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RESEARCH ARTICLE

Vascular Cell Adhesion Molecule 1 and Adiponectin Receptor 1 in Plasma-Derived Extracellular Vesicles of Type 2 Diabetes Patients

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ABSTRACT

Background: Patients with diabetes are susceptible to accelerated vascular damage associated with increased circulating extracellular vesicles (EVs) from endothelial tissue. Adiponectin is a cytokine with anti-inflammatory and insulin-sensitivity properties decreased in the plasma of patients with type 2 diabetes (T2D). This study aimed to assess the content of receptor 1 for adiponectin (AdipoR1) in both total and endothelial EVs from plasma samples of T2D patients and normoglycemic subjects.

Methods: Insulin and soluble vascular endothelial molecule-1 (sVCAM-1) in plasma were measured by ELISA, and we used flow cytometry to determine the content of AdipoR1, CD106, and CD144 in plasma-derived EVs from 14 patients with diabetes and 34 normoglycemic subjects.

Results: Compared to normoglycemic subjects, the percentage of vesicles CD144+ ($p = 0.0041$) and CD106+ ($p = 0.0011$) were higher in patients with diabetes. Compared to T2D patients, the percentage and medium fluorescence intensity from the total EVs/AdipoR1+ ($p = 0.041$ and $p = 0.0220$) were higher in normoglycemic subjects. Plasmatic sVCAM-1 was negatively correlated with plasma adiponectin ($b = -14.415$, $p < 0.0001$), along with the percentage of EVs/AdipoR1+ ($b = -8.209$, $p = 0.001$); and correlated positively with the percentage of EVs/CD144+ ($b = 6.768$, $p < 0.0001$) and HOMA-IR ($b = 67.919$, $p < 0.0001$).

Conclusions: Our results show that the content of AdipoR1 in plasma-derived VEs is lower in patients with T2D than in subjects with normoglycemic. AdipoR1 content in EVs is negatively associated with the endothelial damage marker sVCAM-1 level in plasma and with the HOMA-IR value.

Keywords: Adiponectin, type 2 diabetes, AdipoR1, extracellular vesicles

Introduction

Adipocytes and macrophages in adipose tissue produce and secrete adiponectin, a plasma protein present in the circulation as a dimer, trimer, hexamer, and in the oligomeric form (globular form) and whose levels decrease in obesity and type 2 diabetes (T2D).[1,2] Adiponectin stimulates the fatty acids oxidation, decreases plasma triglyceride levels, and improves glucose metabolism by increasing insulin sensitivity by activating its cell receptors AdipoR1 and AdipoR2, both cell surface receptors.[3,4] The anatomical location of AdipoR1 is ubiquitous, with predominant expression in skeletal muscle cells, whereas AdipoR2 is primarily present in liver cells. Several studies in murine and in vitro models demonstrated that adiponectin, by interaction with AdipoR1, induces the activation of several cellular signals that translocate the Glucose Transporter-4 to the cell surface, activating the oxidation of fatty acids and increase insulin signaling.[2,4]

Diabetes is a chronic disease whose one of the main characteristics is hyperglycemia, which occurs due to failure to produce and/or use insulin at the tissue level.[5] In patients with T2D, the number of plasma extracellular vesicles (EVs) increases, which also occurs in atherosclerosis, coagulopathies, and obesity, among other conditions.[6] Extracellular vesicles are a heterogeneous population of plasma membrane micro-fragments (100 - 1000 nm) released to body fluids after stimulation and activation of most cell types, including circulating cells and endothelial cells *in vivo* and *in vitro*, and they participate as vehicles of intercellular communication by their ability to transfer proteins, nucleic acids, and other biomolecules.[7,8]

Plasma-derived EVs from endothelial cells are considered potential markers of endothelial dysfunction, and endothelial markers such as VE-Cadherin (also called CD144) and activation markers such as CD106 (also called vascular cell adhesion molecule-1, VCAM-1) have been used for the EVs identification and analysis.[8–10]

However, the study of the expression of AdipoR1 has been limited to analysis of its RNAm, using murine models or cell cultures, so it is essential to demonstrate the content of AdipoR1 at the protein level in cells from patients with T2D; therefore, it is feasible to study their plasma-derived EVs to have an approximation for the molecular content and state of the cells from which they come.[8] Hence, the objective of this study was to determine the presence of the AdipoR1 at the surface of the total EVs and EVs from the endothelial origin, EVs/CD144+, of patients with T2D and explore its possible association with the degree of insulin resistance evaluated by HOMA-IR and endothelial activation measuring the plasma VCAM-1 marker.

Materials and methods

Materials

Fluorescent latex beads of 1.0 and 0.1 µM particle size (Cat. L2778 and L9904, respectively) were obtained from SIGMA-Aldrich® (Saint Louis, MO). Annexin V-FITC (Cat. 640905), Annexin V binding buffer (Cat. 422201), anti-human monoclonal antibody CD144-PE (Cat. 348505), anti-human monoclonal antibody CD106-PE/Cy5 (Cat. 305808), isotype anti-human IgG2a-PE controls (Cat. 400213) and human IgG1-PE/Cy5 (Cat. 400117) were obtained from BioLedend® (San Diego, CA). The goat anti-human adipoR1 polyclonal antibody M-18 (Cat. Sc-46749) and the anti-goat IgG-PerCP antibody (Cat. Sc-45091) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The ELISA kit for human adiponectin/Acrp30 (Cat. DY1065) was used by R & D Systems® (Minneapolis, MN), the ELISA kit for human insulin (Cat. IS130D) from Calbiotech Inc. (Spring Valley, CA) and the ELISA kit for the detection of sVCAM-1/CD106 human kit (Cat. 440308) was from LEGEND MAX ™ (San Diego, CA). The rest of the used reagents were of the highest grade.

Study population

Fourteen subjects previously diagnosed, with no more than 10 years of evolution with T2D, and 34 normoglycemic control subjects who attended the Clinical Chemistry Laboratory of the Department of Medicine and Health Sciences in the University of Sonora conform to the study groups; we made the diagnosis following the criteria established by the American Association of Diabetes.[11] Patients with cancer and/or infectious process, abnormal liver, kidney, or thyroid function, and steroid therapy within two months of sample capture were excluded.

The protocol has the approval of the Bioethics and Research Committee of the Department of Medicine and Health Sciences of the University of Sonora; all the procedures follow the International Ethical Standards set out in the Helsinki Declaration, and we obtained informed written consent from each participant.

Anthropometric measurements, blood sampling, and laboratory analysis

Weight was measured using a Defender 3000 series electronic scale (Ohaus, Pine Brook, NJ), and height with a wall-mounted stadiometer from Holtain Ltd. (Dyfed, United Kingdom). The body mass index (BMI) was calculated as equal to the weight divided by height squared (kg/m^2).

Blood samples were collected after 8 - 12 h overnight fasting in BD Vacutainer® EDTA added, sodium citrate, and additive-free tubes. The serum was used to determine glucose, total cholesterol, HDL, LDL, triglycerides, uric acid, creatinine, urea, and total proteins using standardized enzymatic and/or colorimetric kits (Randox Laboratories Ltd). The determinations of soluble VCAM-1, adiponectin, and insulin in plasma were performed using the ELISA technique from plasma samples with EDTA. The HOMA-IR values were estimated according to Matthews *et al.*, 1985.[12,13] The citrate blood samples were used for flow cytometric determinations.

Platelet-poor plasma obtaining

Blood samples in tubes with sodium citrate were centrifuged at 1,500 x g for 15 min; the plasma was separated and centrifuged at 3,000 x g for 30 min to eliminate apoptotic bodies, and third centrifugation at 15,000 x g for 30 min of the plasma at 4 °C was performed to obtain platelet-poor plasma, the supernatant (vesicle-enriched plasma) was aliquoted and stored at - 80 °C until analysis of the EVs by flow cytometry.

Flow cytometry analysis of plasma-derived EVs

The flow cytometry analysis (FACS) of the vesicles was executed using calibrated fluorescent latex beads of 1.0 and 0.1 µm diameter to select the capture area of the EVs.[14] 20,000 Annexin V+ events were evaluated in the 0.1 - 1.0 µm area per assay using a FACSCalibur™ cytometer from Becton Dickinson (San Diego, CA) and the Summit Software Informer® program (Figure 1).

The expression of the endothelial marker CD144,[24] the activation marker CD106, and AdipoR1 on the surface of the plasma EVs/Annexin V+ was evaluated by staining of the EVs. Briefly, 25 µL of platelet-free plasma previously blocked by incubation with PBS/human IgG (50 µg/µL of Beriglobina P, CSL Behring®, Mexico City, Mexico) for 30 min at room temperature.[15] Annexin V-FITC and Annexin V binding buffer were added and incubated for 30 min in the dark, and anti-CD144-PE primary antibodies, anti-CD106-PE-Cy5, and anti-AdipoR1 were 30 min incubated. The secondary antibody coupled to PerCP was added and incubated for 30 min in the dark, and finally perform the cytometry acquisition (Figure 1).

Data analysis

The Student *t*-tests for independent samples, U Mann Whitney, and chi-square were used to analyze the differences between the groups for the clinical and biochemical variables, depending on the normality and type of the variable studied. We evaluated differences in AdipoR1, CD144, and CD106 content in plasma-derived EVs between groups by student t-tests. Simple linear regression analysis of plasmatic sVCAM-1 and the HOMA-IR values with the adiponectin values and the percentage of plasma-derived EVs containing CD144, CD106, AdipoR1, and other variables as appropriate were performed. All statistical analyzes were executed using the 21.0 version of the SPSS software for Windows (Chicago, USA).

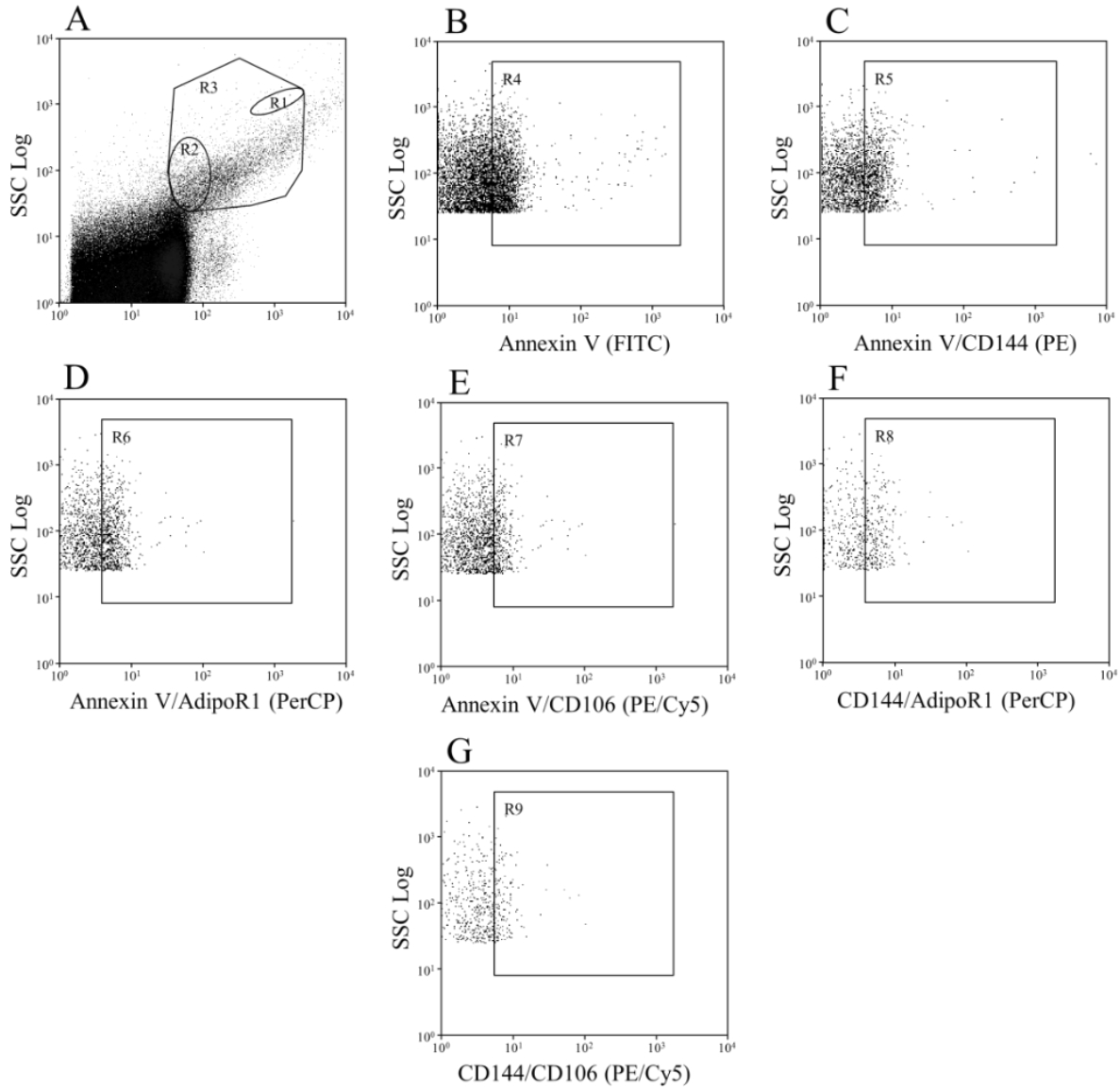

Figure 1. *FACS analysis of plasma extracellular vesicles.* A)'R1' region of beads of 0.1 µm, 'R2' of 1.0 µm, and 'R3' total EVs region (0.1 - 1.0 µm). B)'R4' are EVs/Annexin V+. C)'R5' are EVs/CD144+ taken from 'R4'. D)'R6' are EVs/AdipoR1+ taken from 'R4'. E)'R7' are EVs/CD106+ from 'R4'. F)'R8' are EVs/CD144+/AdipoR1+ from 'R5'. G)'R9' contain EVs/CD144+/CD106+ from 'R5'.

Results

Clinical and biochemical characteristics of the study groups

Differences between groups were found in fasting glucose (p = 0.0001), fasting plasma insulin (p = 0.0001), plasma sVCAM-1 (p = 0.0340), and HOMA-IR values (p = 0.0001), which were higher in patients with T2D (Table 1). Adiponectin concentrations were higher in the normoglycemic group than in the T2D subjects (p = 0.0001). The total cholesterol, LDL, HDL, and triglyceride concentrations show no significant differences between groups.

VCAM-1, VE-Cadherin, and AdipoR1 detected in plasma vesicles surface

The region for the cytometry analysis of the EVs was designated using calibrated fluorescent beads of 0.1 and 1.0 µm in diameter (Figure 1A), and the EVs/Annexin V+ in this size region were analyzed (Figure 1B). The CD144+ endothelial marker, the VCAM-1/CD106 marker of cell activation, and the receptor AdipoR1 were detected on the plasma EVs/Annexin V+ surface by flow cytometry (Figure 1B to 1G).

The percentage of EVs positive for the analyzed molecules was compared between the study groups (Figure 2). The T2D group plasmas showed a higher percentage of EVs/CD144+ (p = 0.0041) and of EVs/CD144+/CD106+ (p = 0.0017) compared to the control group (Figure 2A

and 2D). The percentage of EVs/AdipoR1+ from the total plasma EVs in T2D subjects was lower than in control subjects (p = 0.0098) (Figure 2C). The percentage of total EVs/CD106+ was higher in the group of T2D compared to control subjects (p = 0.0011) (Figure 2B).

The mean fluorescence intensity (MFI) of AdipoR1 is higher in the control group than the T2D group both in total EVs (p = 0.0220) as in the population of EVs derived from endothelial cells (EVs/CD144+) (p = 0.0306) (Figure 3A and 3B). On the other hand, the MFI of the CD106 marker is higher in T2D patients both in total EVs (p = 0.0143) and in the population of endothelial plasma-derived EVs/CD144+ (p = 0.0209) (Figure 3C and 3D).

Univariate analysis of the plasma sVCAM-1 marker and HOMA-IR

The linear regression analysis results using VCAM-1 as the dependent variable show a positive association with HOMA-IR (b = 66.663, p < 0.0001), percentage of EVs/CD144+ (b = 6.768, p < 0.0001), EVs/CD106+ (b = 8.832, p < 0.0001), EVs/CD144+/CD106+ (b = 4.8121, p = 0.0439) and the endothelial origin EVs/CD144+/AdipoR1+ (b = 4.3211, p = 0.0481); and a negative association with plasma adiponectin concentration (b = - 14.415, p = 0.0001), as well as for the total percentage of plasma EVs/AdipoR1+ (b = - 8.209, p = 0.001) (Table 2).

The univariate regression analysis for HOMA-IR value shows a negative association with plasma adiponectin (b = - 0.1581, p < 0.0001) and with the percentage of total EVs/AdipoR1+ (b = - 0.0929, p < 0.0001) (Table 3). The HOMA-IR value shows a positive association with the percentages of plasma-derived EVs/CD144+ (b = 0.0703, p < 0.0001), EVs/CD106+ (b = 0.080, p < 0.0001), and EVs/CD144+/CD106+ (b = 0.054, p = 0.0218) (Table 3).

Table 1. Clinical and biochemical characteristics of the study subjects.

Parameter (n = 48)	**T2D** (n = 14)	**Control** (n = 34)	***p***
Age (years)	53.78 ± 15.20	54 ± 12.20	0.9583
Gender (% male)	57.10	44.10	0.4116
BMI (kg/m²)	33.92 ± 12.74	27.08 ± 3.93	0.0591
Blood pressure			
Systolic	119.0 (105.0, 170.0)	126.50 (111.0, 180.0)	0.5563
Diastolic	80.00 (70.0, 90.0)	80.00 (70.0, 103.0)	0.2832
Fasting plasma glucose (mmol/L)	6.44 (8.5 – 5.8)	4.97 (5.2 – 4.4)	0.0001
Fasting plasma insulin (µU/mL)	5.83 (14.2 – 4.5)	3.94 (4.8 – 2.7)	0.0001
HOMA-IR	1.80 (4.2 – 1.2)	0.83 (1.1 – 0.5)	0.0001
Total cholesterol (mg/dL)	225 ± 70.96	204 ± 44.71	0.3558
Triglycerides (mg/dL)	136.5 (182.5 – 100.2)	126.5 (164.5 – 86.5)	0.4472
LDL (mg/dL)	132.5 (191.4 – 92.5)	134 (147.4 – 102.7)	0.6746
HDL (mg/dL)	40.50 (54.7 – 32.5)	46 (56.2 – 37.5)	0.6809
Male HDL (mg/dL)	58.33 ± 16.36	49.76 + 10.01	0.1422
Female HDL (mg/dL)	35.62 ± 8.65	44.73 ± 17.16	0.1764
Adiponectin (µg/mL)	5.33 (7 – 3.55)	10.66 (15 – 9.5)	0.0001
VCAM-1 (ng/mL)	497.09 ± 170.31	406.04 ± 111.53	0.0340
No treatment (%)	64.28	0	< 0.0001
Hypoglycemic agents use (%)	57.1	0	< 0.0001
Insulin use (%)	14.28	0	0.0243
Insulin + hypoglycemic agents use (%)	7.14	0	0.1152
Biguanides use (%)	7.14	0	< 0.0001
Sulfonylureas use (%)	57.14	0	0.1152
TZD use (%)	7.14	0	0.1152
Fibrates / statins use (%)	21.42	2.94	0.0351
ACEI or ARB use (%)	14.28	14.70	0.0777

U Mann-Whitney, *t*-test and/or square chi were performed according to the distribution and type of data. The results are expressed as mean ± SD, median (range), or %. T2D, type 2 diabetes; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VCAM-1, vascular cell adhesion molecule 1; TZD, thiazolidinedione; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

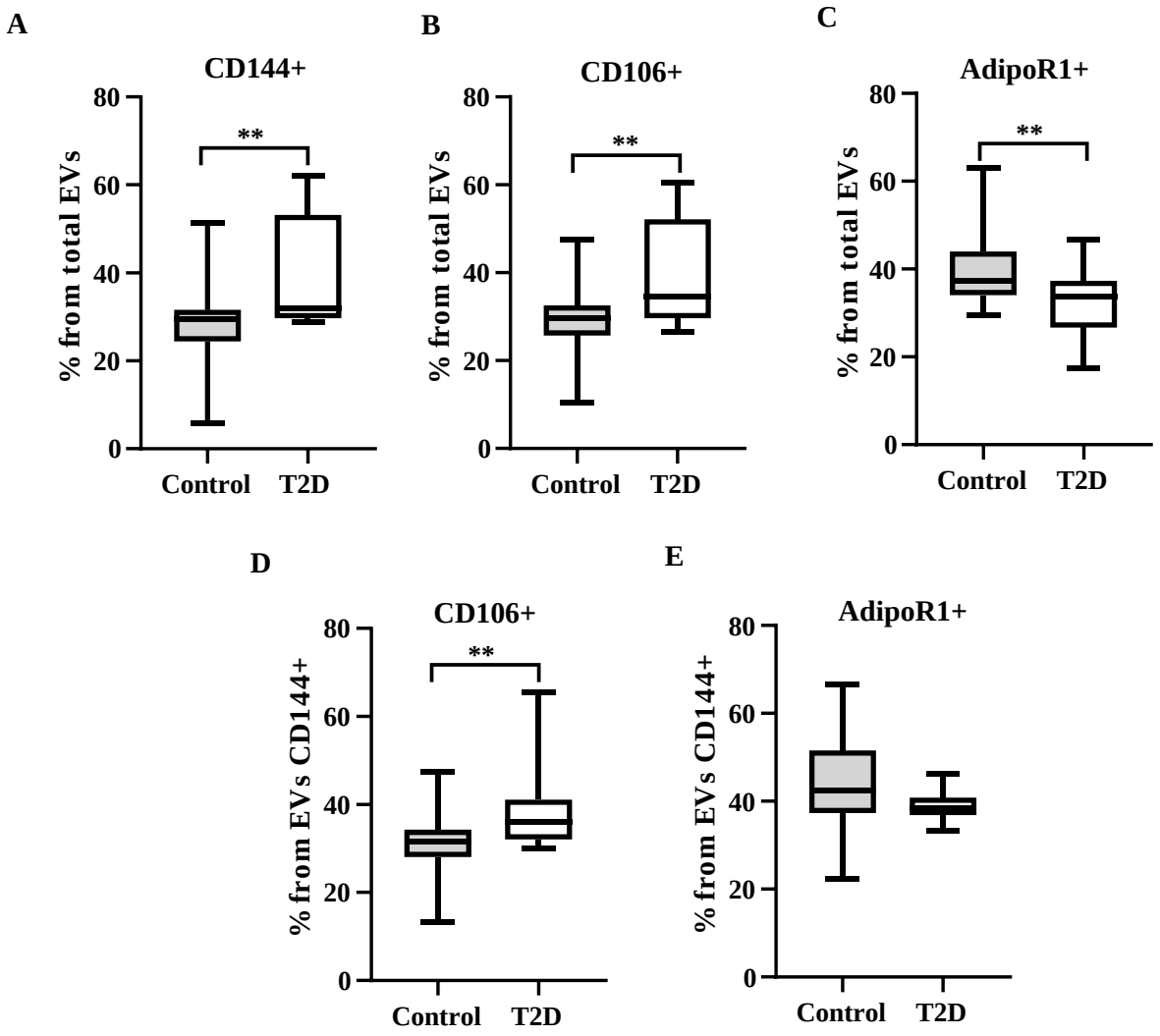

Figure 2. *Vesicles AdipoR1+, CD106+, and CD144+ from the plasma*. The graph represents the EVs percentage that carries A) CD144, B) CD106, C) AdipoR1, D) CD144 and CD106, and E) CD144 and AdipoR1 from the total EVs/Annexin V+ ('R4', Figure 1B). *$p < 0.05$ and **$p < 0.005$ by *t* Student test. T2D, Type 2 Diabetes; EVs, extracellular vesicles.

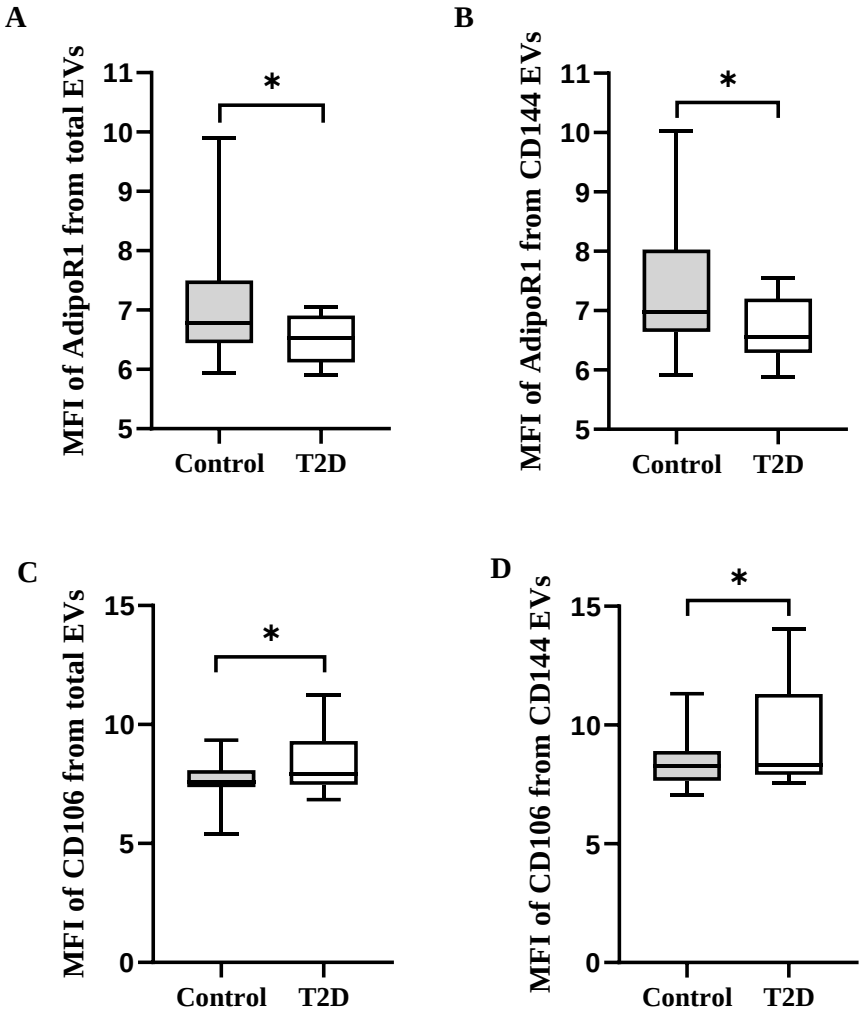

Figure 3. *Mean fluorescence intensity of AdipoR1 and CD106 in EVs*. The graph represents the MFI of AdipoR1 and CD106 from total EVs (A, C) and EVs/CD144+ (B, D) ('R4', Figure 1B). *$p < 0.05$ by *t* Student test. T2D, type 2 diabetes; EVs, extracellular vesicles; MFI, mean fluorescence intensity.

Table 2. Linear regression relationship analysis between soluble VCAM-1 with the percentage of plasma EVs and other variables.

Variable (n = 48)	**VCAM-1**		
	β	**S. E.**	***p***
Group (T2D yes)	91.700	43.040	0.0390
Fasting P-glucose (mg/dL)	1.080	0.506	0.0370
HOMA-IR	66.633	11.128	<0.0001
Adiponectin (µg/mL)	-14.415	3.524	0.0001
% EVs/CD144+	6.768	1.616	<0.0001
% EVs/AdipoR1+	-8.209	2.349	0.0010
% EVs/CD106+	8.832	1.677	<0.0001
% EVs/CD144+/AdipoR1+	4.321	2.128	0.0481
% EVs/CD144+/CD106+	4.812	2.322	0.0439
ACEI or ARB use	-56.553	55.615	0.3145
BMI (kg/m^2)	5.802	2.476	0.0240

VCAM-1, a vascular cell adhesion molecule 1; T2D, type 2 diabetes; HOMA-IR, homeostatic model assessment for insulin resistance index; EVs, extracellular vesicles; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index.

Table 3. Linear regression relationship analysis between HOMA-IR with the percentage of plasma EVs and other variables.

Variable (n = 48)	HOMA-IR		
	β	S. E.	*p*
Group (T2D group, yes)	1.890	0.3323	< 0.0001
Fasting P-glucose (mg/dL)	0.023	0.0036	< 0.0001
Total cholesterol (mg/dL)	- 0.001	0.0037	0.6484
Triglycerides (mg/dL)	0.005	0.0019	0.0048
HDL (mg/dL)	- 0.022	0.0134	0.1036
LDL (mg/dL)	- 0.004	0.0041	0.2717
Adiponectin (µg/mL)	- 0.158	0.0325	< 0.0001
% EVs/CD144+	0.0703	0.0153	< 0.0001
% EVs/AdipoR1+	- 0.092	0.0180	< 0.0001
% EVs/CD106+	0.080	0.0168	< 0.0001
% EVs/CD144+/AdipoR1+	0.002	0.0221	0.8972
% EVs/CD144+/CD106+	0.054	0.0228	0.0218
ACEI or ARB use	0.052	0.5585	0.9259
BMI (kg/m^2)	0.092	0.0214	0.0001

HOMA-IR, homeostasis model assessment for insulin resistance index; T2D, type 2 diabetes; LDL, low-density lipoprotein; HDL, high-density lipoprotein; EVs, extracellular vesicles; TZD, thiazolidinedione; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Discussion

Adiponectin (also called Acrp30, AdipoQ, GBP-28, and apM1) is a cytokine produced by resident macrophages and adipocytes in the white adipose tissue.[1] Adiponectin is a circulating protein anti-inflammatory that enhances fatty acids catabolism and regulates blood glucose levels by improving insulin sensitivity in humans and animals.[16] Disturbances in adiponectin production indicate dysfunction in adipose tissue, and a negative association between high BMI and low adiponectin levels has been proved;[16,17] On the other hand, physical activity strategies for weight loss increase adiponectin values.[17] Here, we observe that the adiponectin levels are lower in patients with T2D who present higher BMI than normoglycemic subjects.

Adiponectin is encoded by the ADIPOQ gene, of which 3 functional isoforms are produced: low-molecular-weight (LMW), middle-molecular weight (MMW), and high-molecular-weight (HMW).[16] The biological effects of adiponectin are variable since it presents multiple isoforms in blood circulation that their receptors recognize.[16] Adiponectin and its receptors, AdipoR1 and AdipoR2, have been linked to the pathophysiology of various chronic diseases associated with obesity.[3] The receptors AdipoR1 and AdipoR2 are significantly homologous in their amino acid sequences and structures; however, they present differences in their affinities for adiponectin isoforms;[16] Preferentially AdipoR1 binds globular adiponectin, and AdipoR2 binds the HMW isoform, and consequently, their responses pathways to adiponectin are not similar.[4,16]

The differential effects of adiponectin by AdipoR1 or AdipoR2 in liver mice have evidence since AdipoR1 gene disruption decreased adiponectin-induced AMPK activation, and AdipoR2 gene disruption resulted in decreased PPARα activation.[18] On the other hand, the *in vitro* high insulin levels repressed the promoter activity of the AdipoR1 gene, resulting in down-regulate the transcription of AdipoR1 without affecting the expression of AdipoR2 in C2C12 mouse myoblast cells cultures,[19] and high mRNA levels of AdipoR1/AdipoR2 from human muscle biopsies is associated with hyperglycemia, hyperlipidemia, obesity, and insulin resistance states.[20] Similarly, in human peripheral blood monocytes, mRNA levels of AdipoR1 and AdipoR2 increase in obese and T2D patients, in contrast to plasma levels of adiponectin reduced in this condition.[21] We appreciate that most human AdipoR1 studies at the endothelial level have focused on studying the mRNA and not on exploring the protein form on the surface cell membrane.

The EVs analysis is a helpful tool that allows us to obtain an approach to the expression of receptors in the vascular endothelial component

cells without using highly invasive and risky methods such as tissue biopsy.[22] An increase in the number of EVs derived from endothelial cells in the plasma of patients with T2D has been evidenced, and it has been promising as a potential marker of vascular injury in T2D and its complications.[8]

Endothelial EVs are small vesicles shed from endothelial cells upon apoptosis or cell activation. Endothelial EVs express some antigens on the membrane surface, such as vascular cadherin/CD144, CD62E, and CD105.[23] Li P. and Qin C. (2015) proposed that the EVs/CD144+ may represent a valuable marker for detecting endothelial dysfunction in ischemic cerebrovascular disease.[23] Here, we show a higher percentage of plasma endothelial plasma-derived EVs/CD144+ in T2D subjects than normoglycemic subjects and positively associated with plasma sVCAM-1 level.

The adhesion molecule CD106 or VCAM-1 is expressed on the surface of activated endothelial cells, tissue macrophages, bone marrow fibroblasts, and dendritic cells, as well as ICAM-1 and E-selectin, which occur after pro-inflammatory stimuli such as TNF-α, the increase of these molecules in their soluble form, sVCAM-1, increase in circulation is associated with the presence of various vascular diseases, such as the increase of endothelial-derived EVs in circulation.[9,24] Our data show an increase in CD106 both in plasma soluble form and bound to EVs in patients with T2D compared to control, and exert a significant and positive relationship with the HOMA-IR value, as previously documented in conditions that present with chronic inflammation like obesity and T2D.[25,26]

Blüer M. et al. (2006) demonstrated a positive association between increased AdipoR1 mRNA levels from muscle biopsies with insulin sensitivity and other metabolic parameters.[27] We found a negative association between total plasma-derived EVs/AdipoR1+ percentages with the HOMA-IR value and plasma sVCAM-1 level.

Several studies show that plasma adiponectin high levels are associated with a low risk of developing T2D and that these levels decline significantly when this condition is established and, therefore, could contribute to the de-regulation of carbohydrate and lipid metabolism;[4,28] that is consistent with our results, which show that adiponectin levels are lower in T2D patients. *In vitro* studies using Aorta Human Endothelial Cells (HAECs) suggest that adiponectin provides a vascular protective effect by inhibiting the NF-kB pathway.[29] Likewise, Jager A. *et al.* (2000) evidence that plasma levels of VCAM-1 in patients with T2D are higher compared to healthy subjects and are positively associated with the risk of cardiovascular mortality.[30]

The expression of CD31, CD105, and CD106 activation markers on the plasma endothelial-derived EVs of patients with T2D has been previously reported;[31] CD31 expression is associated with angiogenesis, platelet activation, and thrombosis, CD105 is expressed in microvascular atheroma plaques, and CD106 has been identified in tissues under inflammation stimulus and atherosclerotic plaque.[31,32]

Here we show that the plasma levels of sVCAM-1 are negatively associated with the adiponectin plasma levels and with the content of the AdipoR1 on the total plasma-derived EVs. Also, we showed that HOMA-IR values are associated with the plasma sVCAM-1 concentration, which is consistent with the anti-inflammatory effects of adiponectin through its receptors at the level of the vascular endothelium. Also, high HOMA-IR values are associated with producing pro-inflammatory cytokines and adhesion molecules that guide endothelial dysfunction and insulin resistance.[33,34]

Conclusions

This study evidence a lower AdipoR1 protein content in the plasma-derived EVs of T2D patients compared to normoglycemic subjects. The AdipoR1 expression level in the endothelial cells, which was indirectly studied by analyzing the expression of AdipoR1 in endothelial plasma-derived EVs/CD144+, could be associated with insulin sensitivity improvement and endothelial activation. However, it is crucial to conduct more extensive studies to elucidate the role of the adiponectin receptors in the endothelium. Therefore, plasma-derived EVs are excellent sources for exploring biomarkers of endothelial damage in chronic dysglycemic and inflammatory conditions.

Conflicts of interest

The authors do not report conflicts of interest.

Founding statement

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