the expression of inflammatory cytokines in adipose tissue. However this hypothesis must be investigated further.

3.5. Interrelationships among variables

Multiple regression analysis (model 1), which included all the enzyme regulators as predictors, showed that LPL activity was negatively associated with insulin concentration, but not with BMI or CRP, supporting the hypothesis that it is insulin resistance as such what interferes with LPL activity. The general linear model (model 2), which was used to evaluate the influence of the Pro12Ala polymorphism taking into account the

Table 5
Anthropometric and biochemical parameters stratified according with the presence of the *PPARγ2* Pro12Ala polymorphism[a].

	Carriers, n = 14	non-Carriers, n = 34	p-value[b]
LPL activity, nmol/g	27.74 (6.01, 105.95)	30.18 (3.60, 70.45)	0.402
Body mass index, kg/m^2	27.50 (22.5, 30.9)	26.90 (19.1, 40.0)	0.700
Body fat, %	40.95 (32.8, 46.9)	40.35 (25.3, 57.5)	0.548
Insulin, μU/ml	13.95 (4.63, 29.52)	13.15 (5.27, 27.46)	0.700
Glucose, nmol/ml	4.67 (3.91, 5.41)	4.64 (3.02, 6.09)	0.901
Free fatty acids, nmol/ml	0.273 (0.086, 0.710)	0.284 (0.075, 0.661)	0.910
Interleukin-6, pg/ml	3.41 (2.49, 7.31)	3.52 (2.42, 128.12)	0.563
Tumor necrosis factor-α, pg/ml	10.85 (9.68, 14.04)	11.14 (8.61, 55.17)	0.910
C-reactive protein, μg/ml	3.02 (0.99, 8.60)	3.54 (0.40, 147.99)	0.748
Leptin, ng/ml	17.74 (5.20, 25.38)	17.85 (5.99, 47.76)	0.427
Adiponectin, μg/ml	8.86 (4.64, 16.11)	8.82 (0.14, 17.47)	0.700
Triglycerides, nmol/ml	1.38 (0.59, 2.33)	1.22 (0.57, 2.81)	0.768

[a] Values are: Median (minimum, maximum).
[b] Mann Whitney U-test.

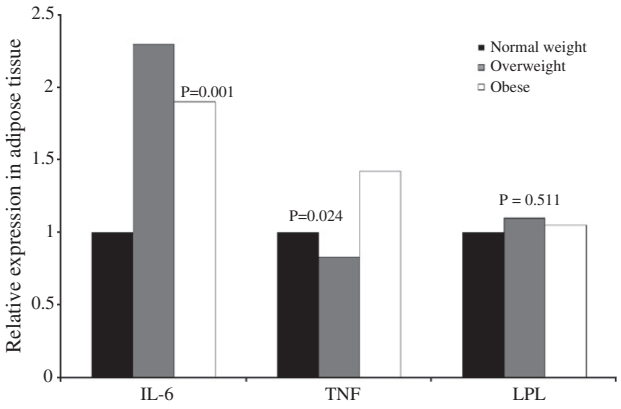

Fig. 1. Gene expression of IL-6, TNF-α and LPL mRNAs in adipose tissue determined by real-time PCR assays is presented stratified by nutritional status (Normal: BMI ≤ 25 k/m^2, overweight: BMI > 25 ≤ 30 k/m^2, obese: BMI > 30 k/m^2). IL-6 and TNF-α expression was higher in obese than in normal women. Expression of LPL was not different. Comparisons were conducted with Kruskal–Wallis test.

presence of abnormal conditions (inflammation, overweight/obesity and insulin resistance), LPL activity was found higher in carriers than in non-carriers (Table 6). Such difference remained significant after adding to the model the enzymatic regulators as predictors (data not shown). A scatter plot of fasting insulin and LPL activity, in which each woman was labeled according with the presence of the polymorphism showed that although for same insulin level there was no difference in LPL activity between carriers and non-carriers, all but one of the carriers presented the lowest concentrations of insulin and the highest LPL activity (Fig. 3), corroborating that the genetic variant is associated with better LPL activity.

In summary, we are presenting reliable evidence that insulin resistance is associated with low abdominal LPL activity independently of obesity and inflammation, in the adipose tissue of reproductive-age women. This association was consistent throughout analyses, as observed in between-groups comparisons (Table 3) and in the regression models, which demonstrated that LPL activity decreased as insulin concentration increased taking into account BMI and CRP (model 1), as well as considering obesity and inflammatory status (model 2).

In addition, we are presenting results suggesting that carriers of the *PPARγ2* Pro12Ala polymorphism are protected against the harmful effects of obesity and insulin resistance on the performance of LPL. This information is relevant to identify pathophysiological

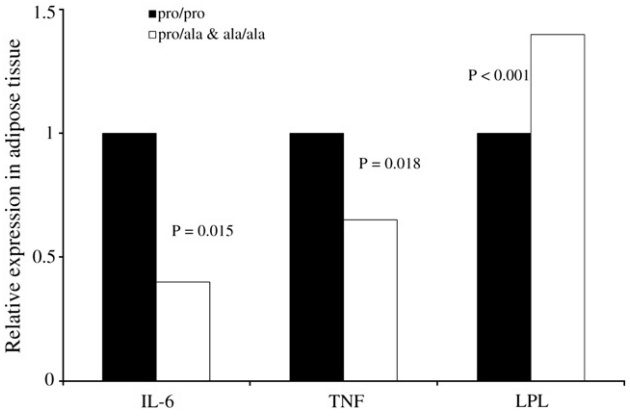

Fig. 2. Gene expression of IL-6, TNF-α and LPL mRNAs determined by real-time PCR assays in adipose tissue is presented stratified by the presence of *PPARγ2* Pro12Ala polymorphism (non-carriers: pro/pro, carriers: pro/ala and ala/ala). IL-6 and TNF-α were less expressed, while LPL was more expressed, in carriers of the polymorphism. Comparisons were conducted with Mann–Whitney test.

Table 6
Predictors of LPL activity in subcutaneous adipose tissue of 48 women.

	Model 1[a]		Model 2[b]	
	Coefficient ± SE	p-value	Δ means ± SE	p-value
Intercept	10.14 ± 28.24	0.721	38.91 ± 6.92	<0.001
FFA	3.72 ± 22.47	0.869		
CRP	−0.172 ± 0.176	0.335		
Glucose	9.06 ± 6.21	0.151		
Leptin	0.347 ± 0.32	0.290		
Adiponectin	−0.020 ± 0.89	0.983		
Insulin	−1.53 ± 0.66	0.024		
BMI	−0.880 ± 1.06	0.411		
Inflammation[c]			−11.35 ± 11.78	0.170
Overweight/obese[d]			−15.97 ± 7.38	0.018
Insulin resistant[e]			−11.43 ± 6.62	0.046
PPARγ2 Pro12Ala[f]			11.96 ± 7.11	0.049

[a] Multiple regression model.
[b] General linear model.
[c] Compared to CRP<10 mg/ml.
[d] Compared to normal nutritional status.
[e] Compared to non-insulin resistant.
[f] Compared to non-carriers of the polymorphism.

mechanisms likely involved in the development of type 2 diabetes mellitus and cardiovascular disease. To our knowledge, the favorable influence of the Pro12aAla polymorphism on adipose LPL activity has not been described before. In fact, our findings are opposite to those of others reporting negative associations between LPL activity and the polymorphism. For instance, in a study that reported reduced LPL activity in carriers of the Pro12Ala genetic variant in patients with cardiovascular disease or diabetes, the LPL activity was measured in plasma instead of adipose tissue (Schneider et al., 2002); therefore, the interpretation of this study may be different because the source of LPL found in post-heparin plasma may be adipose tissue or other tissues including macrophages and muscle (Van Eck et al., 2000). In addition, since the expression of the isoform *PPARγ2* predominates in adipose tissue, it is expected that its role is precisely in such tissue (Auwerx, 1999).

Yet our results are consistent with those of others who have detected other beneficial roles of the *PPARγ2* Pro12Ala polymorphism on metabolism. A protective role of the polymorphism on the risk to develop diabetes mellitus was reported (Stumvoll and Haring, 2002), improved insulin sensitivity (González Sánchez et al., 2002), as well as a significant increase in HDL cholesterol and decrease in TG associated with the polymorphism (Huang et al., 2011). Unfortunately, in these studies the LPL activity was not investigated, but our results add to those findings in that likely an improved LPL functionality is the mechanism through which the polymorphism contributes to all those beneficial roles.

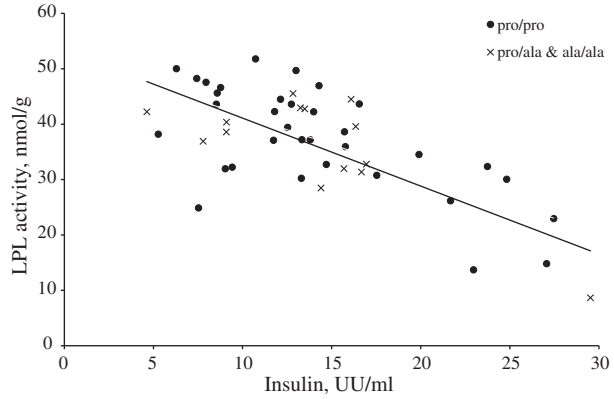

Fig. 3. Scatter plot of fasting insulin and adipose tissue LPL activity in carriers and non-carriers of the Pro12Ala polymorphism (non-carriers: pro/pro, carriers: pro/ala and ala/ala). All but one carrier presented low fasting insulin and high LPL activity.

In conclusion, our results suggest that the *PPARγ2* Pro12Ala polymorphism exert a protective role on the performance of LPL, likely by increasing LPL expression and decreasing inflammatory cytokines expression in adipose tissue of obese women with insulin resistance. The main strength of our study is the simultaneous analysis of clinical characteristics such as obesity, insulin resistance, and systemic and tissue inflammation that usually coexist, which allowed us untangling the effect of each factor on LPL performance.

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