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Pancreas fat content, insulin homeostasis and circulating endothelial microparticles in male essential hypertensive patients

Verónica Alfaro-Lara MD, PhD[1] | **Rocío Muñoz-Hernández PhD**[2,3] | **Luis Giménez-Miranda MD, PhD**[1] | **Luis Beltrán-Romero MD, PhD**[1] | **Francisco Javier Castell-Montsalve MD, PhD**[4] | **Pablo Stiefel MD, PhD**[1]

[1]Unidad clínica de Atención Médica Integral (UCAMI) y Laboratorio de Epidemiología Clínica y Riesgo Vascular, Instituto de Biomedicina de Sevilla (IBiS), Servicio Andaluz de Salud/CSIC/Universidad de Sevilla, Seville, España, Spain

[2]SeLiver Group, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, España, Spain

[3]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spaino, Spain

[4]Unidad de gestión Clínica de Radiología, Hospital Universitario Virgen del Rocío, Sevilla, España, Spain

Correspondence
Pablo Stiefel MD, PhD, Unidad Clínica de Atención Médica Integral (UCAMI) y Laboratorio de Epidemiología Clínica y Riesgo Vascular, Instituto de Biomedicina de Sevilla (IBiS), Servicio Andaluz de Salud/CSIC/Universidad de Sevilla, Seville, Espana 41013, Spain.
Email: pablostiefelgj@gmail.com

Abstract

The pancreas fat content has been poorly investigated in essential hypertension. The authors aim to relate pancreas and liver fat content with parameters measuring insulin resistance, beta-cell function and also with markers of endothelial dysfunction and platelet or endothelial cell destruction. The authors studied a group of 40 male hypertensive patients with well-controlled blood pressure, maintaining a stable weight, and having not changed their medication during the last year. Pancreas fat content was correlated with HOMA-IR ($r = .616$, $p < .001$), HOMA-S ($r = -.439$, $p < .005$), beta cell function parameter ($r = .457$, $p < .005$), and QUICKI ($r = .412$, $p < .01$), whereas liver fat was not patients in the highest quartile of pancreas fat content had more circulating endothelial microparticles than patients in the other quartiles (median 129 [94.3–200] vs. 60.9 [49.4–88.8], $p = .002$). However, patients in the highest quartile of the pancreas fat content distribution did not differ from the lowest in hyperemic response after ischemia nor circulating platelet microparticles count. Liver fat content was not related to any of the parameters studied. In a multivariate stepwise binary logistic regression analysis (Wald Method) circulating endothelial microparticles remain significantly associated with pancreas fat content after adjusting for confounding factors, such as tobacco, diabetes mellitus, hypercholesterolemia, or metabolic syndrome. Our results reflect that in essential hypertension, pancreas fat content is superior to liver fat to study beta-cell functionality and insulin resistance. Moreover, the authors described for the first time that pancreas fat content is related to endothelial cell destruction. Further studies are needed to confirm this point.

KEYWORDS
endothelial dysfunction, endothelial microparticles, HOMA, pancreas fat content, QUICKI

Alfaro-Lara and Muñoz-Hernández contributed equally to this paper.

1 | INTRODUCTION

Currently, not only obesity but also body fat distribution is considered to be more and more important. The visceral adiposity is a hormonally active component of total body fat, which possesses unique biochemical characteristics that influence several pathological processes. Several reviews and meta-analyses have found that visceral adiposity is related to increased risk for cardiovascular disease.[1]

Hepatic steatosis can be observed in a variety of clinical settings and has universally been linked to insulin resistance.[2] However, in addition to liver fat, other manifestations of visceral adiposity have been linked to pathological conditions. In this respect, the pericardial fat has been associated with coronary disease after additional adjustments for other confounding factors.[3] Pancreatic fat is an emerging research topic of particular interest given the organ role in glycemic homeostasis.

Pancreatic steatosis is a commonly observed, but often neglected finding by radiologists. The estimated prevalence of pancreatic steatosis from population-based studies in Asia is approximately 16%. A higher prevalence has been noted in patients with and obesity and previous research has been focused on the association among pancreas fat content obesity, hypertension, metabolic syndrome, diabetes and cardiovascular risk.[4–8]

In essential hypertension, the prevalence of metabolic syndrome is high. Nevertheless, the reason why some hypertensive patients have metabolic syndrome and others do not, as well as the role of different forms of visceral fat distribution on this phenomenon, has been poorly studied.[9]

The aim of the present study was: (a) to make a comparison between pancreas fat content and liver fat content in essential hypertension, (b) to assess the influence of these parameters on beta-cell function, insulin resistance, and insulin sensitivity, and (c) to investigate the relationship among these parameters and endothelial function measured by Laser-Doppler flowmetry and several markers of vascular damage, such as platelet or endothelial microparticles.

2 | PATIENTS AND METHODS

We recruited forty male essential hypertensive patients from our outpatients clinic, who had maintained a stable weight, not changed their usual medication, and have an absent or moderate alcohol intake (less than six drinks per week). None of them were taking drugs able to affect the fat content in the pancreas and liver and microparticles.[10] Table 2 lists all the drugs that the patients were taking for hypertension or its comorbidities. As it can be seen in the Table 2, and as is logical, only oral antidiabetics were taken more frequently patients with metabolic syndrome. However, the flow-mediated dilation in the forearm after ischemia measured by Laser-Doppler technique or the number of circulating microparticles was not influenced by the intake of any of these drugs.

Twenty of them met the criteria for metabolic syndrome according to The National Cholesterol Education Program's Adult Treatment Panel III Report and twenty did not.[11]

All participants completed a comprehensive health-related questionnaire that included lifestyle information (ie, physical activity, tobacco, alcohol, tea, and coffee consumption, and dietary habits), medical and family history (especially those related to premature cardiovascular disease), and the use of medications, nutritional supplements, and vitamins. Exclusion criteria were: use of subcutaneous insulin therapy, one or more previous episodes of acute pancreatitis, any kind of biliopancreatic disease, short bowel syndrome, history of gastric or jejunoileal bypass, bariatric surgery, any kind of hepatic or systemic disease that in the opinion of the investigators may influence on the results of the study and inability to undergo magnetic resonance imaging.

The patients were attended in the clinic after a 12 h overnight fast. After which, blood samples were drawn. Serum and plasma samples were processed and stored until analysis. Thyroid function was routinely tested and was normal in all the studied patients.

After revision of inclusion and exclusion criteria, patients underwent a routine history and physical examination in our research clinic. Brachial systolic and diastolic blood pressure was measured using an automated oscillometric device (Omron M6 Comfort; Omron Healthcare, Amsterdam, Netherlands) in the right arm, with participants lying in the supine position for 10 min by a trained observer. Three blood pressure readings were taken at 2-min intervals, and the mean was used for data analysis, Waist circumference in cm was measured and body mass index calculated and expressed in kg/m^2.

Prior to the study, the Human Investigation Review Committee of the Virgen del Rocio University Hospital approved all protocols, and all participants provided written informed consent. The study was conducted according to the guidelines of good clinical practice and principles expressed in the Helsinki Declaration by the World Medical Association.

2.1 | Assessment of insulin resistance and β-cell function

Insulin resistance and β-cell function[12] were calculated using the homeostasis model assessment HOMA for insulin resistance and insulin sensitivity and beta-cell function (HOMA- β). The software designed by the Diabetes Trials Unit of the Oxford Center for Diabetes, Endocrinology and metabolism was downloaded in http://www.dtu.ox.ac.uk/homacalculator/. The quantitative insulin sensitivity checks index (QUICKI), was also calculated.[13]

2.2 | Assessment of endothelial dysfunction by Laser-Doppler flowmetry

A Laser-Doppler linear Periflux System 5000 (Perimed SA, Järfälla, Sweden) was used to measure flow-mediated dilatation. The test was performed in the morning after 12 h overnight fast. The participant was taken into a quiet room at our Day Hospital with only the researcher and a nurse present. The room temperature was maintained at 22°C

and the technique and possible symptoms were explained in detail. The blood pressure cuff was placed on the patient's arm, with the participant in the supine position and after 15 min of resting. The laser was attached to the forearm at 15 cm from the wrist. Then, the blood pressure cuff was inflated to 40 mm Hg above the systolic blood pressure and maintained at this pressure for 4 min. During this period, the monitoring system showed how perfusion units decreased and reached biological zero. Afterward, the blood pressure was rapidly deflated and the Perfusion Units rise above the pre-ischemic. The data were recorded and analyzed using the software Perisoft for Windows. The values of hyperemic response after the ischemia and the Peak flow were automatically calculated. The same researcher performed all measurements to avoid variability.

2.3 | Microparticles

Blood samples were centrifuged at 3000 rpm for 10 min within 2 h from collection, and serum was immediately stored in aliquots at 280°C until analysis as follows: Fifty microliters Platelet-poor plasma heparin was incubated with a monoclonal antibody anti-CD31-FITC antibody (BD Pharmingen. BD Bioscience, CA, USA), and anti-CD41-Pacific blue, followed by 20 min incubation with PE-conjugated Annexin V (AV) kits according to the manufacturer's instructions (BD Pharmingen, BD bioscience, CA, USA). AV+ was used to determine apoptotic microparticles, CD31 FITC and CD41-Pacifiblue were used to differentiate between CD31 + CD41 + PMPs and CD31 + CD41 – EMPs. The negative control (zero value) was obtained using the isotype antibodies. Flow Count Beads (Beckman Coulter, Marseille, France) were added. MPs were identified as events with a .1–1 μm diameter on forward light scatter (FSC) and side-angle light scatters (SSC) intensity dot plot representation, by comparison to flow cytometry calibration beads (Flow count ® calibrator beads, Beckman Coulter, Marseille, France). Microparticles were analyzed by flow cytometry in duplicate (BD LSR-Fortessa; BD Biosciences, San Diego, CA, USA). Data represent the mean (± SEM) of two independent experiments.

2.4 | Magnetic resonance imaging

The methodology used in our study to calculate liver and pancreas proton density fat fraction was based on performing mDixonQuant sequence. All Magnetic Resonance Imaging were done with a clinical 3 Tesla whole-body system (Ingenia 3.0 T; release 5.1.7.2; Philips Healthcare, Best, the Netherlands) using a torso coil. All patients were asked to be fasting 4 h before and were subjected to a questionnaire to rule out contraindications to perform Magnetic Resonance Imaging study. The mDixonQuant sequence was obtained in apnea for 20 s, obtaining 20 sections of 7 mm thickness including liver and pancreas. mDIXON Quant, enables accurate and reproducible quantification of fat deposition in the liver in a single breath hold. In addition to the quantification result, which can be shown in convenient color maps, the corresponding T2*/R2* (parametric maps of the T2* value (T2 relaxation value), R2* value (inverse of T2*), water (image in which water-bound protons predominate), fat (image in which fat-bound protons predominate), in-phase image (water-bound and fat-bound protons add their signal intensities), and opposed phase images (bound protons to fat and bound to water subtract their signal intensities), could also be provided without the need for additional scanning.[14] On the parametric fat quantification map, several regions of interest were drawn, outlining the liver and head, body, and tail of the pancreas, respectively. The value obtained represents the percentage proton density fat fraction in liver and pancreas.

2.5 | Statistical analysis

Categorical variables were represented by absolute frequencies and percentages (*n*, %). Non-categorical variables were shown as mean ± SEM in case of normal distribution of the variables, and as median and interquartile range otherwise. The normality of the distributions was studied using the test of Kolmogorov-Smirnov. Student's t and Mann–Whitney's U tests were applied to compare quantitative variables in the different groups in the case of normal and non-normal distributions, respectively. Categorical variables were compared with Pearson $\chi 2$ tests or Fisher's exact test as appropriate. A binary logistic regression analysis using a stepwise (Wald) approach was performed to determine the factors influencing (tobacco, diabetes mellitus, hypercholesterolemia and metabolic syndrome) the relation between microparticles and pancreas fat content. A p-value < .05 was considered significant. Statistical analyses were performed using IBM SPSS software (version 23.0; IBM, Armonk, NY, USA).

3 | RESULTS

We have studied 40 male essential hypertensive patients with an average age of 59 years, 22.5% were smokers, 22.5 had hypercholesterolemia, and 25% were diabetic (Table 1). The type of recruitment was from consecutive patients referred to our outpatients' office for hypertension. To increase the percentage of patients with higher visceral fat, 50% of them met criteria of metabolic syndrome. As expected, patients who -in addition to hypertension- had metabolic syndrome, had greater waist circumference, higher frequency of diabetes, lower blood levels of HDL cholesterol and higher levels of triglycerides and glucose. We divided patients by quartiles regarding pancreas and liver fat content. We found that there was a higher percentage of patients in the highest quartile of liver fat content but not in the highest quartile of pancreas fat content among patients with metabolic syndrome (Table 1). Therapy of the studied patients was shown in Table 2. No statistically significant differences were found between patients with and without metabolic syndrome with logical exclusion of antidiabetic drugs.

We also studied the degree of correlation of all these indices with the amount of fat in the different pancreatic areas and we observed that all of them were significantly correlated except QUICK index

TABLE 1 Characteristics of the male hypertensive studied

	All ($n = 40$)	Patients with metabolic syndrome ($n = 20$)	Patients without metabolic syndrome ($n = 20$)	*p* value
Age (years)	59.3 ± 1.5	59.2 ± 1.9	59.4 ± 2.4	NS
Body mass index (kg/m^2)	30.7 ± .5	31.3 ± .7	30.1 ± .8	NS
Waist perimeter (cm)	110.0 ± 1.8	110.4 ± 2.8	101.0 ± 3.1	<.05
Systolic blood pressure (mm Hg)	136.9 ± 2.3	134.0 ± 2.8	140.2 ± 3.7	NS
Diastolic blood pressure (mm Hg)	80.7 ± 2.2	77.0 ± 3.4	83.0 ± 2.3	NS
Tobacco (*n*, %)	9; 22.5%	5; 25.0%	4; 20.0%	NS
Diabetes (*n*, %)	10; 25.0%	7; 35.0%	3; 15.0%	<.01
Hypercholesterolemia (*n*, %)	9: 22.5%	4; 20.0%	5; 25.0%	NS
Total cholesterol (mg/dl)	181.1 ± 5.4	179.1 ± 8.9	183.3 ± 5.9	NS
LDL cholesterol (mg/dl)	101.8 ± 5.0	93.7 ± 7.4	110.3 ± 6.3	NS
HDL cholesterol (mg/dl)	44.8 ± 2.1	37.8 ± 1.8	52.5 ± 3.2	<.000
Triglycerides (mg/dl)	169.7 ± 18.2	232.9 ± 27.6	100.0 ± 8.2	<.000
Glucose (mg/dl)	106.8 ± 3.6	109.0 ± 5.5	93.3 ± 2.2	<.001
Upper quartile liver fat content (*n*, %)	10; 25%	8; 80%	2; 20%	<.005
Upper quartile pancreas fat content (n, %)	10; 25%	6; 60%	4; 40%	NS

Comparison between patients with and without metabolic syndrome.
Quantitative variables are represented as mean ± SEM and qualitative variables as absolute number and percentage.

TABLE 2 Therapy of the male essential hypertensive studied patients

	All patients	Patients with metabolic syndrome	Patients without metabolic syndrome	*p* value
Angiotensin –converting enzyme inhibitors/angiotensin II receptor blocker; *n*, (%)	36 (90.0%)	15 (88.2%)	21 (91.3%)	.574
Diuretics; *n*, (%)	27 (67.5%)	12 (70.6%)	15 (65.2%)	.720
Beta-blockers; *n*, (%)	14 (35.0%)	6 (35.3%)	8 (34.8%)	.973
Calcium channel blockers; *n*, (%)	14 (35.0%)	6 (35.3%)	8 (34.8%)	.973
Statins; *n*, (%)	24 (6.0%)	9 (52.9%)	15 (65.2%)	.433
Ezetimiba; *n*, (%)	1 (2.5%)	0 (0%)	1 (4.3%)	.575
Fibrates; *n*, (%)	6 (15.0%)	1 (5.9%)	5 (21.7%)	.175
Metformin; *n*, (%)	8 (20.0%)	0 (0%)	8 (34.8%)	.006
Other antidiabetic drugs; *n*, (%)	5 (12.5%)	0 (0%)	5 (21.7%)	.051

and the fat content in the head of the pancreas. However, liver fat content was not correlated with the HOMA2 insulin resistance parameter, insulin sensitivity, or beta cell functionality. There was also no relationship with the QUICK index of insulin resistance (Table 3).

We failed to find any significant relationship between pancreas fat content or liver fat content and endothelial dysfunction measured by the hyperemic area after ischemia or by circulating platelet microparticles count. Nevertheless, the number of endothelial microparticles was significantly higher in patients in the highest quartile of pancreas fat content, while this relation was no present regarding liver fat content (Table 4). In this table we can see that the relationship between endothelial microparticles and pancreatic fat is significant both in patients with metabolic syndrome and in those who do not have it. Finally, a multivariate analysis by binary logistic regression stepwise (Wald's method) showed that the positive relationship between pancreas fat content and the number of endothelial circulating microparticles remain being significative after the inclusion of

TABLE 3 Relationship among liver fat content and fat content of different segments of the pancreas with parameters measuring insulin homoeostasis

	HOMA-IR	HOMA-S	HOMA-β	QUICKI
Liver fat content (%)	$r = -.004$ p = NS	$r = -.108$ p = NS	$r = -.082$ p = NS	$r = -.007$ p = NS
Head of pancreas fat content (%)	$r = .383$ $p < .05$	$r = -.310$ $p < .05$	$r = .332$ $p < .05$	$r = .159$ p = NS
Body of pancreas fat content (%)	$r = .527$ $p < .001$	$r = -.383$ $p < .05$	$r = .404$ $p < .01$	$r = -.371$ $p < .05$
Tail of pancreas fat content (%)	$r = .557$ $p < .000$	$r = -.383$ $p < .05$	$r = .450$ $p < .005$	$r = -.368$ $p < .05$

TABLE 4 Markers of endothelial dysfunction and intravascular cell destruction according to the pancreas and liver fat contents; highest versus the three lowest quartiles, and according to the presence or absence of metabolic syndrome

	Pancreas fat (%)			Liver fat (%)		
Metabolic syndrome ($n = 20$)	**High ($n = 5$)**	**Low ($n = 15$)**	***p*-value**	**High ($n = 8$)**	**Low ($n = 12$)**	***p*-value**
Hyperemia area (UP/seg)	200 (115.4–1361)	671.6 (262.2–1119)	.333	671.6 (204.8–850)	655 (144.1–1361)	.800
Endothelial microparticles (microparticles/μl)	127 (98.5–145.4)	65.6(52.4–86.3)	.026	88.8 (58.6–174.1)	69.4 (53.4–99.5)	.210
Platelet microparticles (microparticles/μl)	1401 (70.1–1649)	244.3 (39.6–1084)	.403	1041 (204.6–1601)	85.8 (42.3–981)	.121
	Pancreas fat (%)			**Liver fat (%))**		
No metabolic syndrome ($n = 20$)	**High ($n = 5$)**	**Low ($n = 15$)**	***p*-value**	**High ($n = 2$)**	**Low ($n = 18$)**	***p*-value**
Hyperemia area (UP/seg)	346 (45.1–993.8)	111.6 (17.8–323.3)	.765	114.5 (109.1–119.9)	119.5 (20.3–401.3)	.595
Endothelial microparticles (MPs/μl)	131 (78.4–382.7)	58.2 (37.6–111.6)	.042	256.3 (77.3–435.3)	60 (45.2–141.8)	.184
Platelet microparticles (MPs/μl)	3462 (619.5–5133)	1082 (364.4–5553)	.691	10771 (3462–18079)	908 (459.7-4542)	.111

TABLE 5 Multivariate regression showed that smoking, diabetes, metabolic syndrome, hypercholesterolemia and endothelial microparticles were independent predictors of pancreas fat content (rank-ordered by Wald)

	β	*p*-value	Odds ratio [IC 95%]
Tobacco	−.931	.378	.390 [.047–3.22]
Diabetes mellitus	.143	.878	1.132 [.14–9.113]
Metabolic syndrome	.314	.653	.953 [.053–7.12]
hypercholesterolemia	−.945	.321	.387 [.057–2.487]
Endothelial microparticles	.018	.031	1.015 [1.002–1.029]
Constant		.002	.73

other confunding factors in the equation (tobacco, diabetes mellitus, hypercholesterolemia, and metabolic syndrome) (Table 5)

Figure 1 shows that there was no statistically significant relationship between pancreas and liver fat content.

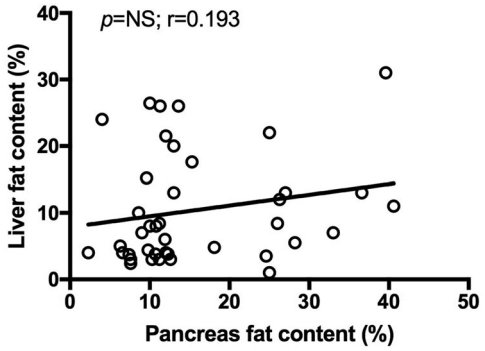

FIGURE 1 Correlation between liver and pancreas fat content.

Figure 2 shows that there was an important relationship between pancreas fat content and different parameters of beta cell function and insulin resistance.

Figure 3 shows how, unlike liver fat, pancreatic fat is related to the functionality of beta cells and insulin resistance or insulin

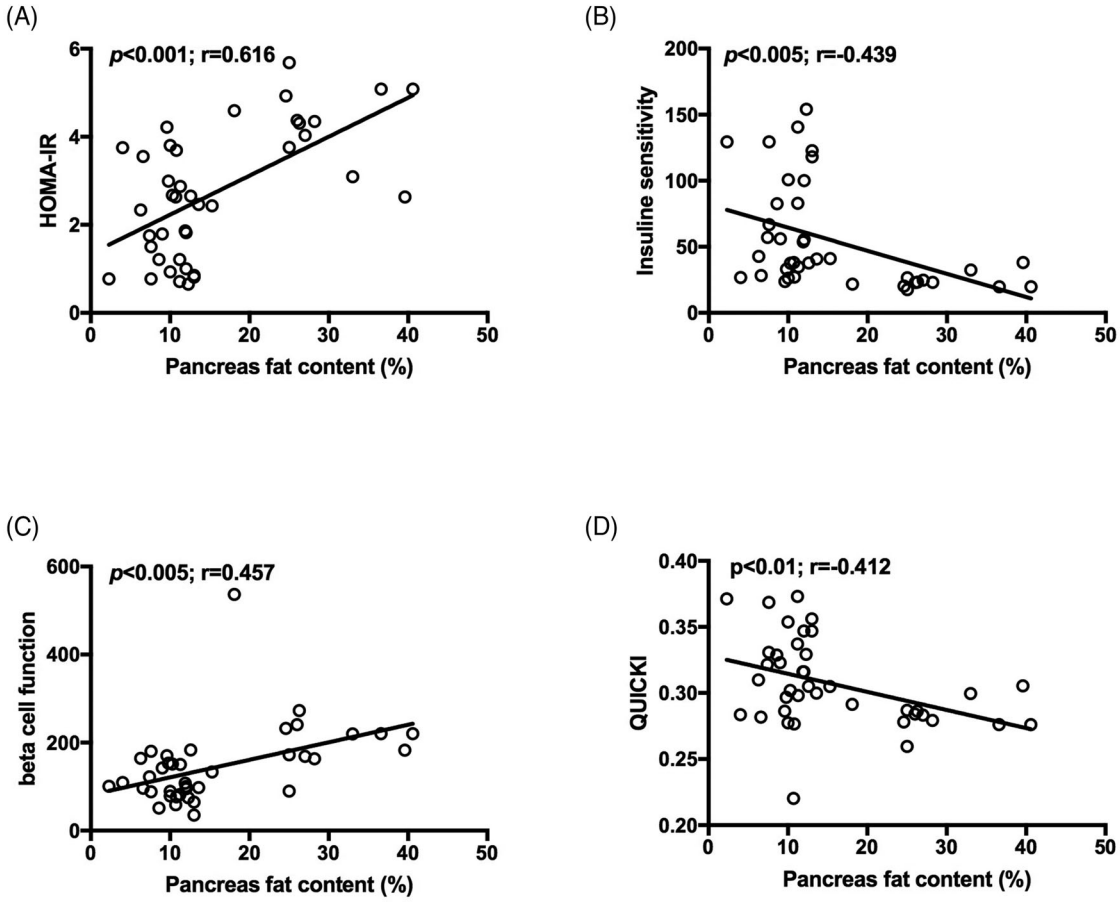

FIGURE 2 Correlation among HOMA-IR values, insulin sensitivity, beta cell function, and QUICKI with pancreas fat content.

sensitivity parameters (HOMA-IR, HOMA-S, β cell function parameter and QUICKI).

4 | DISCUSSION

Since the first description of fatty pancreas in 1933, the effects of pancreatic steatosis have been poorly investigated, compared with that of the liver. Pancreas fat accumulation, associated with obesity, type 2 diabetes mellitus, and metabolic syndrome has been defined as "fatty infiltration" or "nonalcoholic fatty pancreas disease".[5]

To avoid bias, in our study we recruited only male essential hypertensive patients since men show higher ectopic fat deposition in pancreas than women, despite the same body mass index.[15] Furthermore, values of the HOMA-IR and the QUICKI are also different between men and women.[16]

Singh and coworkers[6] carried out a systematic review, meta-analysis, and meta-regression on the clinical relevance of ectopic accumulation of fat in the pancreas and found that this was significantly related to the risk of hypertension (risk ratio 1.67 confidence interval 1.32–2.00, $p < .0001$). In this respect, and more recently, in a prospective study including 267 consecutive patients who were referred to abdominal Magnetic Resonance Imaging (using our technique) and underwent a standard clinical assessment with body mass index, blood pressure measurement and waist circumference, the pancreatic and hepatic fat was evaluated and there was found a significant relationship between the fat content in muscles, pancreas and liver, and the incidence of hypertension.[17] Despite all these facts, pancreatic fat has not been studied selectively in a population of essential hypertensive patients until now.

Now we have studied a sample of male essential hypertensive patients and we have not found a positive relationship between pancreas and liver fat content. Moreover, we have observed a statistically significant and positive relationship among HOMA-insulin resistance ($r = .616$, $p < .001$), and HOMA β cell function parameter ($r = .457$, $p < .005$) and negative with HOMA-insulin sensitivity ($r = -.439$, $p < .005$) and QUICKI ($r = -.412$, $p < .01$) with pancreas but no with liver fat content. Our results are partially in agreement and partially in disagreement with other studies most of them performed in type 2 diabetes mellitus patients, obesity, or even in pediatric populations.[4,18–23]

One factor that may contribute to explain the differences between our results and previous studies is that our study included male hypertensive patients with heterogeneous representation of comorbidities like obesity, diabetes mellitus or metabolic syndrome in comparison to other studies. The other one, may be that we have used a more sensitive

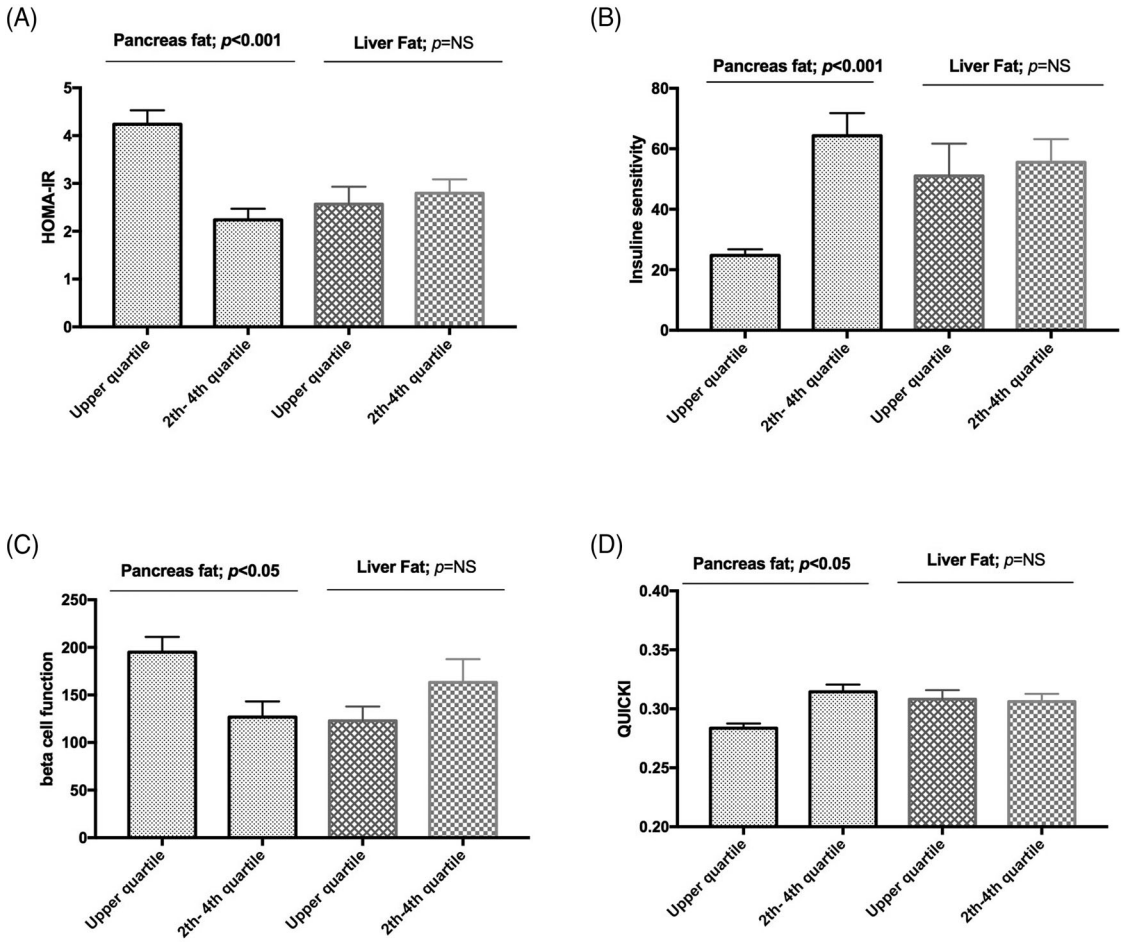

FIGURE 3 Relationship between the amount of pancreas and liver fat content (comparison of patients in the highest quartile vs. 2th–4th quartile) with several marker of insulin resistance and beta cell function.

technique for measuring pancreas and liver fat content than other radiological procedures, even better than histology for measuring total fat, since this is usually distributed in a patchy way, which makes it difficult for the pathologist to quantify.[14]

We observed a positive and not a negative relationship between pancreas fat content and HOMA β cell function parameter. It might seem logical to think that fat infiltration in the pancreas could lead to worsening beta cell function and this could lead to a deterioration in insulin secretion. However, Auerval and coworkers[24] studied Male Wistar rats that were randomly divided into two groups, the normal diet group (2.8 kcal/g) and the high fructose diet group (4.6 Kcal/g). After 2 months, the high fructose diet induced an increase in body weight, insulin, and triglycerides. Liver steatosis was also observed in the high fructose diet group, which was associated with an increase in glycogen storage. In the pancreas, the high fructose diet induced islet hyperplasia. Histological analysis of the pancreas demonstrated a preservation of the pancreas structure without any fibrosis. Moreover, significant staining of islets with insulin was observed, reflecting functional islets. Moreover, islets from high fructose diet rats were bigger than those from normal diet rats.

More recently, in vivo studies further showed that Fibroblast growth factor 21 is critical for islet insulinogenic capacity and normal function in the context of high fructose diet-treated animals.[25] These observations in experimental animals could explain our finding that when pancreas fat content increases, the HOMA-beta parameter also increases rather than decreases (see Figure 2C).

A novelty of our study is that the amount of fat in the pancreas and in the liver has been studied in conjunction with parameters measuring endothelial dysfunction and intravascular cell damage.

We have measured for first time pancreas fat content and hyperemic response after ischemia in the forearm by Laser-Doppler flowmetry and we have found that patients in the highest quartile of the pancreatic fat distribution had a smaller hyperemic area after ischemia than patients in the remaining quartiles, whether this difference was not statistically significant, perhaps in relation to the small sample size. Our work is also the first study to compare pancreas fat content and different markers of intravascular cell destruction, such as microparticles of platelet origin and those of endothelial origin.

Circulating microparticles are small vesicles that are released in response to several injuries. The level of circulating microparticles in peripheral blood has been reported to be increased in

cerebrovascular disease, hypertension, diabetes, smoking, coronary disease and microparticles syndrome existing a positive correlation between circulating levels of obstructive sleep apnea and nocturnal hypoxemia severity.[26] We have also previously reported that changes in microparticles after continuous positive airway pressure in obstructive sleep apnea was greater in those with a more severe disease, defined according to the oxygen desaturation index and the apnea-hypopnea index, thus suggesting that in more severe patients the benefit is greater.[27] Finally, Sinning and coworkers[28] determined CD31+/Annexin V+ microparticles by flow cytometry in 200 patients (age 66.1 + 10.4 years) with angiographically proven stable coronary artery disease and correlated with cardiovascular outcomes. The median follow-up time for major adverse cardiovascular and cerebral events was 6.1 (6.0/6.4) years. A first major adverse cardiovascular and cerebral event occurred in 72 patients (37%). Microparticles levels were significantly higher in patients with major adverse cardiovascular and cerebral events compared with patients without events ($p = .004$). In multivariate analysis (cardiovascular risk factors, number of diseased vessels, use of angiotensin-converting enzyme-inhibitors and statins), microparticles high level were associated with a higher risk for cardiovascular death (Hazard ratio [HR] 4.0, 95% confidence interval (CI) 1.1–14.6; $p = .04$), the need for revascularization (HR 2.4, 95% CI 1.3–4.4; $p = .005$), and the occurrence of a first major adverse cardiovascular and cerebral event (HR 2.3, 95% CI 1.4–3.8; $p < .001$). Inclusion of the microparticles level into a classical risk factor model substantially increased c-statistics from .637 (95% CI: .557–.717) to .702 (95% CI: .625–.780) ($p = .03$). Therefore, they concluded that the level of circulating CD31+/Annexin V+ microparticles is an independent predictor of cardiovascular events in stable coronary patients and may be useful for risk stratification.

One of the most remarkable findings of our study was that we observed that patients who are in the highest quartile of pancreas fat content had a greater number of endothelial microparticles. As far as we know, this finding has never been previously studied, and therefore, we believe that further studies are needed in order to confirm it.

A possible limitation of our study is the small sample size. The main cause for this inconvenience was the difficulty for recruiting male patients (women were excluded) with clinically stable arterial hypertension, who had not undergone significant weight changes and had not modified their medication, or their lifestyle habits, in the last year and agreed to participate.

In summary, we have studied a group of essential hypertensive patients and we have measured pancreas and liver fat content using a highly sensitive technique. We have calculated different parameters of sensitivity and resistance to insulin and beta cell function and assessed endothelium functionality. Our most remarkable finding was that fat in the pancreas correlated better with all these parameters than fat in the liver, and in particular, that patients with more fat in the pancreas had a higher level of endothelial cell destruction, which could imply an increased risk of future vascular events, as previously described by others.[28] Further studies are needed to evaluate the role that Magnetic Resonance Imaging could play in stratifying vascular risk in hypertensive patients.

AUTHOR CONTRIBUTIONS

Alfaro-Lara V and Giménez-Miranda L: Responsible of the recruitment of patients and creation of the database. Dr Alfaro-Lara read his doctoral thesis on this study and obtained the highest possible score. Muñoz-Hernández R: Responsible of microparticles determination. Beltrán-Romero L: Responsible of Assessment of endothelial dysfunction by Laser- Doppler flowmetry. Castell-Montsalve FJ: Responsible of MRI interpretation. Stiefel P: designed he study, responsible of the statistical study and wrote the manuscript.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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