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Original article

Metabolic Score for Visceral Fat (METS-VF), a novel estimator of intra-abdominal fat content and cardio-metabolic health

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SUMMARY

Background & aims: Intra-abdominal and visceral fat (VAT) are risk factors for the development of cardio-metabolic comorbidities; however its clinical assessment is limited by technology and required expertise for its assessment. We aimed to develop a novel score (METS-VF) to estimate VAT by combining the non-insulin-based METS-IR index, waist-height ratio (WHtr), age and sex.

Methods: We developed METS-VF in a sample of 366 individuals with Dual X-ray absorptiometry (DXA). METS-VF was modeled using non-linear regression and validated in two replication cohorts with DXA (n = 184, with n = 118 who also had MRI) and bio-electrical impedance (n = 991). We also assessed METS-VF to predict incident type 2 diabetes (T2D) and arterial hypertension independent of body-mass index (BMI) in our Metabolic Syndrome Cohort (n = 6144).

Results: We defined METS-VF as: $4.466 + 0.011*(\text{Ln(METS-IR)})^3 + 3.239*(\text{Ln(WHtr)})^3 + 0.319*(\text{Sex}) + 0.594*(\text{Ln(Age)})$. METS-VF showed better performance compared to other VAT surrogates using either DXA (AUC 0.896 95% CI 0.847–0.945) or MRI (AUC 0.842 95% CI 0.771–0.913) as gold standards. We identified a METS-VF cut-off point >7.18 in healthy patients which has 100% sensitivity (95% CI 76.8–100) and 87.2% specificity (95% CI 79.1–93.0) to identify increased VAT (>100 cm^2). METS-VF also had adequate performance in subjects with metabolically-healthy obesity. Finally, in our metabolic syndrome cohort, subjects in the upper quintiles of METS-VF (>7.2) had 3.8 and 2.0-fold higher risk of incident T2D and hypertension, respectively ($p < 0.001$). This effect was independent of BMI for both outcomes.

Conclusion: METS-VF is a novel surrogate to estimate VAT, which has better performance compared to other surrogate VAT indexes and is predictive of incident T2D and hypertension. METS-VF could be a useful tool to assess cardio-metabolic risk in primary care practice and research settings.

1. Introduction

Accumulation of visceral adipose tissue (VAT) has been associated with insulin resistance (IR), the number of metabolic syndrome traits and an increased risk of developing type 2 diabetes (T2D), atherosclerosis, dyslipidemia, hypertension and coronary heart disease [1–5]. A gold standard to assess VAT is magnetic resonance imaging (MRI); nevertheless, MRI is expensive and needs to be performed and interpreted by a specialist. Other

Abbreviations: METS-VF, Metabolic Score for Visceral Fat; DXA, dual X-ray absorptiometry; MRI, magnetic resonance imaging; VAA, visceral adipose area; IR, insulin resistance; BIA, bioelectrical impedance; BMI, body-mass index; MS, metabolic syndrome; HR, hazard ratio; 95% CI, 95% confidence interval; AUC, area under the curve; ROC, receiver operating characteristic; VAT, visceral adipose tissue.

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imaging techniques include computerized tomography (CT), dual X-ray absorptiometry (DXA) and Bioelectrical Impedance Analysis (BIA), which are accessible, safe and highly correlated with MRI evaluations [6]. Despite increasing evidence regarding the clinical relevance of evaluating visceral adiposity, its application in everyday clinical practice is limited by equipment and technical difficulties.

Since visceral adiposity has significant metabolic burden and its assessment has been shown to be challenging, surrogate laboratory and anthropometry-based measures have been developed to estimate VAT. Routinely applicable anthropometrical indicators of VAT content include waist circumference (WC), body mass index (BMI), waist-to height index (WHtr) and waist to hip ratio (WHR, [7]); a limitation of these indicators is the challenge of distinguishing between subcutaneous adipose tissue and VAT, especially since these two compartments have opposed clinical and physiological implications. Both subcutaneous and visceral fat have been consistently associated with increased cardio-metabolic risk factors; however, the strongest adverse associations and the higher cardiovascular risk have been attributed to visceral fat depots [8]. This has brought VAT assessment to the spotlight of metabolic research, leading to the development of combined anthropometric and laboratory-based estimators, including the Visceral Adiposity Index (VAI), Lipid Accumulation Product (LAP), Estimated Visceral Area (EVA) and Deep Abdominal Adipose Tissue (DAAT) indexes, which have been validated in different ethnic groups as an effort to translate VAT estimation into epidemiological research and everyday clinical practice [9–12].

Since IR is associated with adipose tissue dysfunction and accumulation of VAT, we hypothesized that using an IR estimate along with anthropometric measures would increase the precision of VAT estimation. METS-IR, a novel non-insulin-based index to estimate IR, has proven to be more accurate to evaluate IR compared to other non-insulin-based indexes; METS-IR is also associated with pathophysiological components of metabolic syndrome and predicts T2D and hypertension [13]. Currently there is no VAT estimator that considers IR as a key component; furthermore, previous data showed a strong predictive capacity for VAT area using METS-IR. Known modifiers of VAT accumulation also include age and male sex; furthermore, body fat distribution could be a reliable marker of VAT accumulation and previous work has demonstrated that VAT accumulation could be accurately assessed using the WHtr [14,15]. In this work, we aimed to develop a novel estimator which combines METS-IR, WHtr, age and sex to estimate VAT using DXA-derived intra-abdominal fat mass estimation and validated against MRI measures of VAT. Furthermore, we evaluated a physiological correlation with adipokines for the index and its capacity to predict cardio-metabolic complications independently of increased BMI.

2. Subjects and methods

2.1. Participants and study setting

2.1.1. Discovery sample and physiological evaluations

In the discovery cohort, we included men and women aged 18–78, with BMI >18.5 kg/m^2 recruited from our clinical facilities. A complete medical and family history, including use of medication was obtained from all subjects from an interview by trained staff. Subjects with history of T2D, whom where not receiving insulin and were only treated with exclusively metformin were included, alongside with subjects with history of essential hypertension and dyslipidemia. No subject had cardiovascular disease, T2D complications, acute infection or any other lipodystrophic syndrome (e.g. HIV). All subjects were invited to participate in the study and signed and informed consent. The Human Research Ethics Committee of Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran (INCMNSZ) approved the study. All procedures were done in accordance with the Declaration of Helsinki.

2.2. Data collection

2.2.1. Biochemical evaluation

Blood samples were obtained after 8–12 h of fasting. Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA), serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2). Lipid concentrations (cholesterol, triglycerides and HDL cholesterol) were measured using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). Plasma adiponectin, leptin, and fibroblast growth factor (FGF-21) concentrations were determined by performing ELISA assays (Merck Millipore). LDL cholesterol was calculated using Martin's equation [16]. METS-IR was calculated using the formula: $(\mathrm{Ln}\,((2{*}G_0) + TG_0){*}\mathrm{BMI})/(\mathrm{Ln}\,(\mathrm{HDL\text{-}C}))$, where G_0 and TG_0 represent fasting glucose and triglyceride concentrations, respectively [13].

2.2.2. Anthropometry and body composition analysis

Anthropometry: Body weight was measured to the nearest 0.1 kg using a seca mBCA 514 medical body composition analyzer with 50 g gradation and height was measured to the nearest 0.1 cm using a seca 284 stadimeter with 1 mm gradation. WC was evaluated using an inelastic seca 201 tape with 0.1 cm precision directly over the skin at the mid-point between the ribcage and the iliac crest with the tape parallel to the floor and collocated after palpation. BMI was calculated as weight in kg divided by the squared product of height in meters. WHtr was calculated WC divided by height, both in centimeters. All anthropometric measures were performed after an 8–12 h fast by trained personnel the day of blood sample evaluations.

DXA evaluation: Whole-body fat, lean mass and visceral adipose tissue (VAT) mass were determined using dual energy X-ray absorptiometry (DXA) (GE Healthcare, CoreScan software version 16) by a certified densitometrist technician. All evaluations were carried out after at least a 4 h fast.

MRI evaluation: Abdominal MRI images were acquired using T1 and T2 sequences in the axial plane using a 3-T MRI scanner (Philips Achieva 3 T). Visceral adipose tissue area (VAA) was measured at the level of the L2–L3, L3–L4 vertebral superior endplate at the umbilicus level, with three slices obtained superior, and one slice inferior at 40 mm intervals (16 cm window); VAA was distinguished from subcutaneous adipose tissue based on the abdominal wall muscle layer. Images were analyzed by a single individual (EEV) using the Analyze software version 2.0.

BIA evaluation: Estimated visceral adipose tissue (EVAT) was measured using 8-point bioelectrical impedance analysis (BIA) with a SECA mBCA 515 medical body composition analyzer calibrated for Hispanic population. Measurements were obtained in subjects with a previous 8–12 h fast in a supine position by applying a low-intensity electric current between two pairs of electrodes placed in both feet of the subjects and in both hands by trained personnel dedicated to body composition analysis. Subjects were indicated to not wear metallic objects and were not consuming medication or had conditions (eg. lipodystrophy, edema) which interfered with the measurements. Body composition measurements were assessed the same day for each patient.

2.3. Development and validation of the index

2.3.1. Mathematical modeling

To develop an estimator for intra-abdominal and visceral fat using DXA, we evaluated non-linear fits of individual crude and log-transformed variables aiming to maximize the explained variance of individual components. The working hypothesis of this work considered that the main predictors for intra-abdominal and visceral fat would include an insulin resistance component (METS-IR), an anthropometric measure of body-fat distribution (WHtr) as well as age, and sex, as suggested by previous research [8,14]. Based on these assumptions, we developed a linear combination of transformed variables which maximized the explained association for log-transformed intra-abdominal fat mass; estimated model coefficients ($\hat{\beta}$) were calculated using the Levenberg–Marquardt algorithm. Model diagnostics were conducted using R^2 and Akaike's information criterion (AIC). The resulting model was termed Metabolic Score for Visceral Fat (METS-VF).

2.3.2. Validation of METS-VF against VAT from DXA, MRI and BIA

METS-VF was validated using three methods to assess VAT (Fig. 1). In the first cohort we included 184 additional subjects who also underwent a total body composition analysis using DXA, aged 20–79 years, without diagnosed cardiovascular disease, smoking history or T2D complications. Furthermore, in a subset of subjects from the validation cohort VAA was quantified in 118 patients using magnetic resonance imaging (MRI). Increased VAA-MRI >100 cm^2 according to previously-established cut-off values, validated for several ethnic groups [17]; in order to identify a cut-off for VAT-DXA mass in our population, we contrasted VAT-DXA and MRI values >100 cm^2 determined the cut-off using the Youden index (VAT-DXA >1389 g, 88.6% sensitivity, 88.9% specificity, AUROC = 0.919 95% CI 0.859–0.978).

A potential limitation of our approach is the assumption that increased in intra-abdominal fat or VAT would be proportional to metabolic disturbances. To mitigate this limitation, an independent sample of 91 metabolically healthy obese individuals (MHO) recruited from other ongoing studies was also assessed. We defined metabolically-healthy obesity as ≤1 NCEP ATPIII criteria for metabolic syndrome except for WC in individuals with BMI ≥30 kg/m^2, in whom VAT-DXA was assessed. This approach would reasonably test the ability of the index to detect VAT independent of significant metabolic abnormalities.

Finally, a third validation was carried out in a cohort of 991 subjects, aged 18–85 years; for validation of METS-VF in the BIA cohort, we defined increased EVAT as values >80th percentile. To contrast our novel score with currently validated models, we calculated the following adipose tissue subrogates: VAI, LAP, EVA and DAAT from fasting and anthropometrical measures in all cohorts [9–12].

2.3.3. Association of METS-VF with adipokine levels

In the subset of the validation cohort in whom VAA-MRI was estimated, plasma adiponectin, leptin, and fibroblast growth factor 21 (FGF-21) concentrations were also assessed. To evaluate the physiological dose–response correlation of adipokine profiles in the MRI sub-cohort, we compared adipokines across METS-VF terciles using trend analysis.

2.3.4. Prediction of incident T2D and hypertension using METS-VF in an open-population cohort

Finally, we evaluated the capacity of METS-VF to predict incident T2D and hypertension in our Metabolic Syndrome Cohort, an open population cohort with the objective to evaluate risk of MS components in the incidence of T2D, hypertension and cardiovascular mortality in an urban population living in 9 different cities in Mexico. The methodology, inclusion criteria and results are described elsewhere [13,15]. Incident T2D was defined as a construct of previous medical diagnosis of T2D, taking hypoglycemic medication and/or fasting glucose levels as determined by current ADA guidelines. Incident hypertension was defined as a construct of previous medical diagnosis of hypertension, taking anti-hypertensive drugs and/or blood pressure >140/90 mmHg as determined by current AHA guidelines. To better evaluate the independent role of METS-VF as a risk factor, analyses were stratified according to BMI category. Time to follow-up was estimated from recruitment up to the last follow-up or occurrence of the incident outcome, whichever occurred first.

2.4. Statistical analysis

2.4.1. Intergroup differences and paired data

To evaluate intergroup differences, we used Student's t-test and Mann–Whitney U where appropriate. Frequency distribution of categorical variables is reported as frequencies and percentages and was compared between groups using chi-squared tests. Logarithmic transformations were applied to approximate normality in variables showing a non-normal distribution. Data are presented as mean ± SD or median and interquartile range.

2.4.2. Validation of METS-VF

We used correlation analysis to evaluate association of VAT subrogates, including METS-VF, against VAT-DXA for the training and validation cohort, VAA-MRI and EVAT. Diagnostic performance was evaluated using areas under the receiving operating characteristic curve (AUROC). To estimate differences between AUROC we performed non-parametric ROC tests using stratified bootstrap sampling with the *pROC* R package. The cut-off point was determined using the Youden index; sensitivity, specificity, positive and negative predictive values and likelihood ratios (PPV, NPV, LR(+), LR(−), respectively) were calculated using the *OptimalCutpoints* R package. To evaluate concordance of VAT estimation using the index with VAT-DXA we performed Bland–Altman analyses.

2.4.3. Prediction of incident T2D, hypertension using METS-VF

To evaluate the association of METS-VF and incident T2D and hypertension we performed survival analysis comparing across METS-VF quartiles, quintiles and cut-off values using Kaplan–Meier curves compared with log-rank tests. Cox proportional-risk regression analyses were used to evaluate risk of incident outcomes adjusting for physical activity, family history of T2D and/or hypertension, prevalent hypertension and smoking. All statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 21.0), R software (Version 3.5.1) and GraphPad Prism (Version 6.0).

3. Results

3.1. Study populations

In the discovery cohort we included 366 individuals with mean age 46.14 ± 14.07 and female predominance (79.1%). Amongst the discovery cohort, 43 subjects were within normal weight (11.7%), 125 were overweight (34.2%) and 198 were obese (54.1%). T2D was present in 62 individuals (17.1%) and hypertension in 54 (14.9%) (Table 1). General assessment of DXA-derived body composition analyses stratified by sex is presented in Supplementary material. In the validation cohort, we included 184 individuals with mean age 46.37 ± 13.98, female predominance (65.6%) and higher T2D

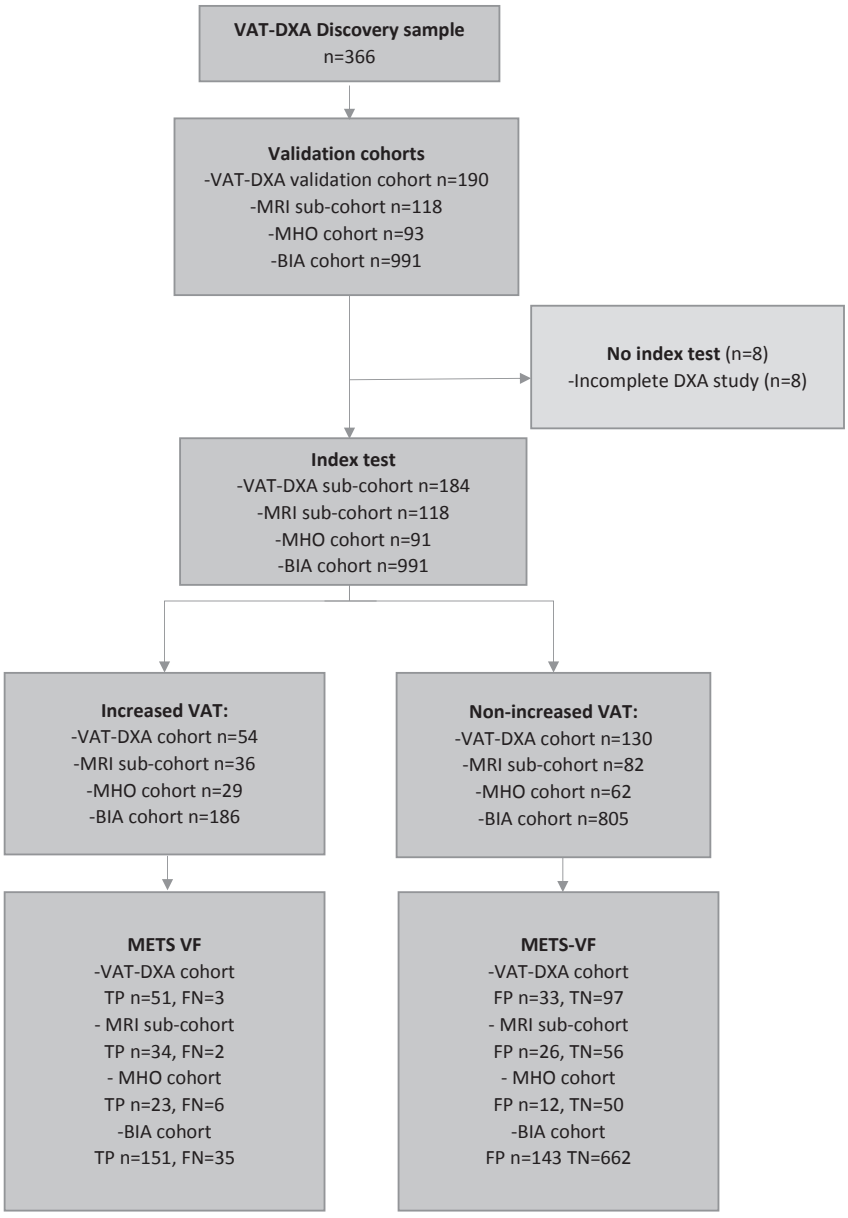

Fig. 1. STARD diagram representing evaluated cohorts for development and validation of the Metabolic Score for Visceral Fat (METS-VF) comparing across validation cohorts according to true and false positive and negative values. Abbreviations: MHO: metabolically healthy obese; MRI: Magnetic Resonance Imaging; VAT-DXA: Visceral Adipose Tissue assessed by dual X-ray absorptiometry, BIA: Bioelectrical impedance analysis; TP: True positive; FP: False positive; TN: True negative; FN: False negative.

Table 1
Biochemical and antropometrical characteristics of training and validation cohorts. Data is presented in mean (±SD) or as median (IQR) according to its distributions.

Parameter	Training DXA-cohort (n = 366)	Validation DXA cohort (n = 184)	MRI subcohort (n = 118)	MHO cohort (n = 91)	BIA cohort (n = 991)
Female sex (%)	287 (79.1)	122 (65.6)	68 (57.6)	81 (89.0)	654 (65.9)
T2D (%)	62 (17.1)	64 (34.4)	64 (54.2)	–	138 (13.8)
Age (years)	46.14 ± 14.07	46.37 ± 13.98	43.07 ± 15.31	40.04 ± 14.29	43.14 ± 14.72
Glucose (mg/dL)	101.84 ± 30.42	99.49 ± 23.06	104.21 ± 26.87	90.01 ± 6.98	106.43 ± 46.29
Insulin (μI/mL)	11.15 (6.87–17.05)	7.00 (5.07–10.12)	7.60 (5.27–11.42)	11.2 (6.9–15.4)	9.0 (5.9–14.3)
Total cholesterol (mg/dL)	179.55 ± 64.21	189.22 ± 37.25	180.67 ± 35.14	177.32 ± 33.44	197.86 ± 43.92
Triglycerides (mg/dL)	161.0 (109.0–261.0)	122.0 (87.5–164.25)	120.0 (85.0–163.25)	115.5 (85.8–134.5)	141.0 (97.0–194.0)
HDL-C (mg/dL)	48.69 ± 27.92	46.48 ± 27.62	45.19 ± 12.34	46.5 ± 7.90	46.01 ± 12.92
BMI (kg/m^2)	32.41 ± 8.14	27.61 ± 4.28	27.32 ± 3.73	36.22 ± 8.46	29.43 ± 6.97
Waist circumference (cm)	100.15 ± 18.24	95.59 ± 14.56	96.75 ± 15.37	105.73 ± 16.55	94.77 ± 16.30
METS-IR	54.06 ± 19.50	42.42 ± 8.56	42.34 ± 7.62	53.75 ± 13.09	46.07 ± 12.82

Abbreviations: T2D: type 2 diabetes; BMI: body mass index; METS-IR: metabolic Score for Insulin Resistance; MHO: metabolically healthy obese; MRI: magnetic resonance imaging; DXA: dual X-ray absorptiometry; BIA: bio-electrical impedance analysis.

prevalence (34.9%). In this cohort, 61 subjects had normal weight (33.1%), 75 were overweight (40.8%) and 48 were obese (26.1%).

3.2. Mathematical modeling of METS-VF

To develop a novel VAT estimate in the discovery cohort, we observed that log-transformed METS-IR and WHtr had the highest observed R^2 using cubic fits for log-transformed VAT mass. Similarly, log-transformed age and male sex were associated to higher VAT mass. Using non-linear regression, we estimated $\widehat{\beta}$ coefficients for each predictor; the overall model explained 59.6% of the variability of log-transformed VAT-DXA (R^2=0.596, Table 2). The model included METS-IR ($\widehat{\beta} = 0.011$), WHtr ($\widehat{\beta} = 3.239$), age ($\widehat{\beta} = 0.546$) and male sex ($\widehat{\beta} = 0.319$) with a resulting equation defined as:

$$\begin{aligned}\mathrm{METS-VF} = {} & 4.466 + 0.011\left[(\mathrm{Ln}(\mathrm{METS-IR}))^3\right] \\ & + 3.239\left[(\mathrm{Ln}(\mathrm{WHtr}))^3\right] + 0.319(\mathrm{Sex}) \\ & + 0.594(\mathrm{Ln}(\mathrm{Age}))\end{aligned} \quad (1)$$

where sex was a binary response variable (male = 1, female = 0) and age expressed in years. Since METS-VF essentially represents log-transformed VAT-DXA mass values, to estimate VAT mass the following transformation is required:

$$\begin{aligned}\mathrm{VAT\,(g)} = {} & e^{4.466+0.011\left[(\mathrm{Ln}(\mathrm{METS-IR}))^3\right]+3.239\left[(\mathrm{Ln}(\mathrm{WHtR}))^3\right]} \\ & +0.319(\mathrm{Sex}) + 0.594(\mathrm{Ln}(\mathrm{Age}))\end{aligned} \quad (2)$$

3.3. Validation of METS-VF, correlation and diagnostic performance against VAT-DXA

We conducted validation for METS-VF in a cohort of 184 patients who underwent DXA evaluation. In comparison to the discovery cohort, the validation cohort had higher rates of T2D and lower BMI. In the discovery cohort, METS-VF had a higher correlation with log-transformed VAT-DXA mass and was superior to other indexes with only EVA having a higher ρ value. These observations were replicated in the validation cohort, where the correlation for METS-VF with VAT-DXA was decidedly superior (Supplementary material). Furthermore, linear regression fits for METS-VF showed the largest decrease in AIC and highest r^2 compared to either METS-IR or WHtr alone or any other VAT surrogate. Finally, we evaluated DXA values >1389 g as analogous to >100 cm^2 in MRI-VAT due to lack of VAT-DXA percentile data in our population. In the discovery and validation cohorts we identified 159 (43.8%) and 54 (29.3%) subjects with increased VAT-DXA mass, respectively. In both cohorts, METS-VF had a higher AUROC compared to other indexes with significantly higher AUROC values in the validation cohort. We performed a semiparametric covariate-adjusted ROC curve analysis to evaluate the role of T2D in modifying performance of METS-VF; after adjustment, the AUROC for all indexes, including METS-VF decreased. Therefore, we estimated cut-offs separately for T2D and non-T2D subjects (Supplementary material).

Concordance of VAT estimated using METS-VF with actual VAT-DXA measures was evaluated using Bland–Altman analyses, demonstrating with <5% outliers. 95% confidence intervals for bias of agreement between VAT-DXA and estimated VAT using METS-VF was +75.38 g (95% CI 10.70–140.06) and stratified by sex comparatively higher in male (+197.72 g 95% CI 60.09–335.34) than female participants (+11.59 95% CI −54.53 to +77.72). Upper and lower 95% limits of agreement in the overall validation cohort were +932.41 g and −781.65 g, respectively (Supplementary material).

Table 2
β-coefficients for estimation of log-transformed visceral fat mass using the Levenberg–Marquardt algorithm for non-linear regression.

Model	Parameter	β	Error	95% CI
$R^2 = 0.596$	Intercept	4.466	0.463	3.697–5.520
Residual = 80.36	Ln (METS-IR)^3	0.011	0.003	0.006–0.016
	Ln (WHtr)^3	3.239	0.286	2.652–3.779
	Ln (Age)	0.594	0.103	0.342–0.749
	Male sex (0/1)	0.319	0.082	0.145–0.467

Abbreviations: WHtr: waist to height ratio; METS-IR: metabolic score for insulin resistance; 95% CI: 95% confidence intervals.

3.4. Validation of METS-VF against VAA-MRI

To evaluate METS-VF against the gold standard for VAT evaluation, we performed a second validation in a subgroup of 118 subjects from the validation cohort with VAA-MRI evaluation; we identified 35 subjects with VAA-MRI >100 cm^2. Subjects in this subgroup were younger and had higher rates of T2D compared to the overall validation cohort, BMI was lower than the discovery cohort but similar to the overall validation cohort; additionally, female predominance was lower compared to both training and validation cohorts (54.2%, Table 2). In this cohort, METS-VF had the highest correlation with VAA-MRI (ρ = 0.697 95% CI 0.597–0.779) compared to other VAT surrogates. We observed the higher AUROC for increased VAA-MRI for METS-VF (AUC = 0.842, 95% CI 0.771–0.913) compared to other VAT surrogates, particularly WHtr and WC. The estimated cut-off points stratified by T2D status were >7.18 for individuals without T2D (AUC = 0.922, 95% CI 0.924–0.993; 88.9% sensitivity, 88.6% specificity) and >7.0 for individuals with T2D (AUC = 0.778, 95% CI 0.654–0.890; 92.0% sensitivity, 63.8% specificity), which were similar to those observed for VAT-DXA.

3.5. Validation of METS-VF against VAT assessed by BIA

To extend our findings to a larger cohort, we performed a third validation in 991 subjects in whom we estimated EVAT using BIA. The rate of T2D in this cohort was significantly lower (13.8%) but subjects had similar age and sex distribution as the DXA validation cohort, except for BMI, which was leaning towards overweight and obese individuals. In this cohort, METS-VF had a high correlation with EVAT (ρ = 0.804 95% CI: 0.771–0.833) compared to VAI, LAP BMI, WtHI and METS-IR alone; EVA, DAAT and WC were slightly superior. Exploring the diagnostic capacity of METS-VF to detect increased EVAT >80th percentile, we found that METS-VF had an AUC of 0.895 (95% CI: 0.870–0.920); only WC and EVA had superior AUC for EVAT.

3.6. Performance of METS-VF in MHO subjects

We evaluated the performance of METS-VF in MHO subjects to investigate whether METS-VF could estimate VAT in subjects without significant metabolic disturbances. This cohort had female predominance (89.0%) with a mean age of 40.04 ± 14.29 years (Table 1). Using the VAT-DXA cut-off described earlier, we identified 29 subjects with increased VAT-DXA(31.9%). As seen in Supplementary Tables 2 and 3, METS-VF showed the higher correlation and AUROC compared to other VAT surrogates, outperforming VAI, LAP, WC and the WHtr.

3.7. Association of METS-VF with adipokine measurements

To explore physiological correlates of increased VAT, we explored associations of METS-VF with adipokine profiles. We observed a correlation between METS-VF values, adiponectin ($\rho = -0.197$ 95% CI −0.349 to −0.012) and FGF-21 ($\rho = 0.230$ 95% CI 0.058–0.399) but not with leptin ($\rho = -0.013$ 95% CI −0.197 to 0.165) which was correlated with BMI ($\rho = -0.223$ 95% CI 0.033–0.390). Across METS-VF terciles, we observed significantly lower adiponectin values in the upper METS-VF tercile compared to middle and lower terciles ($p < 0.01$); in contrast, we observed significantly higher FGF-21 values in the upper METS-VF tercile. These observations of increasing FGF-21 levels and decreasing adiponectin levels across METS-VF terciles were also confirmed by trend analyses ($p < 0.05$, Fig. 2).

3.8. Prediction of incident T2D and hypertension using METS-VF

For prediction of incident T2D and hypertension, we included 9637 subjects from baseline evaluation, from which 6144 completed follow-up. Of note, subjects who developed T2D and/or hypertension had significantly higher METS-VF scores at baseline compared to those who did not ($p < 0.001$). Using Cox proportional-hazard regression analyses, we observed that subjects in the highest METS-VF quintile (METS-VF > 7.2) had a 3.8-fold higher risk to develop T2D. Using the METS-VF > 7.18 cut-off value, subjects had 2.4-fold higher risk of incident T2D (Fig. 3). When stratifying by BMI categories, subjects with METS-VF values >7.18 values and normal weight (BMI 18.5–24.9) had higher additional risk for developing T2D, compared to overweight and obese subjects.

Finally, we assessed prediction of incident hypertension in this cohort. Using Cox proportional-hazard regression analyses, we observed that subjects in the highest METS-VF quintile had 3.7-fold higher risk of incident hypertension. Using the METS-VF > 7.18 cut-off value, subjects had two-fold higher risk of developing hypertension (Fig. 3). As seen with T2D when stratifying by BMI category, individuals with normal-range BMI had higher additional risk of incident hypertension compared to overweight and obese subjects (Supplementary material).

4. Discussion

In the present work, a novel surrogate index to estimate intra-abdominal adipose tissue and VAT using DXA was generated, which incorporates a non-insulin-based IR index (METS-IR), anthropometric measures of body-fat distribution (WtHr), sex, and age. METS-VF had better performance compared to other VAT surrogate indexes compared to VAA-MRI and EVAT-BIA and had the better adjustment to estimate VAT-DXA compared to either of its components alone. In addition, it was proven that VAT estimation using METS-VF correlates with adipokine profiles as those expected in subjects with increased VAT. Finally, the clinical utility of METS-VF to predict incident T2D and hypertension independent of BMI was explored in our Metabolic Syndrome Cohort. METS-VF is a novel estimate of VAT which could be useful for evaluation of cardio-metabolic health primarily in clinical and epidemiological research settings.

As reported, METS-VF had the better correlation and performance with VAT-DXA compared to other surrogate VAT indexes; these observations were replicated in three validation cohorts, which assessed VAT using three distinct methods: DXA, MRI and BIA. The increased performance of METS-VF may be partly due to the fact that most anthropometric indexes including BMI, WC, WHR and WHtr capture variability from both subcutaneous and visceral adipose tissue, thus tending to overestimate visceral adiposity; therefore, including known-modifiers of VAT accumulation could offer more precise estimations and reduce the impact of

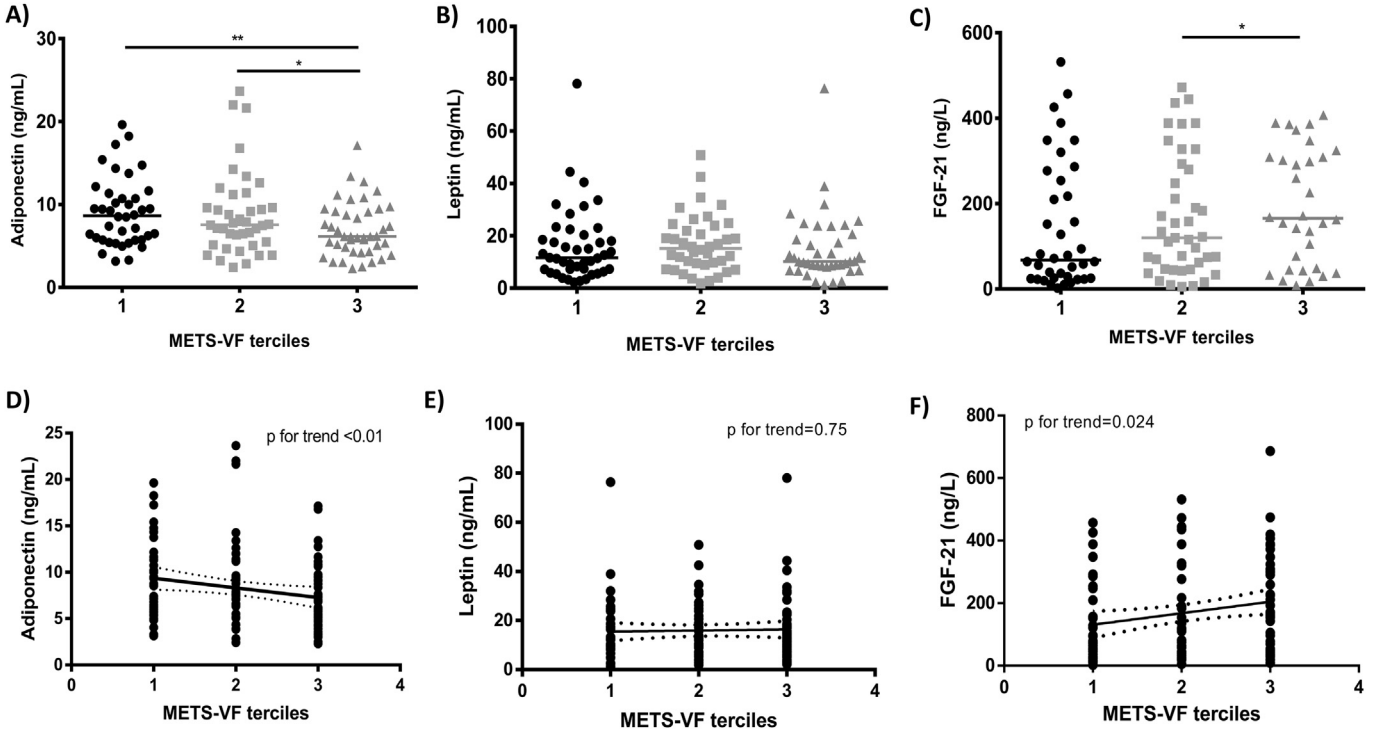

Fig. 2. Tercile comparison and trend analyses of increasing METS-VF terciles for fasting adiponectin (A, D), leptin (B, E) and FGF-21 (C, F) in the DXA validation sample (n = 184). Abbreviations: METS-VF: Metabolic Score for Visceral Fat; FGF-21: Fibroblast growth factor 21. *p-value < 0.05, **p-value < 0.01.

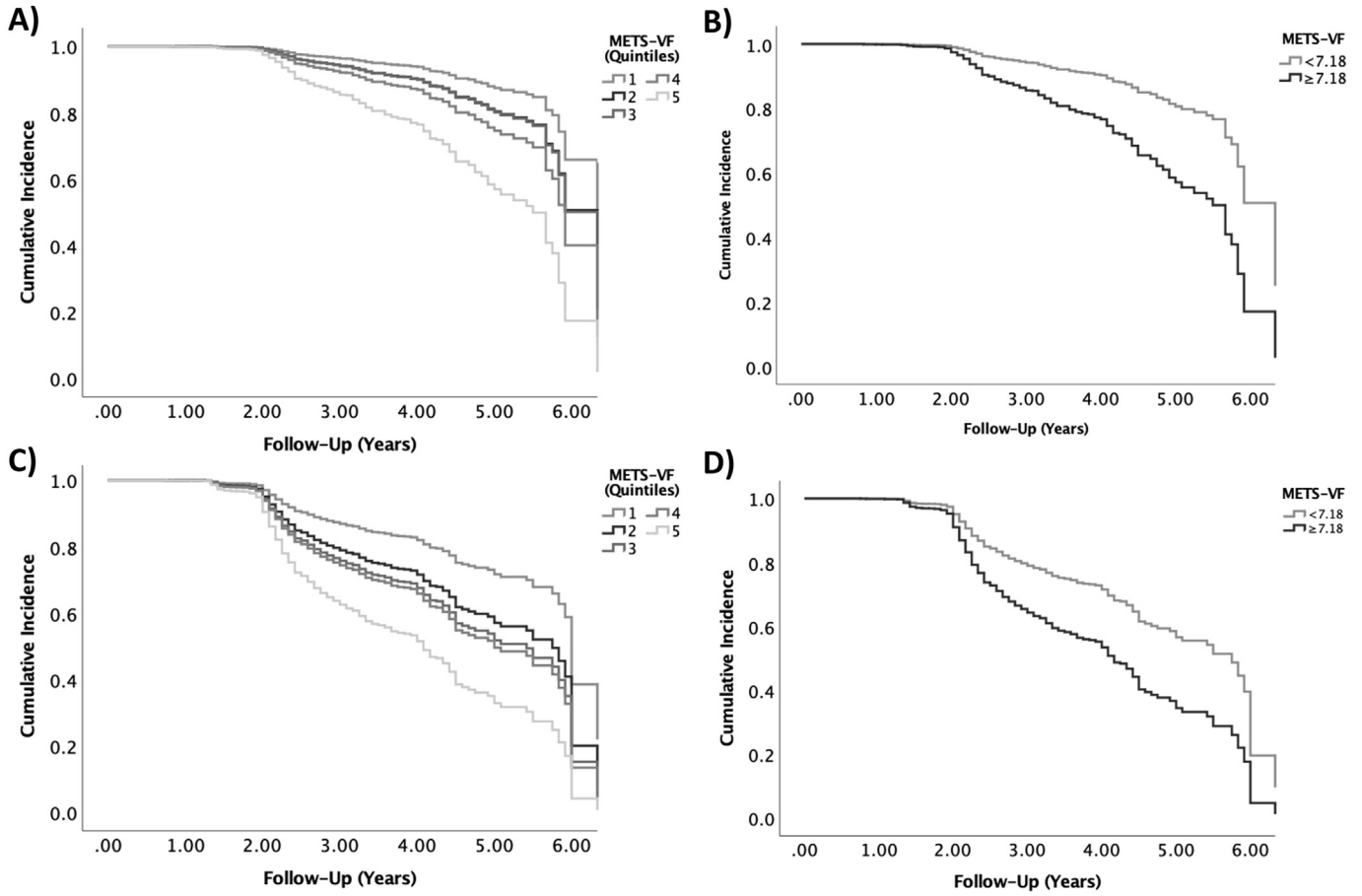

Fig. 3. Incidence of T2D comparing across METS-VF Quintiles (A) and cut-off point (>7.18, B) and incidence of hypertension across METS-VF terciles (C) and the identified cut-off value (D), adjusted for physical activity, family history of diabetes or hypertension and smoking in an open population cohort (n = 6144). Abbreviations: METS-VF: Metabolic Score for Visceral Fat; T2D: Type 2 diabetes.

subcutaneous adipose tissue on VAT estimation [18]. Both VAI and LAP were modeled considering laboratory measures and anthropometric variables; however, modeling methods for those surrogate scores differ and external validity of these indexes might be modified by ethnic-specific variations in body composition. This might also explain the underperformance of both indexes using CT-scan and MRI methods, as was previously reported in Japanese–American population [11]. In their recent study, Wander et al. developed the novel EVA index, which was aimed at improving estimations of visceral adiposity in Japanese–American subjects. In our study, we validated EVA against DXA and MRI; however, the METS-VF index showed superior performance in our population. This could be attributed to two significant differences between both indexes: First, the inclusion of an IR component, which could improve VAT estimation in subjects without significant laboratory disturbances attributable to VAT and the use of an anthropometric index such as WHtr which has demonstrated superior capacity to predict both VAT and cardio-metabolic risk in different ethnic groups [15,19].

To further strengthen the notion that METS-VF in fact estimated VAT, we aimed to demonstrate that adipokine profiles in subjects with increased VAT assessed by METS-VF were like those with increased VAT. Indeed, METS-VF showed a good correlation with lower adiponectin and higher FGF-21 levels, as expected in this group. Endocrine function of VAT mediates the relation between adipokines, IR and body composition [19,20]. Our model is based on the link between gender and age-specific variations in VAT along with IR and whole-body fat distribution. Several mechanisms have been proposed to explain the link between VAT and metabolic disturbances [21,22]. Anatomical disposition of VAT in interaction with IR increase liberation of free-fatty acids directly to the portal circulation, leading to dysregulations in glucose uptake, glycogen synthesis and glucose oxidation [23]. Low adiponectin levels and increased VAT lead to adverse metabolic profiles which could be compensated by increasing FGF-21 levels [24]. Therefore, METS-VF classifies subjects according to expected metabolic profiles of increased VAT which makes it a consistent and reliable estimate of VAT mass.

VAT has been traditionally associated with increased mortality owed to adverse cardio-metabolic outcomes [25,26]. In our study, individuals in the highest METS-VF quintiles had higher risk of incident T2D and hypertension after follow-up. This could be mediated by the consideration of increased age, IR status, pro-coagulative and pro-inflammatory states along with impairment in cytokine production in VAT [5]. Similar to T2D, IR has been proposed as one of the main pathophysiological mechanisms in which VAT may link the atherogenic state seen in subjects with cardiovascular events [27], mainly due to impairments in glucose homeostasis and increased atherogenic dyslipidemia, characterized by high triglycerides and apolipoprotein B and low HDL particles [1]. Subjects who developed T2D and hypertension had higher METS-VF values at baseline, suggesting a clinical application of the novel index as a useful and practical tool for primary care physicians to estimate VAT and its associated risk of cardio-metabolic

outcomes. VAT may be considered as a secondary target to treat patients with metabolic syndrome components to decrease overall cardio-metabolic risk caused by IR [28]. The fact that METS-VF was associated to these outcomes independent of BMI as shown in our study demonstrates the role of estimating VAT in subjects in whom BMI estimation could be insufficient to predict cardio-metabolic risk, especially in young subjects [29]. A precise VAT clinical estimator such as ours could be a useful tool for screening and targeting control in at-risk individuals.

Our study had some strengths and limitations. METS-VF was modeled using a non-linear regression approach considering DXA our gold standard, which can feasibly measure whole-body fat mass and intra-abdominal fat and has been recorded to have substantial accuracy compared to MRI and CT. Furthermore, METS-VF was also validated against MRI, the gold standard for VAT assessment and separate cut-off points were estimated for individuals with T2D to account for the effect of T2D in increasing visceral adiposity [30]. Evaluation of our index went beyond assessment of VAT, it also included prediction of incident cardio-metabolic comorbidities and evaluation of adipokine profiles related to excess VAT. In addition, we demonstrated that the index performs better compared to other measures in MHO subjects in whom increased VAT is not directly correlated with metabolic abnormalities. Nevertheless, some limitations are to be acknowledged. These include a relatively small sample size in the MRI validation cohort and a small number of lean and healthy individuals in both training and validation samples, which could overestimate risk in lower-risk subjects and lead to higher bias in VAT estimation. In addition, since no previous study assessed population percentiles or outcome-driven cut-offs for VAT-DXA mass in Latino populations, we had to identify a cut-off compared to VAA-MRI in our validation cohort which could not reflect the most effective cut-off due to sample size constraints. Furthermore, the differences in T2D prevalence in the training and validation cohorts could impact performance of this measure in T2D patients, which calls for further evaluations in subjects with T2D and validation in a larger sample of healthy individuals to ensure its validity in a clinical setting.

In conclusion, METS-VF is a novel precise estimate of VAT and intra-abdominal fat. METS-VF was validated against three-different methods to estimate visceral adiposity and was shown to replicate the adverse adipokine profile observed in individuals with excess visceral adiposity; furthermore, our index also was shown to predict incident T2D and hypertension independent of BMI. Therefore, METS-VF could be a useful clinical surrogate of visceral adiposity for its use in epidemiological settings and an overall measure of cardio-metabolic health.

CRediT authorship contribution statement

Omar Yaxmehen Bello-Chavolla: Conceptualization, Methodology, Validation, Formal analysis, Software, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Neftali Eduardo Antonio-Villa:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Arsenio Vargas-Vázquez:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Tannia Leticia Viveros-Ruiz:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Paloma Almeda-Valdes:** Investigation, Writing - review & editing, Visualization. **Donaji Gomez-Velasco:** Data curation, Writing - review & editing. **Roopa Mehta:** Investigation, Writing - review & editing. **Daniel Elias-López:** Investigation, Writing - review & editing. **Ivette Cruz-Bautista:** Investigation, Writing - review & editing. **Ernesto Roldán-Valadez:** Investigation, Writing - review & editing. **Alexandro J. Martagón:** Investigation, Writing - review & editing, Visualization, Project administration. **Carlos A. Aguilar-Salinas:** Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing, Visualization, Resources, Supervision, Project administration, Funding acquisition.

Conflicts of interest

Nothing to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2019.07.012.

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