DOI 10.1002/mnfr.201300666 *Mol. Nutr. Food Res.* 2014, *58*, 1852–1860

RESEARCH ARTICLE

Effects of obesity/fatty acids on the expression of GPR120

Francisca Rodriguez-Pacheco[1,2], Sara Garcia-Serrano[1,2*], Eva Garcia-Escobar[1,2**], Carolina Gutierrez-Repiso[1], Juan Garcia-Arnes[1], Sergio Valdes[1,2], Montserrat Gonzalo[1], Federico Soriguer[1,2,3], Francisco J. Moreno-Ruiz[4], Alberto Rodriguez-Cañete[4], Jose L. Gallego-Perales[4], Abelardo Martinez-Ferriz[4], Gemma Rojo-Martínez[1,2,3] and Eduardo Garcia-Fuentes[1,2,3]*

[1] Unidad de Gestion Clinica de Endocrinogía y Nutrición, Instituto de Investigaciones Biomédicas de Málaga (IBIMA), Hospital Regional Universitario, Malaga, Spain
[2] CIBERDEM, Instituto de Salud Carlos III, Malaga, Spain
[3] CIBEROBN, Instituto de Salud Carlos III, Malaga, Spain
[4] Unidad de Gestion Clínica de Cirugía General, Digestiva y Trasplantes, Instituto de Investigaciones Biomédicas de Málaga (IBIMA), Hospital Regional Universitario, Malaga, Spain

Received: September 11, 2013
Revised: February 10, 2014
Accepted: April 2, 2014

Scope: The effects that fatty acids (FAs) exert on G protein-coupled receptor-120 (GPR120) levels, a receptor for FAs, are still unknown. We analyzed the association between GPR120 and obesity, and the FA effects on its expression.

Methods and results: GPR120 levels were analyzed in visceral adipose tissue (VAT) from nonobese and morbidly obese subject. VAT GPR120 mRNA and protein levels were lower in morbidly obese subjects ($p = 0.004$). After, these subjects underwent a high-fat meal. GPR120 mRNA expression in peripheral blood mononuclear cells in the fasting state was lower in morbidly obese subjects ($p = 0.04$), with a decrease 3 h after a high-fat meal only in morbidly obese subjects ($p = 0.043$). Also, incubations of visceral adipocytes from these subjects were made with different FAs. In nonobese subjects, palmitic, oleic, linoleic, and docosahexaenoic acids produced an increase in GPR120 mRNA and protein levels ($p < 0.05$). In morbidly obese subjects, only linoleic acid produced an increase in GPR120 mRNA and protein levels ($p < 0.05$).

Conclusion: Morbidly obese subjects had lower GPR120 mRNA and protein levels in VAT and a lower mRNA expression after a high-fat meal in peripheral blood mononuclear cells. The FAs effect on GPR120 mRNA and protein levels in visceral adipocytes was lower in morbidly obese subjects.

Keywords:
Adipocyte / Adipose tissue / Fatty acids / GPR120 / High-fat meal

1 Introduction

Adipose tissue plays a critical role in glucose homeostasis by releasing adipocytokines that regulate insulin sensitivity in other organs [1]. Unbalanced production of adipocytokines and proinflammatory cytokines seen in visceral obesity contributes critically to the development of many aspects of the metabolic syndrome [2]. This altered metabolism of adipose tissue could be the result of the effect of different signaling

Correspondence: Dr. Eduardo Garcia-Fuentes, Unidad de Gestion Clinica de Endocrinología y Nutrición, Hospital Regional Universitario, Plaza del Hospital Civil s/n, 29009 Malaga, Spain
E-mail: edugf1@gmail.com
Fax: +34-95228670

Abbreviations: **CT**, cycle threshold; **DHA**, docosahexaenoic acid; **FAs**, fatty acids; **GPR120**, G protein-coupled receptor-120; **HOMA-IR**, homeostasis model assessment of insulin resistance index; **PBMC**, peripheral blood mononuclear cells; **SAT**, subcutaneous adipose tissue; **VAT**, visceral adipose tissue

*These authors contributed equally to this work.
**Additional corresponding author: Dr. Eva Garcia-Escobar, E-mail: eyring@gmail.com

molecules, such as fatty acids (FAs). This altered metabolism could induce insulin resistance [3, 4].

The influence of diet and its FA composition on adipose tissue development and distribution is well known [5–7]. FAs are now known to act as endogenous signaling molecules, playing a variety of roles in physiological regulation [8–16]. However, the different type of FAs can have different effects and can be differently regulated [8–12]. While saturated FAs are proinflammatory; omega-3 FAs (docosahexaenoic acid (DHA; 22:6n-3)) and eicosapentaenoic acid (20:5n-3), the major ingredients in fish oil, exert potent anti-inflammatory effects and reduce the risk factors for development of the metabolic syndrome [9, 13]. FAs exert their effects by several mechanisms. Among these, FAs may carry out their functions via G protein-coupled receptors [14–16]. G protein-coupled receptor-120 (GPR120) is an orphan receptor which was isolated from a human genomic DNA fragment [16]. Analyses for the tissue distribution of GPR120 mRNA showed an abundant expression in the mouse and human intestinal tract [17]. GPR120 is a receptor for unsaturated long-chain FAs, such as α-linolenic acid and DHA [13, 15–18]. Furthermore, GPR120 regulates various physiological processes, including gut hormone secretion, islet function, food preference, osteoclastogenesis, anti-inflammation, adipogenesis, and appetite control [17, 19–22]. GPR120-deficient mice fed a high-fat diet develop obesity, glucose intolerance, and fatty liver with decreased adipocyte differentiation and lipogenesis and enhanced hepatic lipogenesis [23]. Insulin resistance in such mice is associated with reduced insulin signaling and enhanced inflammation in adipose tissue [23]. In humans, obesity seems to be significantly associated with an increase in GPR120 expression in both subcutaneous (SAT) and visceral (VAT) adipose tissues [23]. However, little is known on the physiological role of GPR120 in adipocytes. GPR120 regulates the adipocyte development and differentiation [21]. GPR120 activation in 3T3-L1 cells led to an increase in glucose transport and translocation of GLUT4 to the plasma membrane [24]. Also, siRNA-mediated decreased of GRP120 in differentiated 3T3-L1 cells was associated with significant reductions of proteins related to the glucose metabolism, such as IRS-1 and GLUT4 [25]. On the other hand, previous results show that GPR120 is involved in inflammasome inhibition induced by omega-3 FAs in macrophages [26], and that eicosapentaenoic acid, DHA, and arachidonic acid induce the same GPR120-mediated signaling events, but with different kinetics and intensity in Caco-2 cells [27].

These previously mentioned studies have shown different effects of the GPR120 receptor but, to our knowledge, the effects that FAs may exert on human GPR120 expression are still unknown, as is the possibility that these effects could be modulated under different conditions such as obesity. With this background in mind, we investigated in humans (i) the association between obesity and GPR120 expression in adipose tissue, (ii) whether obesity may modify the effect of different types of FAs (palmitic, oleic, linoleic acids, and DHA) on GPR120 expression in visceral adipocytes, and (iii) the acute change in GPR120 expression in human peripheral blood mononuclear cells (PBMC) in response to a high-fat meal.

2 Materials and methods

2.1 Subjects

The study included 33 morbidly obese (BMI >40 kg/m^2) and 13 healthy, nonobese subjects (BMI < 25 kg/m^2). All the morbidly obese subjects underwent laparoscopic gastric by-pass. Subjects were excluded if they had type 2 diabetes mellitus, cardiovascular disease, arthritis, acute inflammatory disease, infectious disease, or were receiving drugs that could alter the lipid profile or the metabolic parameters at the time of inclusion in the study. The weight of the morbidly obese subjects had been stable for at least 1 month before bariatric surgery. The nonobese subjects underwent laparoscopic surgery for hiatus hernia or cholelithiasis, had no alterations in lipid or glucose metabolism, with a similar age and the same selection criteria as the morbidly obese group, and reported that their body weight had been stable for at least 3 months before the study. All subjects (nonobese and morbidly obese subjects) were of Caucasian origin. Samples from subjects were processed and frozen immediately after their reception in the Regional University Hospital Biobank (Andalusian Public Health System Biobank). All the participants gave their written informed consent and the study was reviewed and approved by the Ethics and Research Committee of Regional University Hospital, Malaga, Spain.

2.2 Laboratory measurements

Blood samples from all subjects before surgery were collected after a 12-h fast. The serum was separated and immediately frozen at −80°C. Serum biochemical variables were measured in duplicate. Serum glucose, cholesterol, HDL cholesterol, triglycerides, and free FAs (Randox Laboratories Ltd., Antrium, UK) were measured by standard enzymatic methods. Adiponectin levels were measured by enzyme immunoassay (ELISA) kits (DRG Diagnostics, Marburg, Germany). Leptin levels were measured by ELISA kit from Mediagnost (Reutlingen, Germany). The insulin was analyzed by an immunoradiometric assay (BioSource International, Camarillo, CA, USA). The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated with the following equation: HOMA-IR = fasting insulin (μIU/mL) × fasting glucose (mol/L)/22.5 [28]. Morbidly obese subjects were also divided in two groups (with low HOMA-IR (<4.7) and with high HOMA-IR (>8) [29–32]) to check if there were significant differences in the GPR120 mRNA expression in adipose tissue according to the insulin resistance levels.

2.3 High-fat meal

The high-fat meal was consumed 2 weeks before surgery. The subjects who underwent the high-fat meal had the same biochemical and anthropometric characteristics that the group in which they are included. Samples of venous blood were obtained after a 12-h fast before the meal (baseline). The nonobese ($n = 7$) and morbidly obese subjects ($n = 7$) underwent a 60 g fat overload with a commercial preparation (Supracal, SHS International, Liverpool, UK). Samples of venous blood were also obtained 3 h after the high-fat meal [29, 33]. Human peripheral blood mononuclear cells (PBMC) were isolated from anticoagulant-treated blood by Ficoll standard density gradient centrifugation [30]. The commercial preparation of 125 mL contains 60 g fat, of which 12 g are saturated, 35.35 g are monounsaturated, and 12.75 g are polyunsaturated. Each 100 mL contains less than 1 g lauric acid, less than 1 g myristic acid, 4.8 g palmitic acid, 1.4 stearic acid, 27.7 g oleic acid, 9.6 g linoleic acid, 1.4 behenic acid, and 0.5 g lignoceric acid. Only water was permitted during the process, and no physical exercise was undertaken.

2.4 Adipose tissue samples

VAT SAT samples were obtained during bariatric surgery in the morbidly obese subjects ($n = 33$) and during laparoscopic surgery for hiatus hernia in the nonobese subjects ($n = 13$) [31, 34]. The fasting/anaesthetic protocols were the same for nonobese and morbidly obese subjects. After a 10-h fast, they underwent to a balanced anaesthesia, with an induction phase using sevoflurane and propofol, and a maintenance phase using sevoflurane, remifentanil, and rocuronium. The biopsy samples were washed in physiological saline and immediately frozen in liquid nitrogen. Biopsy samples were maintained at −80°C until analysis. Other VAT samples from nonobese ($n = 5$) and morbidly obese subjects ($n = 5$) were placed in PBS supplemented with 5% BSA to perform the adipocyte isolation and in vitro incubations. These subjects had the same biochemical and anthropometric characteristics that the group in which they are included.

2.5 Adipocyte isolation and incubations

All reagents were from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Palmitic (16:0), oleic (18:1n-9), linoleic (18:2n-6), and DHA (22:6n-3) acids were dissolved in 0.1 M NaOH at 80–90°C. The resulting stock solutions were added to calcium-free DMEM containing 0.5% BSA-FA-free to treat the isolated adipocytes.

VAT adipocytes were isolated by digesting freshly isolated VAT with 1 mg/mL collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA) in DMEM for 1 h at 37°C in a shaking water bath. Digests were filtered and centrifuged at 300 × *g* for 10 min. Adipocytes were washed twice with DMEM and cultured in 24-well plates (50.000 adipocyte/wells) with DMEM (4.5 g/L glucose) supplemented with 10% BSA-FA-free, 1% L-glutamine, 1% penicillin, and streptomycin for 24 h. Also, oleic (18:1n-9), linoleic (18:2n-6), palmitic (16:0), and DHA (22:6n-3) acids were added at 25, 50, and 100 μM doses [35, 36], leaving some wells as controls without FAs but with the same concentration of NaOH (control). Each treatment was performed in triplicate. Following these treatments with FAs for 24 h, adipocytes were collected for mRNA and protein extraction.

2.6 Determination of adipocyte size

Mean visceral adipocytes diameter was determined on isolated adipocytes from nonobese ($n = 10$) and morbidly obese subjects ($n = 15$). In brief, isolated adipocytes were washed twice with PBS and fixed with 4% formaldehyde. The diameter of 100 adipocytes was measured with a calibrated light microscope.

2.7 Adipocytes viability

Before and after these 24 h of incubation, the number of adipocytes per wells was counted to determine the number of intact adipocytes. Also, a cell viability assay was performed in triplicate using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Southampton, UK) according to the manufacturer's instructions.

2.8 Isolation of RNA and quantitative RT-PCR

Total RNA from adipose tissue, adipocytes, and PBMC was extracted by RNeasy lipid tissue mini kit (Qiagen Science, Hilden, Germany), and total RNA was treated with 55U RNase-free deoxyribonuclease (Qiagen Science) following the manufacturer's instructions. The purity of the RNA was determined by the absorbance260/absorbance280 ratio on a Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The integrity of total purified RNA was checked by denaturing agarose gel electrophoresis and ethidium bromide staining.

First, strand cDNA was synthesized by retrotranscription using the M-MLV retrotranscriptase (Promega). Gene expression levels were analyzed in triplicate by quantitative real-time RT-PCR using a RotorGene Q Real Time PCR system (Qiagen Science), as previously is described [37]. The primers used were designed with the online program Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Primers used were GPR120 (Fwd: 5′-CTTCTTCTCCGACGTCA AGG-3′; Rev: 5′-AGAGGGATAGCGCTGATGAA-3′). The cycle threshold (CT) value for each sample was normalized with the expression of β-actin (housekeeping gene; Fwd: 5′-TACAGCTTCACCACCACGGC-3′; Rev:

5′-AAGGAAGGCTGGAAGAGTGC-3′). Standard curves were constructed with serial dilutions (1/2) of a mixture of samples used in each experiment by plotting values of CT (the cycle at which the fluorescence signal exceeds background) versus log cDNA input (in nanograms) [37]. CT values from each experimental sample were then used to calculate GPR120 mRNA gene expression levels, which were expressed as the percentage of relative gene expression referred to the wells named control.

2.9 Western blot analysis

Adipose tissue and adipocytes were homogenized in ice-cold lysis buffer containing 50 mmol/L Tris-HCl (pH 7.5), 1 mmol/L EGTA, 1 mmol/L EDTA, 1% Triton X-100, 1 mmol/L sodium orthovanadate, 50 mmol/L sodium fluoride, 5 mmol/L sodium pyrophosphate, 0.27 mol/L sucrose, 0.1% 2-mercaptoethanol, and complete protease inhibitor cocktail (1 tablet/50 mL; Roche Diagnostics, Mannheim, Germany). Homogenates were centrifuged at 13 000 × *g* for 10 min at 4°C, supernatants were removed, and aliquots were snap-frozen in liquid nitrogen. Protein concentration was determined with the BCA Protein Assay Reagent (Thermo Fisher Scientific Inc., Rockford, IL, USA). Lysate (20 μg protein) was subjected to SDS-PAGE on 10% polyacrylamide gels and electrotransferred on a polyvinylidene fluoride membrane. Membranes were blocked in TBS-Tween 20 (50 mmol/L Tris-HCl (pH 7.5), 0.15 mol/L NaCl, and 0.1% Tween 20) containing 5% skimmed milk for 16 h at 4°C. After washing, membranes were incubated with monoclonal anti-GPR120 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-β-actin (Sigma-Aldrich) at a dilution of 1:500 or 1:1000, respectively, overnight at room temperature. Membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibody (Promega, Madison, WI, USA) at a dilution of 1:1000 or 1:5000, respectively, for 1 h at room temperature. The proteins were visualized and quantified by an ImageQuant LAS 4000 (GE Healthcare UK Limited, Buckinghamshire, England). The results were expressed as GPR120/β-actin ratio.

2.10 Statistical analysis

The statistical analysis was done with SPSS (Version 11.5 for Windows; SPSS, Chicago, IL, USA). Differences between two groups were compared by the Mann–Whitney test. Differences between more than two groups were compared by the Kruskal–Wallis test. Differences between two related variables were analyzed by the Wilcoxon test. The Spearman correlation coefficients were calculated to estimate the associations between variables. Values were considered to be statistically significant when the $p \leq 0.05$. The results are given as the mean ± SD. In figures, the results are given as the mean ± SEM.

Table 1. Anthropometric and biochemical variables in the nonobese and morbidly obese subjects

	Nonobese subjects	Morbidly obese subjects	*p*
Sex (male/female)	4/9	12/21	
Age (years)	42.5 ± 12.4	40.9 ± 10.9	0.847
Weight (kg)	70.6 ± 11.3	150.4 ± 23.4	<0.0001
BMI (kg/m^2)	24.6 ± 3.4	54.0 ± 5.7	<0.0001
Waist (cm)	94.4 ± 12.5	143.7 ± 19.2	<0.0001
Hip (cm)	102.6 ± 7.5	153.1 ± 14.0	<0.0001
Glucose (mg/dL)	84.6 ± 9.4	102.3 ± 16.7	<0.0001
Cholesterol (mg/dL)	205.8 ± 45.1	201.4 ± 42.1	0.447
Triglycerides (mg/dL)	85.4 ± 59.2	122.4 ± 63.8	0.002
Insulin (μIU/mL)	11.1 ± 4.2	24.6 ± 12.6	<0.0001
HOMA-IR	2.25 ± 0.75	6.28 ± 3.50	<0.0001
Free fatty acids (mmol/L)	0.335 ± 0.124	0.578 ± 0.225	<0.0001

The results are given as the mean ± SD. Mann–Whitney test is used to compare the anthropometric and biochemical variables between the nonobese and morbidly obese subjects. HOMA-IR, homeostasis model assessment of insulin resistance index.

3 Results

3.1 Expression of GPR120 in VAT of nonobese and obese subjects

Table 1 summarizes the characteristics of the nonobese and morbidly obese subjects. All the anthropometric variables and glucose, triglycerides, insulin, HOMA-IR, and free FAs were significantly higher in the morbidly obese subjects.

We investigated the presence of the GPR120 in VAT and SAT of nonobese and obese subjects. RT-PCR analysis showed that VAT of nonobese subjects presented higher GPR120 mRNA levels than that of morbidly obese subjects ($p = 0.004$; Fig. 1A). However, no significant differences were detected in SAT ($p = 0.102$; Fig. 1A). Significant differences were also detected between VAT and SAT in nonobese subject ($p = 0.012$), but not in morbidly obese subjects ($p = 0.145$). In previous studies, we have found that there were differences in the mRNA expression of different genes in morbidly obese subjects according to the insulin resistance levels [38, 39]. When we analyzed the GPR120 mRNA expression in morbidly obese subjects according to the insulin resistance levels, we did not observe significant differences, either in VAT or SAT (data not shown). VAT GPR120 mRNA correlated significantly only with BMI ($r = -0.407$, $p = 0.023$). SAT GPR120 mRNA did not correlate with any of the variables studied (data not shown). By performing Western blot analysis on the two adipose tissues using antibodies specific for GPR120, we confirmed the results obtained by quantitative mRNA expression analysis (Fig. 1B).

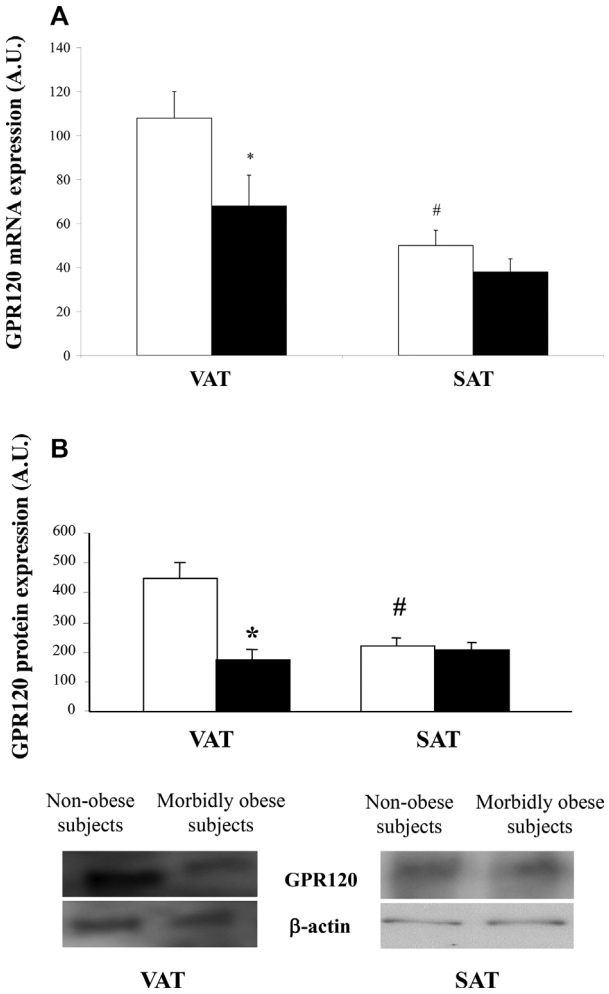

Figure 1. GPR120 in adipose tissue. (A) GPR120 mRNA expression levels in visceral (VAT) and subcutaneous (SAT) adipose tissues from nonobese (□) ($n = 13$) and morbidly obese subjects (■) ($n = 33$). (B) GPR120 protein levels and representative immunoblot in VAT and SAT from nonobese and morbidly obese subjects ($n = 5$ per group). The results are given as the mean ± SEM. $^{*}p < 0.05$: significant differences between nonobese and morbidly obese subjects in VAT. $^{\#}p < 0.05$: significant differences between VAT and SAT in nonobese subjects. A.U., arbitrary units.

3.2 GPR120 after a high-fat meal

As GPR120 regulates various physiological processes [17, 19–22], we wanted to test whether an increase in free FAs levels in response to a high-fat meal [30, 33] produces a change in GPR120 mRNA expression. As we cannot access to human VAT in the feeding state, we used an approach in human PBMC [30]. As with VAT, GPR120 mRNA expression in human PBMC in the fasting state was significantly higher in nonobese subjects than in morbidly obese subjects ($p = 0.04$; Fig. 2). Three hours after the high-fat meal, we observed a significant increase in GPR120 mRNA expression in nonobese subjects ($p = 0.018$), but a significant decrease in

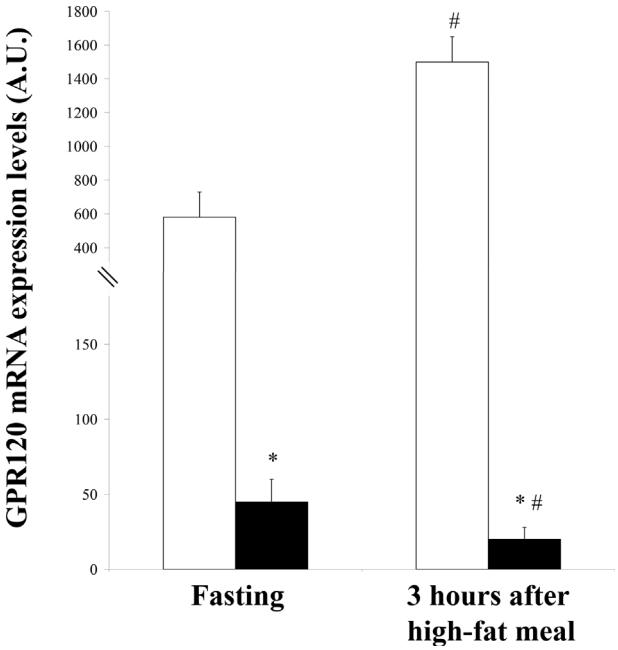

Figure 2. GPR120 mRNA expression levels in human peripheral blood mononuclear cells in fasting and 3 h after a high-fat meal from nonobese (□) and morbidly obese subjects (■) ($n = 7$ per group). The results are given as the mean ± SEM. $^{*}p < 0.05$: significant differences between nonobese and morbidly obese groups. $^{\#}p < 0.05$: significant differences between before and after high-fat meal. A.U., arbitrary units.

morbidly obese subjects ($p = 0.043$; Fig. 2). After the high-fat meal, GPR120 mRNA expression in human PBMC is also significantly higher in nonobese subjects than in morbidly obese subject ($p = 0.03$)

3.3 Regulation of GPR120 mRNA and protein levels by different FAs in VAT

As we found a different change in GPR120 mRNA expression after a high-fat meal, we wanted to examine the effects of each type of FA (saturated, monounsaturated, omega-3, and omega-6) on GPR120 mRNA and protein levels in human visceral adipocytes from both groups of subjects. First, after 24 h of incubation, the number of intact adipocytes per wells was 87.1 ± 3.4% and 85.2 ± 4.9% in nonobese and morbidly obese subjects, respectively. The cell viability measured by the CellTiter-Glo® Luminescent Cell Viability Assay was 89.6 ± 4.6% and 87.1 ± 5.1% in nonobese and morbidly obese subjects, respectively. The mean diameter of visceral adipocytes from morbidly obese subjects are significantly larger than those from nonobese subjects (113.7 ± 13.7 μm versus 79 ± 8.9 μm, $p = 0.001$).

GPR120 mRNA expression in visceral adipocytes from nonobese subjects in response to incubation with FAs was higher than in morbidly obese subjects, independently of doses and type of FA ($p < 0.05$ for all cases; Fig. 3A).

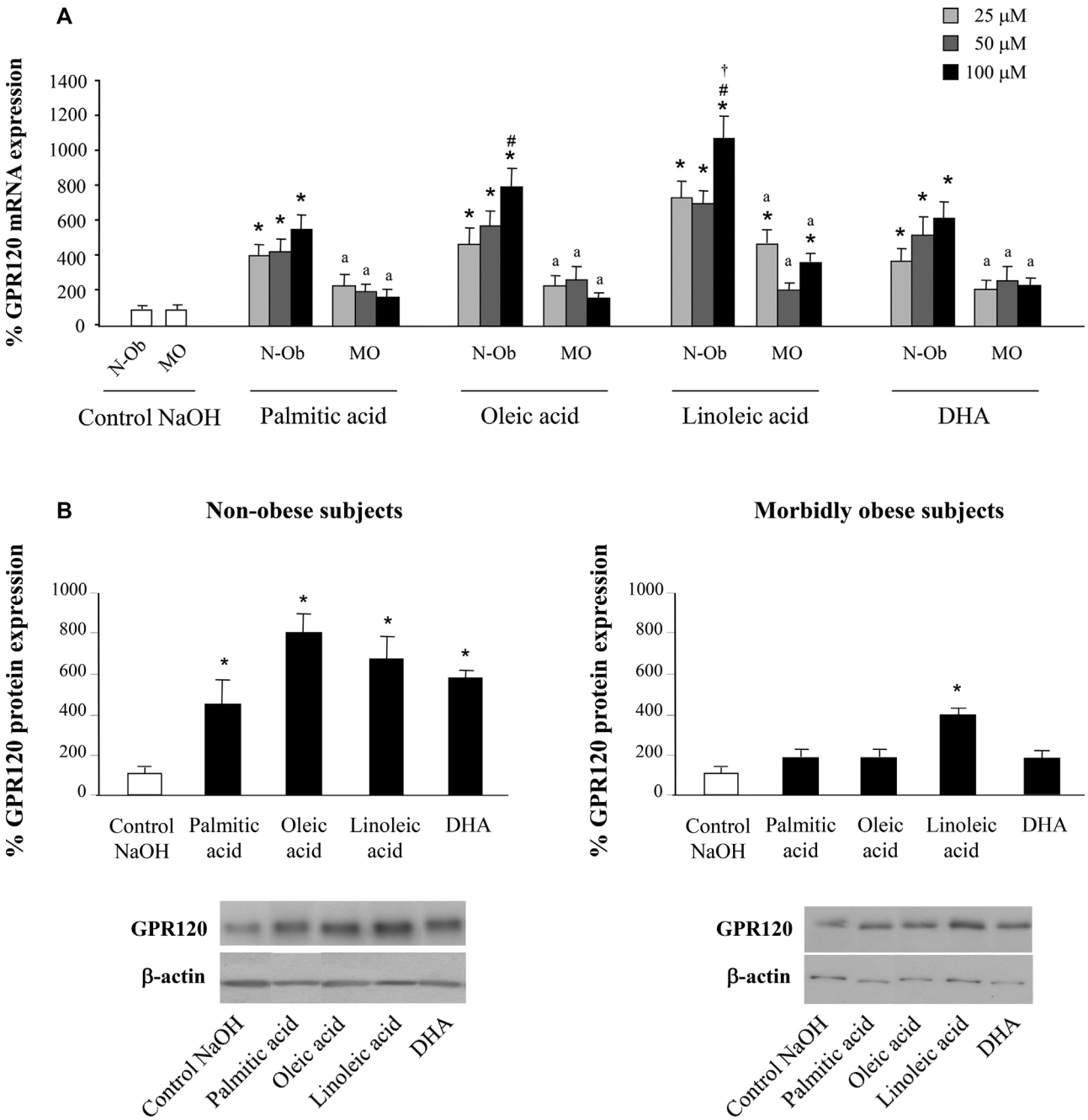

Figure 3. Effect of palmitic (16:0), oleic (18:1n-9), linoleic (18:2n-6), and docosahexaenoic (DHA; 22:6n-3) acids on GPR120 mRNA and protein levels in adipocytes. Adipocytes isolated from human visceral adipose tissue were incubated in DMEM in the presence of palmitic, oleic, linoleic, and DHA (25, 50, and 100 μM) for 24 h. After culture, cells were harvested and GPR120 mRNA and protein levels were determined. The averaged results were then calculated and expressed as a percentage of vehicle-treated control levels (Control NaOH; 100%). Data are the mean ± SEM ($n = 5$ per group). At least three replicate wells were evaluated per treatment in each experiment. (A) Effects on GPR120 mRNA expression levels in visceral adipocytes from nonobese (N-Ob) and morbidly obese subjects (MO). (B) Effects on GPR120 protein levels and representative immunoblot in visceral adipocytes from nonobese and morbidly obese subjects. *$p < 0.05$: significant differences to corresponding control without treatment. #$p < 0.05$: significant differences to 25 and 50 μM doses. †$p < 0.05$: significant differences between 100 μM linoleic acid and all the FA and doses used in the nonobese group. [a]$p < 0.05$: significant differences in GPR120 mRNA expression in visceral adipocytes with each doses and type of FA used between nonobese and morbidly obese subjects.

In nonobese subjects, all FAs and doses used produced a significant increase in GPR120 mRNA expression with respect to controls without treatment (Fig. 3A). Twenty-five micromolar and 50 μM doses had similar effects with all FAs. Only with oleic and linoleic acids, 100 μM showed a significant increase in GPR120 mRNA expression with respect to 25 and 50 μM doses ($p < 0.05$ in all cases; Fig. 3A). Moreover, 100 μM linoleic acid had the most potent effect, being statistically significant compared to the other FAs and doses used ($p < 0.05$). However, in the morbidly obese subjects, only linoleic acid (25 and 100 μM) produced a significant increase in GPR120 mRNA expression with respect to control without treatment ($p < 0.05$ and $p < 0.05$, respectively; Fig. 3A). As 100 μM was the dose with the most significant effect, we confirmed the results obtained by Western blot analysis with this dose (Fig. 3B).

4 Discussion

In this study, we found that nonobese subjects presented more GPR120 mRNA and protein levels in VAT than the morbidly obese subjects, with similar levels between both groups in SAT. GPR120 mRNA and protein levels could be differently regulated by different FAs in adipocytes from nonobese and from morbidly obese subjects. Also, GPR120 mRNA expression in PBMC in the fasting state was lower in morbidly obese subjects, with a decrease 3 h after a high-fat meal only in morbidly obese subjects.

GPR120 has been identified as a receptor for FAs and is mainly expressed in intestinal tissue. However, another tissue highly involved in FA metabolism is adipose tissue because it is the main reservoir of FAs. We showed that GPR120 mRNA and protein levels in VAT, but not in SAT, were higher in nonobese subjects than in morbidly obese patients and independent of the insulin resistance status. However, it has recently been reported that GPR120 expression is significantly higher in obese than in nonobese individuals, in both SAT and VAT [23]. We do not know the reason for this discrepancy. Our results in VAT agree with the results found in PBMC, where GPR120 mRNA expression was also higher in nonobese subjects in the fasting state than in morbidly obese subjects. These results in morbidly obese subjects presenting lower GPR120 mRNA expression in VAT, together with the significant and negative correlation with BMI, suggest that this receptor is associated with obesity, and probably with obesity-associated comorbidities. Different studies report that VAT has a stronger association with metabolic risk factors and the metabolic syndrome than SAT [40, 41]. Also, our results in morbidly obese subjects are in agreement with the results obtained in GPR120-deficient mice [18]. GPR120 KO mice develop obesity, increased inflammation, and insulin resistance, consistent with a role for GPR120 signaling in the metabolic syndrome, obesity, and diabetes mellitus [18]. However, we have not found an association between GPR120 and insulin resistance status.

In previous studies, we have found that a high-fat meal produces an increase in plasma free FAs levels [30, 33]. As GPR120 is an FA receptor, we wanted to determine whether this high-fat meal could have an effect on GPR120 mRNA and protein levels. A previous study found that GPR120 is regulated in a tissue-specific manner in rats in response to a high-fat diet, with GPR120 mRNA expression increasing significantly in cardiac tissue and in extensor digitorum longus skeletal muscle compared to control, but not in soleus muscle or hepatic tissue [42]. In our study, we found a similar increase in GPR120 mRNA expression in nonobese subjects after a high-fat meal. The postprandial results are important because humans are in a postprandial state most of the day [43].

FAs are known to act as endogenous signaling molecules that play a variety of roles in physiological regulation [8–16]. However, little is known about the effect of different type of FAs on GPR120. For this reason, and to confirm the results found in PBMC after a high-fat meal, we incubated visceral adipocytes from nonobese and morbidly obese subjects with saturated (16:0), monounsaturated (18:1n-9), and polyunsaturated FAs (omega-3 and omega-6). Adipose tissue is an organ greatly involved in FA metabolism and storage [44]. Although GPR120 is also expressed in the stromal vascular fraction of adipose tissue [19, 21], the expression of GPR120 mRNA was higher in adipocytes compared to stromal vascular fraction [21]. GPR120 mRNA and protein levels in adipocytes after 24 h of incubation with FAs were higher in nonobese than morbidly obese subjects (Fig 3A and B). This is in concordance with a lower GPR120 mRNA expression in VAT and PBMC in the morbidly obese subjects. In addition, in nonobese subjects, all the FAs tested increased GPR120 mRNA and protein levels, though mainly oleic and linoleic acids, with the linoleic acid being the FA with the most potent effect. In morbidly obese subjects, only linoleic acid had effect on GPR120 mRNA and protein levels. These results seem to suggest that linoleic acid is more selective for this receptor, as has been found in other study [21]. Nevertheless, a limitation of our study concerns the fact that the largest adipocytes are broken in the isolation process. However, even so, the diameter of the isolated visceral adipocytes from morbidly obese subjects is larger than those from nonobese subjects, thereby partially retaining the phenotypic characteristics of these groups of subjects. Other limitation of this study is that doses of FAs used in our in vitro experiments are lower than the human postprandial concentration of free FAs. However, we have used lower FAs doses to avoid the cytotoxic and proinflammatory effects of some FAs (i.e. palmitic and linoleic acids at doses ≥200 μM) [45–48]. Palmitic, oleic, and DHAs at doses lower than 100 μM were unable to induce inflammation on primary culture of human adipose tissue and mature adipocytes [36, 48]. Also, we have used the same dose range for all FAs to made comparisons between the effects of the four FAs used.

Our results contribute to the understanding of the molecular mechanisms by which FAs may be involved in the

regulation of adipocyte metabolism. The role of G-protein-coupled receptors has been described in mediating the effect of FAs on inflammation and insulin sensitivity [49]. These effects could be partially mediated by GPR120. This receptor is an FA receptor with anti-inflammatory and insulin-sensitizing effects [19, 50]. A modification in the levels of this receptor in morbidly obese subjects could potentially participate in the reported adverse consequences of obesity and the metabolic syndrome, and could therefore emerge as a potential new target for treatment of obesity-associated comorbidities [22, 25].

In summary, the results presented here show that morbidly obese subjects have lower GPR120 mRNA levels, mainly after a high-fat meal, and that the direct effect of different type of FAs on GPR120 mRNA and protein levels in visceral adipocytes is altered in this type of subject. These findings may have important implications, since the GPR120 can regulate various physiological processes [17, 19–22] and could be involved in different metabolic disorders [23]. Also, high-fat meals, typical in Western diets, may produce a significant decrease in GPR120 mRNA expression in an obese state and this altered expression of GPR120 mRNA after a high-fat meal may be partially involved in obesity-associated comorbidities. Further studies will be required to define more precisely the relationship between FA-induced changes in GPR120 mRNA and protein levels and the functional consequences of these changes in adipose tissue.

The authors thank P. González López for assistance with technical and administrative support. CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) and CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM) are initiatives of ISCIII. Rodriguez-Pacheco F is supported by a fellowship from Sara Borrell program of the Instituto de Salud Carlos III (CD10/00028). This work was supported in part by a grant from the Instituto de Salud Carlos III (PS09/01060) and Consejería de Economia, Innovacion, Ciencia y Empresa de la Junta de Andalucía (CTS-8081).

The authors have declared no conflict of interest.

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