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Increased visceral fat accumulation modifies the effect of insulin resistance on arterial stiffness and hypertension risk

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Abstract *Background and aims:* Both insulin resistance (IR) and visceral adipose tissue (VAT) are related cardiometabolic risk factors; nevertheless, their joint effect on endothelial functionality is controversial. This study aims to evaluate the joint effect of IR and VAT on endothelial functionality using the pulse-waveform analysis and explore the mediating role of VAT on the effect of IR on arterial pressure, arterial stiffness and incident arterial hypertension.
Methods and results: We measured VAT (n = 586) using two methods (dual-energy X-ray absorptiometry and a clinical surrogate), arterial stiffness (with pulse-waveform velocity), and IR (using three methods: HOMA2-IR (n = 586), a frequently sampled intravenous glucose tolerance test (n = 131) and euglycemic hyperinsulinemic clamping (n = 97)) to confirm the mediator effect of IR on VAT. The incidence of arterial hypertension attributable to the mediating effect of IR related to VAT was evaluated using a prospective cohort (n = 6850). **Adjusted linear regression models, causal mediation analysis, and Cox-proportional hazard risk regression models were performed to test our objective.** IR and VAT led to increased arterial stiffness and increased blood pressure; the combination of both further worsened vascular parameters. Nearly, 57% ($\Delta_{E\rightarrow MY}$ 95% CI: 31.7–100.0) of the effect of IR on altered pulse-wave velocity (PWV) analysis was mediated through VAT. Moreover, VAT acts as a mediator of the effect of IR on increased mean arterial pressure ($\Delta_{E\rightarrow MY}$ 35.7%, 95% CI: 23.8–59) and increased hypertension risk ($\Delta_{E\rightarrow MY}$ 69.1%, 95% CI: 46.1–78.8).
Conclusion: VAT acts as a mediator of IR in promoting arterial stiffness and arterial hypertension. Both phenomena should be targeted to ameliorate the cardiometabolic risk.

Abbreviations: T2D, Type 2 Diabetes; CVD, Cardiovascular Disease; VAT, Visceral Adipose Tissue; PWV, Pulse-wave velocity analysis; AIX75, Augmentation index normalized; IR, Insulin resistance; HOMA-IR, Homeostasis model assessment for insulin resistance; METS-IR, Metabolic Score for Insulin Resistance; METS-VF, Metabolic Score for Visceral Fat; EST-VAT, Estimated Visceral Adipose Tissue; BMI, Body mass index; HR, Heart rate; TC, Total cholesterol; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; IQR, Interquartile range.

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Introduction

Obesity is a major risk factor for the development of type 2 diabetes (T2D), arterial hypertension, cardiovascular events, and all-cause mortality [1,2]. Its prevalence has increased in pandemic dimensions, leading to a public health problem worldwide, particularly in developing countries [3]. The pathogenesis of obesity is multifactorial; however, evidence suggests that insulin resistance (IR) plays a fundamental role in the accumulation of body fat and dysfunction of adipose tissue [4]. Underlying mechanisms by which obesity leads to adverse cardiovascular outcomes have been attributable to the differences in fat distribution and adipose tissue function in particular related to visceral adipose tissue (VAT) [5]. Accumulation of VAT is a known independent risk factor for cardiometabolic diseases, particularly those related to atherosclerosis [6,7]. Evidence suggests that increased VAT accumulation has been linked to impairments in vascular health and has shown to promote vascular damage, proinflammatory states and impairments in glucose and lipid metabolism, causing a stress-induced response leading to IR [8]. Therefore, we can infer that both IR and VAT accumulation interacts to impair vascular health by independent mechanisms [9,10]. Nevertheless, it is not completely understood how both IR and VAT interact in the dysregulation of vascular physiology. An approach to study altered vascular hemodynamics previous to the onset of cardiometabolic events is the pulse wave-form analysis [11–14]. Although both factors have been independently associated with arterial stiffness, the joint effects of both IR and VAT on arterial stiffness remain unclear. Hence, we sought to propose a pathophysiological mechanism, which links the effects of both IR and VAT on arterial stiffness, blood pressure levels, and arterial hypertension risk, thus establishing a connection between sequential metabolic events and subsequent vascular dysfunction. We propose that the effects of IR on arterial stiffness may partially be mediated by VAT, given its stronger association with the cardiovascular risk, and that the presence of both factors leads to more adverse vascular health than either one alone.

Methods

Pulse-wave velocity and EHC cohort

We used an open-population cohort consisting of consecutive subjects recruited between January 2018 and September 2019 at the Metabolic Disease Research Unit of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City. The research team of this facility assesses individuals with metabolic comorbidities including persons living with T2D (HbA1c $\geq$ 6.5%), prediabetes (2-h glucose challenge $\geq$140 mg/dL but $<$200 mg/dL), both primary and secondary dyslipidemias, and obesity (BMI $\geq$30 kg/m^2). All participants were interviewed by trained staff to obtain a complete medical and family history, including use of medication, sociodemographic information, diet, and physical activity habits. A second subset of this PWV cohort (n = 131) was assessed with a frequently sampled intravenous glucose tolerance test (FSIVGTT) to assess insulin sensitivity and its relationship with VAT. Similarly, an independent second cohort of obese and lean women (euglycemic hyperinsulinemic clamp cohort) aged 18–65 years was assessed using an euglycemic hyperinsulinemic clamp (EHC, N = 97) to investigate the effect of VAT on IR and on mean arterial pressure (MAP), a long-term determinant of arterial hypertension [15]. In all our analysis, we excluded patients with previous diagnosis of cardiovascular events or chronic kidney disease. A diagram of the studied cohorts used in this work, along with the complete details of the population studied, is presented in the supplementary material. The Human Research Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán approved the study, and all participants provided written informed consent.

Data collection in PWV and EHC cohorts

Blood sample measurements

Blood samples were obtained after 8–12 h of fasting. Plasma glucose concentration was measured using an automated glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA), serum insulin concentration was measured by chemiluminescent immunoassay (Beckman Coulter Access 2), HbA1c levels were measured by high-performance liquid chromatography (HPLC) (Variant II Turbo, BIORAD), lipid concentrations (total cholesterol (TC), serum triglycerides and HDL cholesterol) were measured by colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter), and LDL cholesterol was calculated using Martin's equation [16]. For the clinical estimation of IR, the HOMA2-IR index was calculated, using the HOMA calculator and metabolic score for insulin resistance (METS-IR) formula (LN((2*G_0)+TG_0))* BMI/(LN(HDL-C)), where G_0 and TG_0 were fasting glucose and triglycerides in mg/dl, respectively [17,18].

Anthropometrical measurements

For anthropometrical evaluation, all subjects were weighed on calibrated scales using a SECA mBCA 514 medical body composition analyzer, and the height was

determined with a floor scale SECA stadiometer. The waist circumference (WC) was evaluated using an inelastic SECA 201 tape with 0.1 cm precision; we placed the tape directly over the skin at the mid-point between the ribcage and the iliac crest with the tape parallel to the floor. The body mass index (BMI) was calculated as weight in kilogram divided by the squared product of height in meters. The waist-to-height ratio (WtHr) was calculated using WC divided by height, both in centimeters.

Body composition analysis

Total adipose tissue (TAT), lean mass, and visceral adipose tissue (DXA-VAT) were determined by dual-energy X-ray absorptiometry (DXA) (GE Healthcare, CoreScan software version) after a 4-h fast. A detailed description of the methodology for the measurement of VAT using DXA is available in the supplementary material. As previously mentioned, VAT estimation is usually reserved in epidemiological and clinical studies; therefore, the use of a clinical surrogate in our findings could complement and validate its estimation of vascular health in a clinical setting. For this purpose, we used the metabolic score for visceral fat (METS-VF). METS-VF was calculated using the following variables: the METS-IR index, the WtHr, sex and age in years [19]. The estimation of visceral adipose tissue (EST-VAT) in grams was obtained by taking the exponential transformation of METS-VF. Increased visceral adiposity phenotype was defined as DXA-VAT $\geq$1.4 kg or EST-VAT $\geq$1.3 kg; in patients living with T2D, this was considered as EST-VAT $\geq$0.9 kg [19].

Pulse-waveform analysis measurements

Participants were instructed not to consume caffeinated beverages and refrain from smoking $\leq$48hrs before evaluation. Upon the pulse-waveform analysis evaluation, all participants were placed in a supine position for 10 min and baseline supine brachial artery blood pressure (BP) and heart rate (HR) were recorded using a semi-automated cuff-based device (SphygmoCore XCEL, AtCor Medical Pty Ltd, USA). Pulse wave velocity (PWV) measurements were taken after achieving hemodynamic stability, defined as two readings within the systolic BP (SBP) of $\pm$9 mmHg, diastolic BP (DBP) $\pm$6 mmHg and HR $\pm$8 beats/min. The mean arterial blood pressure (MAP) was obtained by doubling the DBP, adding this to the SBP and dividing the product by 3. To assess PWV, carotid pulse waves were measured by applanation tonometry and femoral pulse waves were simultaneously obtained by a partially inflated cuff over the femoral artery at the leg midway between hip and knee. PWV was determined by calculating the ratio of corrected distance between pulse measuring sites to time delay between carotid and femoral pulse waves. Distance was measured with a non-stretchable tape from the suprasternal notch to the carotid site, from the femoral artery at the inguinal ligament to the proximal edge of the thigh cuff from the suprasternal notch to the proximal edge of the thigh cuff. Distances 1 and 2 were subtracted from distance 3 and used in the calculation of PWV. The augmentation index (AIX) was also considered for this analysis. The AIX is another parameter delivered from the augmentation pressure in the pulse-waveform analysis. The AIX values estimate the wave reflection from the periphery; an earlier return of the reflected wave occurs with increased PWV. Normalized values of AIX for heart rate at 75 bpm (augmentation index normalized, AIX75) were used to reduce the effect attributable to heart rate [20]. All blood samples, anthropometrical, DXA and pulse-waveform analysis measurements were assessed the same day for each patient.

Metabolic syndrome cohort to assess role of VAT on incident arterial hypertension

A third cohort was included to test our hypothesized mediator effect of VAT attributable to IR on the risk of incident arterial hypertension. We included participants of the Metabolic Syndrome cohort (MS-cohort). The MS cohort was developed to evaluate the risk of MS components on incident T2D, arterial hypertension, and cardiovascular mortality in an urban population from 9 different cities in Mexico [21]. Briefly, all participants were interviewed to obtain a medical history, physical activity habits and anthropometrical measurements as well as biochemical analyses after a 9–12 h fast. None of these patients had arterial hypertension at baseline. These same evaluations were carried out after a minimum of two years follow-up. Time to follow-up was estimated from recruitment up to the last follow-up or incident outcome, whichever occurred first. Incident arterial hypertension was defined as a construct of either diagnosed arterial hypertension, blood pressure values $\geq$ 140/80 mmHg or the use of antihypertensive treatment at follow-up.

Statistical analysis

Frequency distribution of categorical variables are reported as frequencies and percentages. Logarithmic, quadratic and squared root transformations were applied to approximate normality in variables showing non-parametric distribution. Data are presented as mean $\pm$ SD or as median and interquartile range, where appropriate.

Association of visceral adiposity with pulse-waveform analysis measurements

To evaluate the correlation of VAT or IR with pulse-waveform analysis measurements, we used Pearson correlation analysis as well as partial correlation analyses adjusted for age, sex, current smoking status, previous history of arterial hypertension, glycated hemoglobin (to evaluate glucose control, particularly in patients with T2D), and IR [22–24]. Similar to METS-VF, our purpose was to extrapolate our results to a non-experimental setting; hence, we used the HOMA2-IR as a clinical surrogate to estimate IR. To assess the independent effect of VAT on the included predictors, linear regression models were fitted against normalized values of PWV and AIX75 adjusted for covariates. These previous analyses were stratified

considering subgroups of patients with T2D and/or arterial hypertension in the PWV cohort, to assess the independency of these conditions. Furthermore, to support our hypothesis that VAT has an independent effect over TAT, we use the division of the percentage of visceral against the percentage of TAT (%VAT/TAT) to use it as our predictor in our linear regression analysis.

Modifiers of the effect of VAT on PWV

VAT accumulation is also known to be modified by sex, age, and body-composition [25]. Hence, modifiers of the association of VAT with PWV were evaluated using interaction effects (β_{INT}) on vascular health for VAT and EST-VAT considering sex, age $\geq$50 years, BMI (non-obesity vs. obesity), and obesity estimated using DXA-derived total-fat (25% men; 35% women) [26]. To address the heterogeneity of the study sample, mixed linear regression models were fitted including the different clinical phenotypes included in the study as random effects. Model diagnostics for linear models were conducted by assessing model residuals and the Bayesian information criterion (BIC). To explore whether VAT measurements and increased visceral adiposity phenotypes predict arterial stiffness, defined as PWV values $\geq$ 80th for the PWV cohort, logistic regression models were used with DXA-VAT, EST-VAT and its previously reported cut-off values to define increased VAT [19].

Causal mediation analysis

Finally, to explore whether the presence of VAT may act as a mediator of the risk conferred by IR on vascular health, three model-based causal mediation analyses were developed: 1) VAT (DXA-VAT/EST-VAT) as a mediator of the effect of IR (HOMA2-IR/SI-index) on arterial stiffness (PWV cohort, FSIVGTT dataset). 2) Because the concept of arterial stiffness is pathophysiological, the second model investigated whether the effect of IR/VAT on MAP is mediated by arterial stiffness, thus linking both concepts. To confirm that VAT (DXA-VAT/EST-VAT) is a mediator of the effect of IR (MFFM/HOMA2-IR/SI-index) on MAP, the EHC cohort was used. 3) Finally, to explore a long-term consequence of this mediating mechanism, the role of VAT (EST-VAT) as a mediator of the effect of IR (HOMA2-IR) on incident arterial hypertension risk was investigated using the MS-cohort (Fig. 1). As it has been previously described, we sought to explore the association of VAT on IR in all three models to confirm the bidirectional effect of both predictors. All mediation analyses were performed using the *mediation* R package; to permit inference to obtain a 95% confidence interval using bias-corrected accelerated non-parametric bootstrap. To demonstrate the sequential ignorability assumption, a sensitivity analysis was run to demonstrate residual confounding by varying the correlation between the residuals of both the outcome and the moderator models. The third mediation model was tested using causally ordered model-based mediation analyses using beta coefficients extracted from Cox proportional risk regression models. Statistical analyses were performed using R software version 3.6.1 and the Statistical Package for Social Science (SPSS) version 24.0. A p value < 0.05 was considered statistically significant.

Results

Study population

In the PWV cohort, 586 participants were included, with female predominance (70.3%) and a median age of 51 (IQR: 41–59) years. Comorbidities in this sample included 262 patients living with T2D (44.4%), 174 with pre-diabetes (29%), 167 (28.5%) with arterial hypertension, 182 currently smoking (31.1%), and 31 with a primary dyslipidemia (5.4%). The subset assessed with the FSIVGTT included 131 participants, with female predominance (62.6%) a median age of 53 years (IQR: 45–57), 18 with previously diagnosed T2D, and 13 (9.9%) with arterial hypertension. Only 14 (14.4%) patients in the EHC dataset had previously diagnosed arterial hypertension. Complete biochemical and anthropometrical evaluations of all cohorts are presented in Table 1.

Association of VAT (DXA-VAT/EST-VAT) with PWV, AIX75, and HOMA2-IR

We observed a positive association between PWV and AIX75 values with both DXA-VAT/EST-VAT and HOMA2-IR after adjustment for covariates (Fig. 2, Table 2). When we stratified these models by T2D and arterial hypertension, we found that the effect was increased in patients without both conditions. As expected, the effect of VAT was independent of TAT (Supplementary Material). We also observed an association between increasing quartiles of VAT measurements (DXA-VAT/EST-VAT) and PWV, AIX75, and HOMA2-IR. Finally, we confirmed that an increase in DXA-VAT indicated higher odds of arterial stiffness and that increased visceral adiposity (DXA-VAT $\geq$1.4 kg) was also associated with arterial stiffness. These observations were replicated when using EST-VAT obtained with METS-VF. Finally, we confirm that these associations sustained in our mixed-effect models to control for the heterogenicity of our population after adjusted for covariates (Supplementary Material). Interestingly, when including both DXA-VAT and EST-VAT with HOMA2-IR, the latter did not remain significant indicating a potential mediating mechanism of VAT on PWV values.

Modifiers of the association of VAT (DXA-VAT/EST-VAT) with PWV measures

We observed a significant interaction of DXA-VAT with female gender ($\beta_{INT} = -0.217$, 95%CI: -0.324, -0.121) and age; subjects >50 years had a steeper increase in PWV with the increase in DXA-VAT values ($\beta_{INT} = 0.132$, 95%CI: 0.026,0.226). Interestingly, obese participants (BMI $\geq$30 kg/m^2) were shown to have decreased arterial stiffness compared with lean subjects with increased VAT ($\beta_{INT} = -0.069$, 95%CI: -0.076, -0.003). We confirm this finding in obese subjects according to body fat assessed by

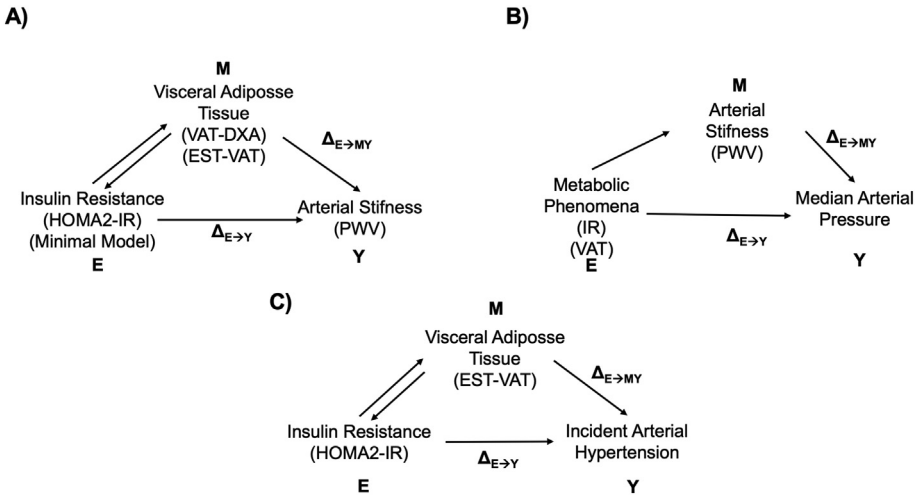

Figure 1 Model-based causal mediation analyses using arterial stiffness (A), median arterial pressure (B), and incident arterial hypertension (C) as our outcomes. *Abbreviations*: DXA = Dual X-Ray Absorptiometry; METS-VF = Metabolic Score for Visceral Fat; HOMA2-IR=Homeostatic Model for Insulin Resistance.

DXA (β_{INT} = −0.131, 95%CI: −0.236, −0.028, Fig. 3), which confirm that VAT has a hazardous effect on vascular health, even on lean subjects. These interactions remained significant even after adjustments (Fig. 3).

VAT and IR phenotypes modifies vascular health parameters

Next, we investigated whether IR status (HOMA2-IR >2.5) [25] could modify the association between increased visceral obesity (DXA-VAT >1.4 Kg) and PWV measures. Using these thresholds in our PWV cohort, 37 subjects (6.6%) were found with IR but normal VAT, 134 subjects (23.9%) with visceral obesity but no IR and 62 (11.1%) subjects with both IR and visceral obesity and the rest had none. We observed significantly higher PWV values in subjects with IR (7.08 ± 1.42 vs. 6.43 ± 1.30, $p < 0.001$) and in those with visceral obesity (7.21 ± 1.40 vs. 6.16 ± 1.17, $p < 0.001$). When comparing SBP, DBP and the pulse waveform analyses measurements, there were significantly higher PWV and AIX75 values in subjects with both IR and visceral obesity than in those with either IR or visceral obesity alone. SBP and DBP were also higher in subjects with both conditions compared to IR and healthy subjects but not compared to those with increased VAT (Supplementary Material).

VAT as a modifier of the effect of IR on PWV

Our first causal-based mediation model showed that the direct effect of HOMA2-IR (E) on PWV (Y) was not significant ($\Delta_{E\rightarrow Y}$ = 0.020, 95%CI -0.002, 0.040) but the effect of DXA-VAT (M) was ($\Delta_{E\rightarrow MY}$ = 0.027, 95%CI 0.017,0.040). Overall, the effect of IR on PWV is modified by DXA-VAT, approximately 57.6%. Interestingly, when we stratified our population according to T2D and arterial hypertension status, we found that the mediated effect of DXA-VAT increased to 78.9% and 58.5%, respectively; similar results were obtained using METS-VF. As expected, we found that VAT predicts HOMA2-IR, settling the bidirectional association. Nevertheless, we found that HOMA2-IR does not act as a mediator on the effect on PWV. To demonstrate that this effect is consistent, the same causal mediation analysis was carried out in the FSIVGTT dataset with minimal model analysis (n = 131, Supplementary Material). In this sample, the direct effect of IR-FSIVGTT (E) on PWV (Y) was not significant ($\Delta_{E\rightarrow Y}$ = 0.031, 95%CI -0.017, 0.070); however, the modifying effect of DXA-VAT (M) persisted ($\Delta_{E\rightarrow MY}$ = 0.0078, 95%CI 0.0010, 0.020), with 20.2% of the association between IR and PWV being explained by the presence of VAT, after adjusting for covariates (Table 3). As previously explored, we do not found a mediator effect of IR-FSIVGTT on PWV, but rather a bidirectional association of IR-FSIVGTT and VAT.

VAT as a modifier of the effect of PWV on MAP

The second model-based causal mediation analysis focused on evaluating whether arterial stiffness (M) was a mediator of the effect of either VAT or IR (E_1 and E_2, respectively) on MAP (Y). In the PWV cohort, the direct effect of DXA-VAT on MAP was significant ($\Delta_{E1\rightarrow Y}$ = 6.81, 95%CI: 3.35,10.50) as was the effect of PWV ($\Delta_{M\rightarrow Y}$ = 5.22, 95%CI: 3.43, 7.13), representing 35.7% of the total effect of VAT on MAP. For HOMA2-IR there was a significant direct effect ($\Delta_{E2\rightarrow Y}$ = 1.88, 95%CI: 0.70,3.30) but not a mediating effect of PWV on MAP. To confirm that VAT was the main moderator, a mediation model was generated whereby VAT (M) was explored as a modifier of the effect of IR (E) on MAP (Y); there was a direct effect of IR on MAP ($\Delta_{E\rightarrow Y}$ = 1.80, 95%CI 1.14−2.49), but more importantly, a modifying effect of VAT on MAP ($\Delta_{M\rightarrow Y}$ = 1.88, 95%CI 0.61−3.24), which accounted for 48.9% (95%CI 30.8−80.0) of the effect of IR on MAP. To confirm the consistency of this finding, this effect was explored using an independent dataset with EHC-derived measures. Using a causality

Table 1 Characteristics of the cohorts included in our study. Abbreviations: BMI: Body Mass Index; WTHr: Waist-to-height ratio; WHr: Waist-to-hip ratio; TC: Total Cholesterol; DXA: Dual-energy X-ray absorptiometry; Augmentation index 75 = heart rate–corrected augmentation index.

Parameter	PWV cohort n = 586	FSIVGTT cohort n = 131	EHC Cohort n = 97	MS-Cohort-Baseline n = 6850
Female sex (%)	412 (70.3%)	82 (62.6%)	97 (100%)	4623 (67.5%)
Age (years)	51 (41–59)	53 (45–57)	33 (24–48)	40.1 (9.8)
BMI (kg/m2)	29.10 (26.38–32.40)	26.4 (24.2–30.7)	41.8 (21.8–47.6)	27.6 (25.2–30.5)
WTHr	0.59 (0.54–0.65)	0.57 (±0.07)	0.7 (0.5–0.8)	0.59 (0.54–0.61)
Glucose (mg/dl)	101.0 (92.00–121.25)	94 (86–102)	91 (84–98)	85.4 (10.7)
Triglycerides (mg/dl)	160 (105–231)	143 (100–292)	121 (86.3–163)	153 (106–223)
TC (mg/dl)	195.0 (168.75–224.0)	195 (168–223)	175.4 (±31.8)	203 (40.8)
HDL-C (mg/dl)	43.0 (36.0–51.25)	42.8 (±13.6)	46.4 (±11.9)	44.3 (11.6)
LDL-C (mg/dl)	120.55 (99.18–141.93)	113 (87–140)	97.8 (88–122)	124.8 (28.8)
Insulin (UI/dl)	9.50 (6.30–14.40)	7.9 (5.1–11.7)	10.7 (5.7–22.4)	9.8 (6.9–14.2)
HbA1c (%)	6.0 (5.70–7.10)	5.7 (5.5–6.0)	5.4 (5.2–5.9)	–
SBP (mmHg)	121 (112–132)	118 (107–128.3)	112 (102–122)	114 (108–119.3)
DBP (mmHg)	73 (66–80)	71 (64.8–77)	74 (69.5–80.5)	75.5 (70–80)
MAP (mmHg)	89.9 (10.9)	86.8 (±11.8)	86.8 (±9.9)	86 (7.2)
EST-VAT (kg)	1.11 (0.77–1.50)	1.1 (±0.5)	1.5 (±1.2)	0.9 (0.6–1.2)
VAT-DXA (kg)	1.13 (0.78–1.61)	1.0 (0.6–1.6)	1.3 (0.02–1.8)	–
Carotidal PWV (mm/s)	6.52 (±1.34)	6.3 (5.6–7.2)	–	–
AIX 75 (%)	34.97 (±13.22)	34.3 (±12.8)	–	–

model in which the M-value adjusted for free-fat mass (M_{FFM}, E) had an effect on MAP (Y) via DXA-VAT, there was a significant direct effect ($\Delta_{E\rightarrow Y} = -8.92$, 95%CI -16.26, −0.91) and a significant modifying effect for VAT ($\Delta_{M\rightarrow Y} = -7.16$, 95%CI -12.62, −2.14); overall, DXA-VAT explained 43.4% of the effect of M_{FFM} on MAP, after adjusting for arterial hypertension. These analyses were replicated using EST-VAT from METS-VF (Table 4).

VAT as a modifier of the effect of IR on incident hypertension

Our analyses suggested that VAT may modify the effect of IR on MAP. In order to assess this prospectively, the role of VAT in predicting incident hypertension via IR was explored in our MS cohort, where METS-VF is known to be a predictor of incident hypertension [19]. Participants with visceral obesity or both IR and visceral obesity had a higher risk of incident hypertension, but not those with only IR (Supplementary Material). Using casually ordered mediators with a weighted Cox-Breslow model, VAT (EST-VAT) was found to be a modifier of the increased risk for incident hypertension associated with IR (HOMA2-IR). Overall, the role of increased visceral obesity explained 69.1% of the risk (95%CI 46.1–78.8) of incident hypertension associated with IR (Table 4).

Discussion

In the present work, IR and VAT accumulation are shown to be interlinked metabolic disturbances. This IR-VAT relationship modifies the effect of both phenomena on altered vascular health, leading to arterial stiffness, increased blood pressure levels, and eventually arterial hypertension. Moreover, we show that VAT acts as a mediator of the effect of IR on altered vascular hemodynamics including arterial stiffness, blood pressure and overall hypertension risk. Our approach is novel since it investigates the association from a pathophysiological perspective, culminating in a direct clinical application which integrates previous evidence into a comprehensive pathway assessed in a diverse cohort of patients. Our results confirm a joint effect of both phenomena in altered vascular health; nevertheless, the controversial role of IR and VAT has not been clearly established. Both mechanisms contributed to their own pathophysiological changes, although both had similar and joint pathways, which could explained the effects of IR/VAT hazard on vascular health. The relationship of visceral adiposity and arterial stiffness has previously been demonstrated [13,14]; this relationship, although not completely understood, is supported by evidence on metabolic impairments underlying the accumulation of visceral adiposity. One of the most reliable hypothesis of this effect may be attributable to altered production of proinflammatory adipokines and impairments in lipolytic activity in VAT [27]. It has been shown that VAT increases the production of leptin and visfatin, both of which increase the risk of cardiovascular disease [28]. Both adipokines promote endothelial dysfunction by exerting downregulation of the nitric oxide synthase (iNOS) activity, increasing the effect of angiotensin II and proliferation of vascular smooth muscle cells in large arteries which reduce compliance of large arteries [29–31]. Furthermore, VAT produces traditional proinflammatory cytokines (IL-6, IL-1 and TNF-alfa), which augments the proinflammatory state in obese patients [32]. Other mechanisms involved are those directly

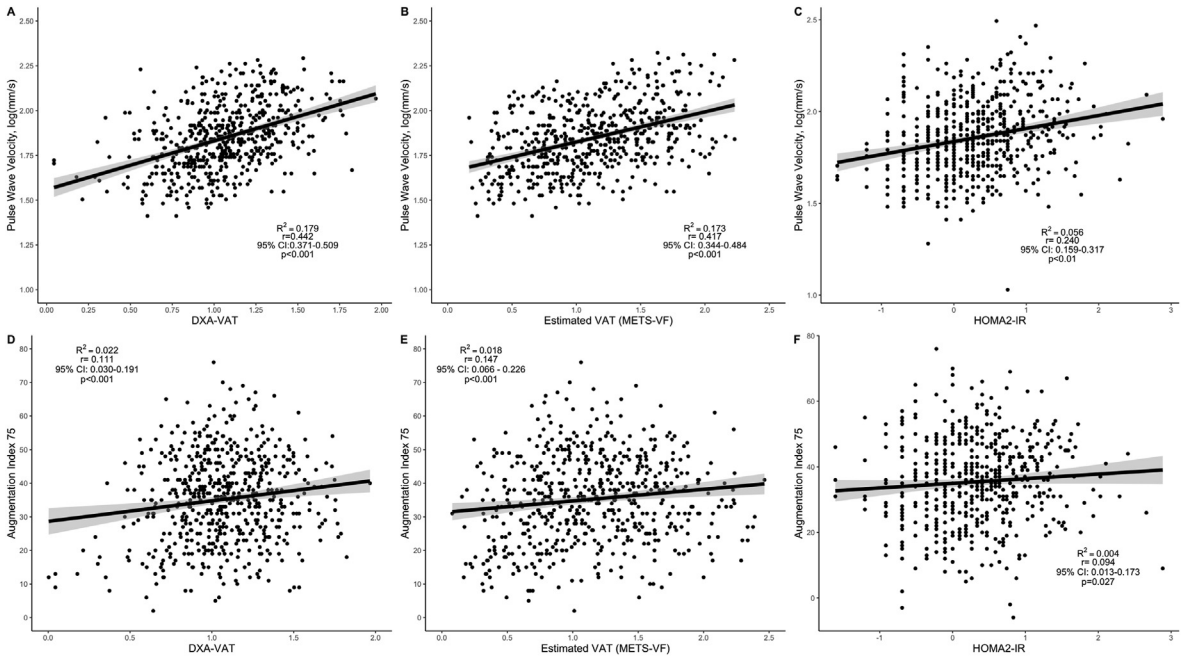

Figure 2 Association of pulse wave velocity (A,B,C) and heart rate−corrected augmentation index (augmentation index 75; D,E,F) with DXA-VAT, EST-VAT and HOMA2-IR, respectively. *Abbreviations*: DXA = Dual X-Ray Absorptiometry; METS-VF = Metabolic Score for Visceral Fat; HOMA2-IR=Homeostatic Model for Insulin Resistance.

related in the renin-angiotensin-aldosterone system (RAAS). It has been proposed that VAT impairs the RAAS through mechanical compression, increased production of RAAS proteins (renin, angiotensin-converting enzyme, chymase, angiotensin 1-7), and induced activation of mineralocorticoid receptors attributable to VAT [33−35]. Additionally, it has been explored in experimental studies that VAT induces the activation of the sympathetic nervous system directly by renal nerves and promoting dysfunction in the melanocortin receptors [35,36]. Moreover, it has been proposed a bi-directional mechanism between VAT and IR. As previously described, increased VAT promotes an proinflammatory state in which there is a release of pro-inflammatory cytokines (e.g. IL-1B, IL-6, TNF-a) and reactive oxygen species, which promotes a stress-induced response, leading to a hyperglycemic state and consequently a decreased insulin action [37]. All these mechanisms related to the deleterious risk properly are attributed to VAT in promoting arterial stiffness, which ultimately impacts vascular hemodynamics.

To further prove that the effect of VAT on vascular health is heterogenous and is modified by certain factors, we showed that sex acts as a modifier on the effect of VAT on PWV, a mechanism which is probably explained by the physiological changes seen in menopause leading to an increased risk of arterial hypertension [38]. It is known that VAT increases with age and we showed that increasing age has a more negative effect of VAT on arterial stiffness. Finally, the interaction observed in lean subjects confirms that VAT accumulation could increase the risk of

Table 2 Linear regression models to test the association of pulse wave velocity and heart rate corrected augmentation index with DXA-VAT, estimated VAT (EST-VAT) using METS-VF and HOMA2-IR. We adjusted for sex, age, HOMA2-IR (in models with VAT-DXA and EST-VAT), HbA1c, fasting cholesterol, fasting triglycerides, smoking status and arterial hypertension. Abbreviations: METS-VF: Metabolic Score for Visceral Fat; DXA-VAT: Dual-energy X-ray absorptiometry used to estimate visceral adipose tissue.

Model	Parameter	β	95%CI	T	P Value
Pulse Wave Velocity (PWV)	DXA-VAT R^2 = 0.476; F = 52.96	0.122	0.113−0.132	5.30	<0.001
	Estimated VAT (METS-VF) R^2 = 0.359; F = 41.19	0.191	0.089−0.149	7.74	<0.001
	HOMA2-IR R^2 = 0.439; F = 51.50	0.044	0.023−0.065	4.17	<0.001
Heart rate-corrected augmentation Index (AIX75)	DXA-VAT R^2 = 0.279; F = 24.61	7.648	3.41−11.88	3.55	<0.001
	Estimated VAT (METS-VF) R^2 = 0.035; F = 3.686	2.927	0.580−5.27	2.45	0.015
	HOMA2-IR R^2 = 0.264; F = 25.57	2.439	2.439−3.923	3.23	0.001

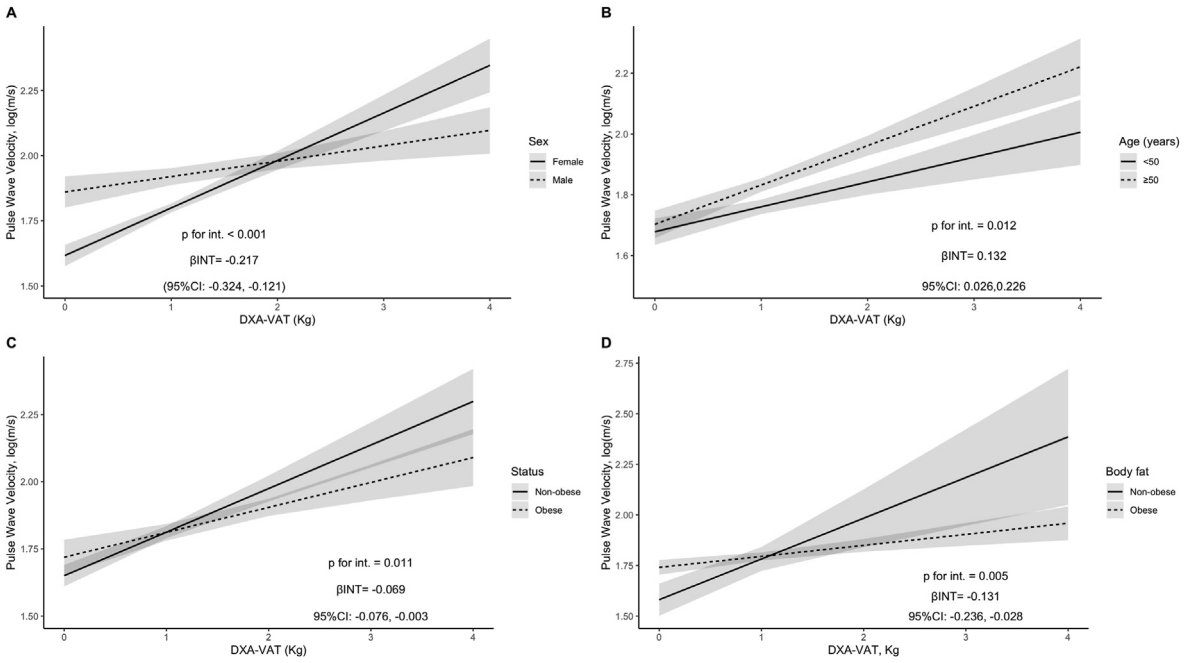

Figure 3 Estimated interaction effect of DXA-VAT with sex (A), age (≥50 years) (B), BMI obesity (C), and Body fat obesity using DXA (D). *Abbreviations*: DXA = Dual X-Ray Absorptiometry; METS-VF = Metabolic Score for Visceral Fat.

cardiometabolic events independently of whole-body fat distribution assessed by BMI, as shown in previous studies [33,39]. Overall, this confirms that the effect of VAT is modified by several patient-specific factors, including the state of whole-body insulin sensitivity.

Although the debate thus far has been focused on the underlying metabolic mechanisms that could explain the phenomena involved in visceral adiposity, IR has been traditionally tagged as the trigger between both visceral adiposity and vascular health [40]. IR promotes accumulation of VAT by increasing the release of free-fatty acids directly to the portal circulation, leading to dysregulation in glucose uptake, glycogen synthesis and glucose oxidation; as shown by our data, this indirect effect could lead to VAT-induced adverse vascular effects. In addition, IR impairs vascular health directly via the activation of non-enzymatic glycation of matrix proteins, causing sub-endothelial accumulation of advanced end glycation products, proving that these mechanism lead to arterial stiffening and altered vessel hemodynamics [41]. Studies have proven that IR also impairs the RAAS, thought to be adipose tissue dysfunction, and thus, we could hypothesize that these mechanisms confirm that IR has both direct and VAT-mediated effects on vascular health and that the metabolic disturbances associated with IR might lead to endothelial impairments and increase arterial hypertension risk [42].

Our suggested hypothesis driven by our results is that IR causes an increased accumulation of VAT. Nevertheless, VAT accumulation could be responsible for an increased hazard effect on vascular hemodynamics in patients with IR by the mechanisms above mentioned. As these effects sustain in subjects at risk for both conditions (e.g. metabolic syndrome, prediabetes or T2D), these will eventually cause arterial stiffness in an irreversible way, leading to arterial hypertension [43,44]. Targeted interventions to ameliorate both IR and VAT accumulation should be assessed to identify long-term benefits of improving both phenomena and promoting vascular health.

Our results also indicate that the potential application of these mechanisms would demand routine examination of both IR and VAT to design interventions, which might delay or prevent vascular dysfunction. Although several anthropometrical and biochemical indexes have been proposed for the estimation of VAT, the lack of inclusion of an IR estimator is a potential limitation of such indexes [19]. Estimation of VAT using METS-VF has been suggested as a novel approach to evaluate the cardiometabolic risk attributable to VAT, with a capacity to predict T2D and arterial hypertension, with the additional benefit of including an IR estimator (METS-IR). Here, we observed that the associations with direct DXA-VAT measurements were replicated using METS-VF, providing evidence for its use in a clinical setting where the availability of imaging methods and trained personnel for interpretation may be limited. The addition of a VAT estimator could complement the evaluation of cardiometabolic risk in primary care settings and represent a low-cost strategy for the evaluation of cardiovascular and metabolic health in at-risk subjects.

This study has some strengths and limitations. A strength of this approach is the use of several methods to validate findings using surrogates including HOMA2-IR and METS-VF and by using rigorous measurements to evaluate IR, such as the EHC, FSIVGTT and DXA for VAT assessment in diverse cohorts with various risk profiles, which increases the external validity of the findings [31].

Table 3 Causal mediation analysis predicting the mediating effect of VAT-DXA and EST-VAT, on pulse wave velocity and arterial stiffness via insulin resistance. Abbreviation: ACME: average causal mediation effects; ADE: average direct effects.; IR as defined as HOMA2-IR >2.5.

Causal Mediation Model	Outcome (Y)	Effector (E)	Mediator (M)	ACME	ADE	Total Effect	Proportion Mediated
1	Pulse-wave velocity	HOMA2-IR (PWV Cohort)	DXA-VAT	0.027 (0.017−0.040)	0.020 (−0.002−0.040)	0.047 (0.027−0.070)	57.6% (31.7−100.0)
			EST-VAT	0.014 (0.007−0.020)	0.026 (0.006−0.050)	0.041 (0.018−0.060)	36.5% (17.8−75.0)
	Arterial stiffness (PWV > p80)	IR (PWV Cohort)	DXA-VAT >1400 g	0.036 (0.015−0.070)	0.034 (−0.036−0.110)	0.70 (0.002−0.160)	51.4% (−138.1−353.0)
			EST-VAT (METS-VF >7.18)	0.016 (0.0002−0.030)	0.041 (−0.040−0.130)	0.057 (−0.029−0.140)	27.8% (−193.1−220.0)
	Pulse-wave velocity	Minimal Model (FSIVGTT-Subset)	DXA-VAT	0.0078 (0.0010−0.020)	0.031 (−0.017−0.070)	0.038 (−0.008−0.020)	20.2% (−881.8−143.0)
			EST-VAT	0.022 (0.005−0.040)	0.010 (−0.036−0.060)	0.032 (−0.014−0.080)	59.5% (−283.4−567.0)

Table 4 Causal mediation analysis predicting the mediating effect of DXA-VAT/EST-VAT on mean arterial blood pressure (MAP) and incident arterial hypertension via insulin resistance. Abbreviations: ACME: average causal mediation effects; ADE: average direct effects; FFM: Fat-free mass.

Causal Mediation Model	Outcome (Y)	Mediator (M)	Effector (E)	ACME	ADE	Total Effect	Proportion Mediated
2	MAP	PWV (PWV Cohort)	DXA-VAT	3.78 (2.42, 5.43)	6.81 (3.35−10.50)	10.59 (7.07, 14.36)	35.7% (23.8−59.0)
			HOMA2-IR	0.42 (−0.08, 0.94)	1.46 (0.30−2.79)	1.88 (0.70−3.30)	22.2% (2.3−93.0)
	MAP	DXA-VAT (EHC cohort)	M-value adjusted for FFM (EHC cohort)	−7.16 (−12.62, −2.14)	−8.92 (−16.26, −0.91)	−16.08 (−21.31, −10.42)	43.4% (13.6−91)
		EST-VAT (EHC cohort)		−5.07 (−11.21, 0.48)	−10.77 (−18.60, −2.96)	−15.84 (−21.25, −10.49)	32.3% (−0.02,75.0)
3	Incident arterial hypertension	HOMA2-IR (MS-Cohort)	EST-VAT	0.00037 (0.00006−0.00068)	0.061 (0.039−0.083)	0.062 (0.039−0.084)	0.60% (0.15−0.81)
			EST-VAT (METS-VF >7.18)	0.047 (0.018−0.082)	0.021 (0.011−0.032)	0.068 (0.039−0.104)	69.1% (46.1−78.8)

The METS-VF index was used as a clinical surrogate of visceral adiposity; this is reliable compared with DXA evaluation, leading to an optimal clinical surrogate that also correlates with increased arterial stiffness. Finally, IR was evaluated using three different methods, in which the mediation results were replicated, providing evidence that visceral adiposity is a partial modifier of IR on vascular health, even after adjusting for conditions that also increase cardiovascular risk (e.g. T2D, arterial hypertension, and dyslipidemia). Moreover, we replicate our findings using clinical surrogates, which endorses the hypothesis that cardiometabolic health estimation should be assessed in a clinical setting.

Limitations must be acknowledged, including the ethnic composition of the cohorts; the results are restricted to Mexican population with diverse metabolic conditions given the variability of body composition, which might influence numeric estimates. Furthermore, other conditions that are known to modify vascular hemodynamics were not included, such as participants with previously known cardiovascular events or subjects with chronic kidney disease. We do not perform additional VAT measurements with other imaging methods, such as MRI or CT; nevertheless, the correlation with DXA has been proven to be sufficient in previously performed studies ([19,45]). Finally, because the associations with PWV were only conducted in a cross-sectional setting, the role of direct and indirect effects of IR on arterial hypertension risk via PWV remains an area of opportunity for future research.

In conclusion, we explore a potential mediation mechanism that links IR and VAT on arterial stiffness, increased blood pressure levels and arterial hypertension risk. The role of VAT as a known modifier of vascular health and cardio-metabolic risk should be interpreted considering the overall IR status to fully address its role on vascular health. Given the increasing epidemic of obesity and IR-associated phenomena, a complete understanding of the mechanisms involved and their impact on vascular health should shed light on the benefits of interventions targeting both phenomena.

Authors′ contributions

NEAV, OYBC, AVV, CAFM, and DABG developed the conceptualization of the study and performed and interpreted statistical analyses and developed predictive models. RM, AVV, and AJM performed data collection. NEAV, OYBC, RM, and CAAS participated in manuscript drafting and processing. CAAS provided mentorship and supervision. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors read and approved the final version of this manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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Appendix B

METABOLIC SYNDROME STUDY GROUP: The metabolic syndrome study group is composed of: Olimpia Arellano-Campos, Donaji V. Gómez-Velasco, Omar Yaxmehen Bello-Chavolla, Tania Viveros-Ruiz, Alexandro J. Martagón-Rosado, Ivette Cruz-Bautista, Marco A. Melgarejo-Hernandez, Paloma Almeda-Valdés, Liliana Muñoz-Hernandez, Daniel Elias-Lopez, Fabiola Mabel Del Razo-Olvera, Bethsabel Rodríguez Encinas, Renán Fernando Fagoaga Ramírez, Luz E. Guillén, José de Jesús Garduño-García, Ulices Alvirde, Yukiko Ono-Yoshikawa, Ricardo Choza-Romero, Leobardo Sauque-Reyna, Ma. Eugenia Garay-Sevilla, Juan M. Malacara-Hernandez, María Teresa Tusié-Luna, Luis Miguel Gutierrez-Robledo, Francisco J Gómez-Pérez, Rosalba Rojas, and Carlos A. Aguilar-Salinas.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2020.09.031.

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