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Low CD36 monocyte expression levels are associated with overweight in Mexicans with type 2 diabetes

Baja expresión de CD36 en monocitos se asocian con sobrepeso en mexicanos con diabetes tipo 2

Beatriz T. Martín-Márquez[1,2*], Mónica Vázquez-Del Mercado[1,2,3], Pedro E. Sánchez-Hernández[4,5], Erika A. Martínez-García[1,2,4], Fernanda I. Corona-Meraz[6], Flavio Sandoval-García[1,2,7], Soraya A. Zavaleta-Muñiz[8,9], Ma. Aurora Maravilla-Domínguez[8,9], Ana L. Fletes-Rayas[10], and Antonio de J. García-Estrada[1]

[1]Department of Molecular Biology and Genomics, Instituto de Investigación en Reumatología y del Sistema Músculo Esquelético (IIRSME), Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jal.; [2]Research Group UDG-CA-703 "Immunology and Rheumatology", Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jal.; [3]Rheumatology Service, Division of Internal Medicine, Hospital Civil de Guadalajara Dr. Juan I. Menchaca, Guadalajara, Jal.; [4]Physiology Department, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jal.; [5]Immunology Laboratory, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jal.; [6]Department of Biomedical Sciences, Division of Health Sciences, Centro Universitario de Tonalá, Universidad de Guadalajara, Tonalá, Jal.; [7]Neuroscience Department, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jal.; [8]School of Health Sciences, Universidad Juárez del Estado de Durango (UJED), Gómez Palacio, Dgo.; [9]School of Health Sciences, Research group UJED-CA-135, "Environmental nutritional elements in the disease process", Universidad Juárez del Estado de Durango (UJED), Gómez Palacio, Dgo.; [10]Department of Comprehensive Applied Clinical Nursing, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jal. Mexico

ABSTRACT

Objective: To analyze the association of body weight, glucose and oxidized LDL (ox-LDL) on the expression of CD36 in monocytes in Mexican patients with type 2 diabetes mellitus (T2D). **Methods:** 115 individuals without diabetes and patients with TD2 with normal weight and overweight were included in a cross-sectional study. **Results:** Low expression of CD36 was observed in subjects without diabetes and patients with T2D who were overweight compared to normal-weight. **Conclusion:** Patients with T2D and subjects with overweight without diabetes presented low levels of CD36 expression.

Keywords: Type 2 diabetes. CD36. Overweight. Normoglycemic. Ox-LDL. Glucose.

RESUMEN

Objetivo: Analizar la asociación del peso corporal, glucosa y LDL oxidada (ox-LDL) sobre la expresión de CD36 en monocitos en pacientes mexicanos con diabetes mellitus tipo 2 (DM2). **Métodos:** Se incluyeron 115 individuos sin diabetes y pacientes con DM2 con normo-peso y sobrepeso en un estudio transversal. **Resultados:** Baja expresión de CD36 se observó en sujetos sin diabetes y pacientes con DM2 con sobrepeso en comparación con normo-peso. **Conclusión:** Pacientes con DM2 y sujetos sin diabetes con sobrepeso presentaron bajos niveles de expresión de CD36.

Palabras clave: Diabetes tipo 2. CD36. Sobrepeso. Normoglucémico. Ox-LDL. Glucosa.

Correspondence:
*Beatriz T. Martín-Márquez
E-mail: bethymar@hotmail.com

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INTRODUCTION

CD36 is an 88kDa transmembrane class B2 scavenger receptor that binds with high affinity to long-chain fatty acids (FAs), oxidized low-density lipoprotein (ox-LDL), collagen, thrombospondin, and degrades advanced glycation end products involved in diabetic vascular and renal complications[1]. CD36 is expressed in monocytes/macrophages, adipocytes, smooth muscle cells, platelets, and enterocytes among others cells and has influence in glucose homeostasis affecting insulin signaling pathways that may cause pancreatic β-cell dysfunction observed in type 2 diabetes mellitus (T2D)[2]. CD36 expression in monocytes has been shown to be upregulated by hyperglycemia in a post-transcriptional manner and its expression is associated with induction of insulin resistance (IR) contributing to atherogenesis[3]. T2D is a chronic metabolic non-contagious disorder characterized by hyperglycemia, IR, and abnormal FA metabolism associated with cardiovascular complications. T2D is the leading cause of death in México according to the World Health Organization, having a prevalence of diagnosed diabetes in Mexican adults of 10.3% in accordance to "The National Health and Nutrition Survey 2018" (ENSANUT 2018)[4]. The condition of hyperglycemia, hyperinsulinemia, and dyslipidemia among Mexican population is a conditioning factors that can modulate molecules related with lipid clearance and atherosclerotic process, in which, CD36 may contribute to the pathogenesis of T2D and its complications. The role of CD36 in IR and T2D has been controversial; some studies reported that increased circulating CD36 levels were observed in IR states including T2D[3,5]; meanwhile, studies in Japanese patients with diabetes with CD36 deficiency showed highest levels of glucose, hemoglobin (Hb) A1c, high-density lipoprotein cholesterol (HDL-c), and body mass index (BMI) compared to non-diabetic CD36 deficient subjects[6]. With the purpose of complementing our previous findings, where we found that the *CD36* rs3211938 TG genotype is associated with high glucose, ox-LDL, HDL-c levels, and IR in T2D Mexican patients[7], we conducted an analysis to evaluate whether the adiposity-related parameters and lipids are associated with peripheral blood mononuclear cells (PBMCs) CD36 expression in T2D and non-T2D normoglycemic (NT2D) individuals with normal weight (NW) and obesity (OW) from West México.

MATERIAL AND METHODS

Subject assessment

We analyze 115 subjects in a cross-over study classified in NT2D adults and patients with T2D (n = 58 and n = 57, respectively) from a previous study. The protocol was carried out in a period of 8 months (June 2018-February 2019)[7]. The NT2D subjects were recruited from open urban population and the T2D from the external consultation of the Endocrinology Specialty Service of the Hospital Civil Dr. Juan I. Menchaca, at Guadalajara, Jalisco, Mexico. The treatment of the patients with T2D was metformin in a dosages that ranging from 500 to 1000 mg/day. The exclusion criteria for all recruited subjects included malignancy, renal, hepatic, or thyroid disease, pregnancy, smoking, and statin and lipid-lowering drugs treatment use. In addition, we considered exclusion criteria of insulin treatment for patients with T2D. We employed a structured questionnaire that was applied to each NT2D and patients with T2D to obtain demographical and clinical information. The study design and protocol were conducted in accordance with the guidelines of the Declaration of Helsinki and were approved by the Institutional Review Board committee of the Hospital Civil Dr. Juan I. Menchaca, with the number 957/09 and by Investigation, Ethics and Biosafety Committee of Centro Universitario de Ciencias de la Salud of Universidad de Guadalajara with the ethical certificate number: CI-7108.

Anthropometric measurements

Anthropometric measurements were taken in duplicate as follows: height was measured by using a stadiometer (seca Gmbn & Co. Kg™); body weight

and total body fat were determined using bioelectrical impedance analysis (TANITA BC-577F). Waist–hip circumference (WC) was measured in the midway between the inferior margin of the ribs and superior border of iliac crest and the hip circumference (HC) at the fullest point around the buttocks. BMI was calculated as weight (kg) divided by height (m^2) and waist–hip radio (WHR) was calculated with WC divided by HC. HOMAR-IR index was calculated with the formula HOMA-IR = fasting insulin level (μUI/mL) × fasting glucose level (mg/dL)/405. Blood pressure was measured twice with certified sphygmomanometer after 15 min of rest in supine position. We based on the World Health Organization (WHO) criteria to classify individuals with NW < 25.0 kg/m^2 and OW ≥ 25.0 kg/m^2.

Metabolic profile determinations

Basal glucose (mg/dL), HDL-c, low-density lipoprotein cholesterol (LDL-c), and HbA1c serum levels were determined with routine colorimetric, enzymatic, and immunoturbidimetry methods (Randox™ Laboratories). Normoglycemia was defined as fasting basal glucose ≤ 100 mg/dL. Basal insulin and ox-LDL levels were performed by ELISA (Immundiagnostik™).

CD36 PBMC membrane expression

PBMC were isolated by density gradient centrifugation using Lymphoprep™ (Nycomed Pharma™). Once we obtained 5 × 10^6 cells in 50 µL of phosphate-buffered saline (PBS), we performed the flow cytometry using fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibodies against human CD36 and phycoerythrin (PE)-conjugated anti-human CD14 (BioLegend™). The PBMCs were incubated with FITC- or PE-conjugated monoclonal antibodies for 30 min at 4°C in darkness at room temperature, then were washed twice and fixed with 1% paraformaldehyde before being assayed with a flow cytometer (Beckman Coulter™) and analyzed with the software WinMDI v2.9. The results were expressed as median fluorescence intensity (MFI).

Statistical analysis

Anthropometric measurements, lipid profile, and CD36 expression levels were analyzed with Kruskal–Wallis test or Mann–Whitney *U*-test as appropriate. Correlations were assessed by Spearman test and multiple linear regression was also designed to establish predictor T2D variables of CD36. Results are given as mean ± standard deviation (SD) or median with 25 and 75 percentiles. Data were analyzed by statistics software SPSS v23 (IBM Inc., Chicago, IL, USA). A two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

NT2D and subjects with T2D anthropometrical and metabolic profile determinations

We included 115 individuals classified in NT2D adults (n = 58) and patients with T2D (n = 57); 69.8% and 67.7% were female aged 42 ± 16.5 and 47.7 ± 17.6 years, respectively. The NT2D and subjects with T2D anthropometrical and metabolic profile differences are observed in figure 1. When comparing subgroups with NW and OW, we observed that subjects with OW presented the higher measurements in BMI, WHT, weight, total body fat %, and weight/fat, as well as in LDL-c levels. Regarding ox-LDL, we noticed that NT2D and T2D with NW showed the highest levels compared to NT2D and individuals with T2D.

CD36 PMBC expression and metabolic markers

When we analyzed the CD36 MFI monocyte expression in PMBC in the study subjects, we observed that the subjects with T2D showed a low CD36 expression compared to NT2D subjects ($p < 0.001$; Fig. 2). Consequently, we performed an analysis taking account the WHO criteria classifying the NT2D and subjects with T2D in NW and OW; and we noted that the CD36 MFI monocyte expression showed a decrement in NT2D and T2D with OW compared to NW subjects (Fig. 3). Regarding metabolic parameters,

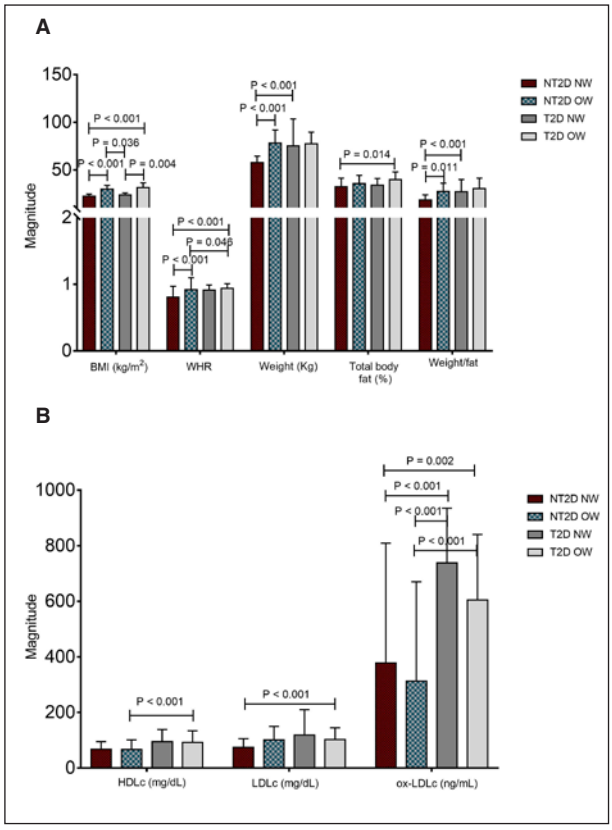

Figure 1. Comparison of adiposity-related parameters and lipid profile in study subjects. **(A)** Comparison of adiposity-related parameters among NT2D and subjects with T2D with NW and OW. **(B)** Comparison of lipid profile among NT2D and subjects with T2D with NW and OW. NT2D: non-type two diabetes mellitus; T2D: diabetes patients with type 2 diabetes; NW: normal weight; OW: overweight; WHR: waist–hip ratio; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; ox-LDL: oxidized low-density lipoprotein cholesterol. Data are presented as means ± standard error of the mean (SEM). Kruskal–Wallis test was used.

we observed that the glucose basal levels and HOMA-IR in patients with T2D with NW showed the higher measurements compared to T2D with OW. We do not observe differences between individuals in insulin basal levels despite the OW (Fig. 3).

CD36 PMBC expression is negative associated with body dimensions and metabolic markers

We performed a Spearman test with the variables in contrast to CD36 PMBC data expression, and we noticed that CD36 is conversely associated with

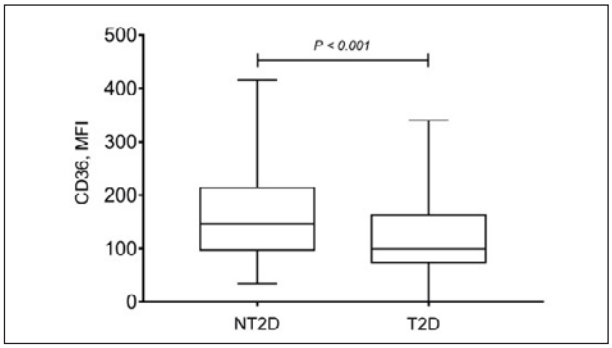

Figure 2. CD36 peripheral monocyte expression in NT2D adults and patients with T2D. n = 115. NT2D: non-T2D adults; T2DM: patients with type 2 diabetes; MFI: mean fluorescence intensity. Data are presented as quartiles, minimum, maximum, and median. Mann–Whitney *U*-test was used.

Table 1. Multiple regressions between CD36 PBMC membrane levels and T2D variables

Total R	0.529 β-coefficient (95% CI)	p
Constant	464.499 (179.370-749.628)	0.002
ox-LDL (ng/mL)	−0.115 (−0.214-[−0.016])	0.025
Glucose (mg/dL)	−0.336 (−0.686-0.013)	0.050
Waist–hip ratio	−232.349 (−523.778-59.080)	0.114

Adjusted model: β estimates and adjusted R^2 was 0.280. β-coefficients are given as value (95% confidence interval).
PBMC: peripheral blood mononuclear cells; T2D: type 2 diabetes; CI: confidence interval

BMI, WHR, glucose, and ox-LDL (*rho* = −0.2135 to −0.2486; Fig. 4).

Ox-LDL, glucose, and WHR predict variations in CD36 PMBC expression levels

We performed multiple logistic regression analysis and noticed that ox-LDL, glucose, and WHR predict 28% of the variation on the levels of CD36 PMBC membrane expression (Table 1).

DISCUSSION

CD36 is a scavenger receptor expressed in a wide variety of cells that are sensitive to lipids and carbohydrate metabolism alterations and inflammatory

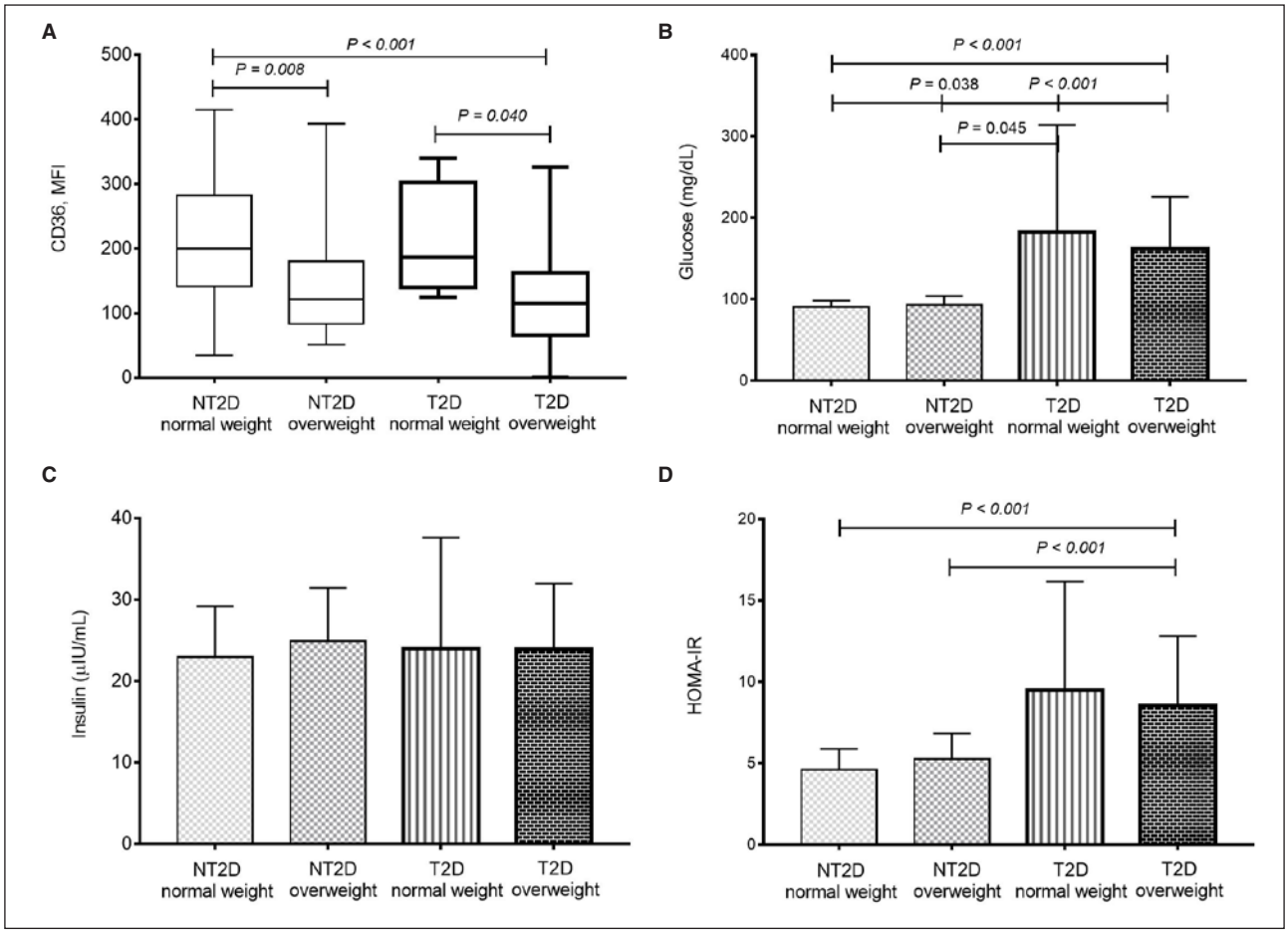

Figure 3. CD36 expression and metabolic markers in NT2D and T2D in with NW and OW. n = 115. **(A)** CD36 expression in individuals with normal weight and overweight. Data are presented as quartiles, minimum, maximum, and median. **(B)** Basal glucose levels in individuals with NW and OW. **(C)** Basal insulin levels in individuals with NW and OW. **(D)** HOMA-IR in individuals with NW and OW. Data are presented as means ± standard deviations. Mann–Whitney *U*-test was used. NT2D: non-T2D; T2D: patients with type 2 diabetes; NW: normal weight; OW: overweight; MFI: mean fluorescence intensity.

response modulation. Due to its participation in the development of β-cell dysfunction, CD36 is a key receptor involved in T2D immunopathology[2]. In this regard, we conducted a study in patients with T2D and normoglycemic NT2D individuals with NW and OW from West México with the objective of determine an association of adiposity-related parameters and lipids with the expression levels of CD36 in PBMC. IR is one of the main characteristics of pathological manifestation associated to T2D. In our study, we observed that NT2D and T2D have high insulin levels and present higher HOMA-IR classified as IR. This impaired metabolic homeostasis could be related to dyslipidemia that compromised liver metabolism, even in NT2D with NW[8]. When we determined the CD36 MFI in monocytes in NT2D and subjects with T2D, we observed that the patients with T2D showed the low CD36 expression levels compared to NT2D. When performing a patient classification in NW and OW, we noticed that the NT2D and patients with T2D with OW showed the lower CD36 expression levels compared to NW. These observations are in concordance with the results obtained in a study performed by Madrigal-Ruiz et al. in Mexican mestizo individuals ≥ 30 years old with abdominal obesity, where they observed a CD36 mRNA subexpression (54% less) and decreased levels of soluble CD36 (sCD36) in a condition of abdominal fat and higher fasting glucose, compared with individuals with abdominal

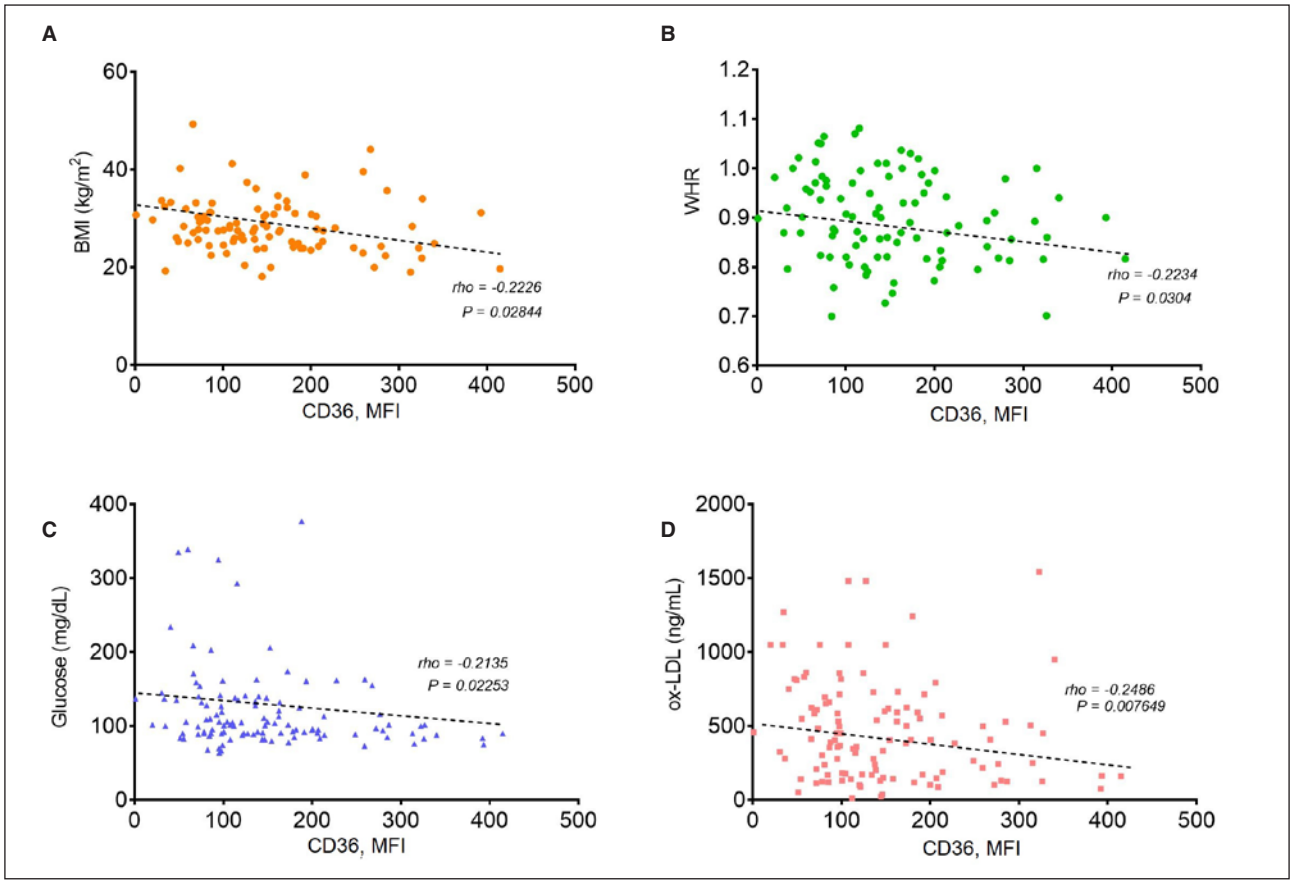

Figure 4. CD36 correlations with body dimensions and metabolic markers. **(A)** CD36 correlation with BMI. **(B)** CD36 correlation with WHR. **(C)** CD36 correlation with glucose. **(D)** CD36 correlation with ox-LDL. Spearman test. MFI: mean fluorescence intensity; BMI: body mass index; WHR: waist–hip ratio; ox-LDL: oxidized low-density lipoprotein cholesterol.

obesity < 30 years[9]. However, although sCD36 levels reflect the monocytes and macrophages CD36 expression in adipose tissue, arteries, and liver, it is possible that specific population factors such as size population, age, T2D time duration, and *CD36* genetic variations may affect sCD36 concentrations[10]. On the other hand, we observed that our observations agree to Gomez-Bañuelos et al. results, where they reported a low CD36 PMBC receptor levels in Mexican women with obesity and pharmacologically treated rheumatoid arthritis patients with subclinical atherosclerosis[11]. We also noticed that CD36 PBMC expression conversely associate with BMI, WHR, glucose, and ox-LDL. These results reinforce the findings of Madrigal and colleagues where sCD36 conversely associates with WC, total body fat mass, body fat index, and LDL-c, among other metabolic parameters in Mexican populations[9]. Some studies suggested that CD36 deficiency could be detrimental on human health, indeed, it was observed that CD36 deficient individuals present higher HbA1c plasma levels, IR, and significantly increased rate of morbidity by cardiovascular complications[12]. Low CD36 expression levels can be result of the CD36 internalization in adipose tissue in response to excess of FA during lipolysis in an IR state, accompanied by the decrease of adiponectin production[10]. Moreover, in our study, we also notice that ox-LDL, glucose, and WHR can predict 28% the variation in CD36 PBMC expression levels. Since CD36 is consider as a central scavenger receptor for the detection, accumulation, and metabolism of lipids and FA in different tissues and cells, these results strengthen the central role of ox-LDL and the hyperglycemia as a triggers for metabolic complications associated with long-term obesity[13]. CD36 binds

ox-LDL with high affinity and its responsible for the transformation of the monocyte-macrophages into foam cells in the 60-70% of cases, being a key element for the formation of atheroma[14]. In a diabetes microenvironment, ox-LDL disrupts insulin signaling in adipocytes and macrophages inducing metabolic abnormalities such as impaired glucose uptake, decreased secretion of adiponectin, and increased lipolysis[10]. There has been observed that in a lipolysis scenario, lipolytic products can induce CD36 internalization in a feedback loop that might protect against excessive depletion of intracellular triacylglycerol[15]. In our study, we observed that NT2D and subjects with T2D with NW showed the highest levels of ox-LDL compared to OW individuals and could be attributable to the intrahepatic fat and to the development of dyslipidemia and hepatic and peripheral IR in individuals with NW[8]. On the other hand, hyperglycemia is an important modulator of *CD36* mRNA and protein *in vitro* and hyperglycemic conditions have been found to be responsible for the synthesis of CD36 both mRNA and protein in healthy subjects[14]. However, the hyperglycemia in a dysfunctional metabolic state could change CD36 expression and contributes to an increase of FA uptake[10]. Furthermore, the T2D treatment could influence of CD36 expression; some studies confirm that metformin treatment inhibit lipid uptake through downregulation of CD36 by suppressing β-catenin and nuclear factor kappa B (NF-κβ), attenuating the inflammatory response by suppression of tumor necrosis factor alpha (TNF-α) production that impairs CD36 expression[16,17].

CONCLUSION

We conclude that NT2D and subjects with T2D with OW showed the lowest CD36 PBMC expression levels compared to NW individuals of Mexican population from West México. Ox-LDL, glucose, and WHR predict 28% of the variation on the levels of CD36 PMBC membrane expression. Further research is required to evaluate the biological role of CD36 PBMC expression in the development and progression of T2D and its complications.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL DISCLOSURES

Protection of people and animals. The authors declare that the procedures followed were in accordance with the ethical standards of the responsible human experimentation committee and in accordance with the World Medical Association and the Declaration of Helsinki.

Confidentiality of the data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the corresponding author.

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