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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