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Relationship of visceral adipose tissue with surrogate insulin resistance and liver markers in individuals with metabolic syndrome chronic complications

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Abstract

Background: Visceral adipose tissue (VAT) has a hazardous influence on systemic inflammation, insulin resistance and an adverse metabolic profile, which increases the risk of developing non-alcoholic fatty liver disease (NAFLD) and chronic complications of diabetes. In our study we aimed to evaluate the association of VAT and the triglyceride glucose (TyG) as a proxy of insulin resistance surrogated with metabolic and liver risk factors among subjects diagnosed with metabolic syndrome (MetS).

Methods: A cross-sectional study was performed including 326 participants with MetS (55–75 years) from the PREDIMED-Plus study. Liver-status markers, VAT and TyG were assessed. Participants were stratified by tertiles according to VAT ($n = 254$) and TyG ($n = 326$). A receiver operating characteristic curve was used to analyse the efficiency of TyG for VAT.

Results: Subjects with greater visceral fat depots showed worse lipid profile, higher homeostatic model assessment for insulin resistance (HOMA-IR), TyG, alanine transaminase (ALT), fibroblast growth factor-21 (FGF-21), fatty liver index (FLI) and hepatic steatosis index (HSI) compared with participants in the first tertile. The multi-adjusted linear-regression analyses indicated that individuals in the third tertile of TyG (>9.1–10.7) had a positive association with HOMA-IR [β = 3.07 (95% confidence interval (CI) 2.28–3.86; p trend < 0.001)], ALT [β = 7.43 (95% CI 2.23–12.63; p trend = 0.005)], gamma glutamyl transferase (GGT) [β = 14.12 (95% CI 3.64–24.61; p trend = 0.008)], FGF-21 [β = 190.69 (95% CI 93.13–288.25; p trend < 0.001)], FLI [β = 18.65 (95% CI 14.97–22.23; p trend < 0.001)] and HSI [β = 3.46 (95% CI, 2.23–4.68; p trend < 0.001)] *versus* participants from the first tertile. Interestingly, the TyG showed the largest area under the receiver operating curve (AUC) for women (AUC = 0.713; 95% CI 0.62–0.79) compared with men (AUC = 0.570; 95% CI 0.48–0.66).

Conclusions: A disrupted VAT enlargement and impairment of TyG are strongly associated with liver status and cardiometabolic risk factors linked with NAFLD in individuals diagnosed with MetS. Moreover, the TyG could be used as a suitable and reliable marker estimator of VAT.

Keywords: insulin resistance, metabolic syndrome, non-alcoholic fatty liver disease, triglyceride glucose index, visceral adipose tissue

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Introduction

The metabolic syndrome (MetS) encompasses a cluster of cardiometabolic features like impaired glucose metabolism, dyslipidaemia, abdominal obesity, and elevated blood pressure.[1] The strong association between MetS and an increased risk of cardiovascular disease (CVD) as well as all-cause mortality is well documented.[2,3] On the other hand, non-alcoholic fatty liver disease (NAFLD) is recognized as the hepatic manifestation of MetS[4] that is related with insulin resistance and diabetes type 2 (T2DM).[5] NAFLD is a highly prevalent chronic liver illness, whose incidence linearly increases with body mass index (BMI) and adiposity.[6] This condition is quite common in obese individuals with central adiposity.[7,8] The distribution of adipose tissue is of great importance since abdominal obesity is a key factor in the development of the MetS[9] and NAFLD.[10] Insulin resistance is considered the primary triggering mechanism for the development of T2DM, NAFLD and MetS when fat accumulates in intra-abdominal depots[9,10] Thus, body-fat distribution in older adults is critical for determining how susceptible they are or will be to developing NAFLD and/or other CVD[11–14] being partly attributed to sex differences in fat content.[12,14] Central obesity is often quantified using waist circumference. But, it can be confounded by varying levels of subcutaneous fat in the waist, and may not accurately reflect visceral fat in all individuals.[15] The dual-energy X-ray absorptiometry (DXA) is a practical and valuable tool to assess visceral fat mass.[16] Nevertheless, the DXA equipment is expensive and might not be easy to access. In this sense, the identification of non-invasive markers able to discriminate subjects with higher visceral adiposity and higher susceptibility for developing NAFLD would be of great interest, as well as relating to T2DM complications. Indeed, liver biopsy is the gold standard for NAFLD diagnosis,[7] but it is an invasive technique not suitable for routine screening and monitoring.[7] Several non-invasive markers related to liver status and insulin resistance have been proposed in characterizing NAFLD.[7,17–19] A novel potential marker is triglyceride glucose (TyG), which has demonstrated a better predictive value compared with fasting plasma glucose (FPG) for the risk of T2DM in normoglycaemic individuals, as well as being associated with insulin resistance.[20] In the present study, the hypothesis was that subjects with a larger amount of VAT and increased TyG levels have higher susceptibility for showing adverse manifestations related to T2DM and development of NAFLD. Therefore, our primary objective was to assess the potential association of TyG with VAT, cardiometabolic risk factors, serum and NAFLD markers in overweight/obese individuals with MetS.

Materials and methods

Study population and design

This research is a cross-sectional study concerning baseline data from participants of the Navarra-Nutrition Centre within the PREDIMED-Plus trial (ISRCTN89898870; http://www.isrctn.com/ISRCTN89898870). PREDIMED-Plus is a multicentre, parallel-group, randomized trial carried out in Spain, aiming to evaluate the effectiveness of an energy-restricted traditional Mediterranean diet, physical activity promotion and behavioural support (intervention group) on the primary prevention of CVD, in comparison with general advised energy-unrestricted Mediterranean diet (control group). Detailed methods and protocols of the study have been published previously.[21,22] In brief, 6874 individuals were recruited in 23 Spanish centres. Eligible participants were men (55–75 years) and women (60–75 years) with a BMI $\geqslant$27 kg/m^2 and $<$40 kg/m^2 and fulfilling at least three criteria for the MetS: waist circumference (WC) in White people $\geqslant$102 cm for men and $\geqslant$88 cm for women, elevated triglycerides levels $\geqslant$150 mg/dl or drug treatment for hyperlipidemia; reduced high-density lipoprotein cholesterol (HDL-c) $<$40 mg/dl in men and $<$50 mg/dl in women or drug treatment; elevated blood pressure systolic $\geqslant$130 mmHg and/or diastolic $\geqslant$85 mmHg or current use of antihypertensive medication; elevated fasting glucose $\geqslant$100 mg/dl or drug treatment, according to guidelines from the International Diabetes Federation/National Heart, Lung and Blood Institute/American Heart Association (2009).[23] As described elsewhere, exclusion criteria included a background of alcohol overuse, liver injury, history of previous CVD, gastrointestinal or other disorders, infectious processes, therapy with immunosuppressive drugs, cytotoxic agents or systemic corticosteroids. The protocol and procedures were approved by the Research Ethic Committee for clinical investigations of the University of Navarra (053/2013) according to the Declaration of Helsinki. All participants provided written informed consent. At Navarra-Nutrition Centre, 331 were included in

the study, of which 326 participants had available data to calculate TyG, and 254 patients were assessed by DXA.

Study assessment

Clinical and biochemical measurements

At baseline, participants completed an administered survey, which included questions about socio-demographic characteristics, lifestyle behaviours, disease history and medication. Smoking habits were classified into 'never', 'former' or 'current smoker', as described elsewhere.[21] Blood pressure was measured in triplicate using a validated semiautomatic oscillometer (Omron HEM-705CP, Netherlands). T2DM was established as previous diagnosis of diabetes or glycated haemoglobin (HbA1c) $\geqslant$6.5%, use of antidiabetic medication or fasting glucose $\geqslant$126 mg/dl according to the American Diabetes Association guidelines.[24] After overnight fasting for at least 12 h, a blood sample was obtained from each participant. Serum and plasma were collected and frozen at −80°C. All biochemical measurements, including plasma glucose, HbA1c, insulin, total cholesterol, HDL-c, triglyceride, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) were performed using standard laboratory enzymatic methods and following validated protocols.[21] The fibroblast growth factor 21 (FGF-21) plasma concentrations were measured using human FGF-21 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA) with an autoanalyzer system (Triturus, Grifols SA, Barcelona, Spain) following the manufacturer's instructions. Low-density lipoprotein cholesterol (LDL-c) concentration was calculated by Friedewald's formula and the very-low-density lipoprotein cholesterol (VLDL-c) was calculated as triglycerides / 5.[25] Also, homeostatic model assessment for insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin (mIU/l) × fasting glucose (nmol/l)/22.5.[26]

Dietary variables

Trained dietitians face-to-face administered a semi-quantitative 143-item food-frequency questionnaire to estimate energy intake and alcohol consumption.[27] Also, a 17-item questionnaire was implemented, which is a modified version of the previously validated questionnaire used in the PREDIMED study to assess the participant's adherence to the Mediterranean diet.[28]

Physical activity measurement

Physical activity was assessed using the short Registre Gironi del Cor questionnaire that showed high reliability and sensitivity in detecting changes in moderate and vigorous intensity.[29,30] This tool was validated in the Spanish adult population, which is a version of the Minnesota Leisure Time.[29] This questionnaire evaluated the total energy expenditure in leisure-time physical activity using Metabolic Equivalent Tasks (METs) in minutes/week. Physical activities were classified into light-intensity (<4 METs), moderate intensity (4.0–5.5 METs), and vigorous intensity ($\geqslant$6 METs) as detailed in the report.[29] Sedentary lifestyles were evaluated using a validated Nurses' Health Study questionnaire.[31] For the present study, physical activity was expressed as MET hours/week.

Anthropometry and body composition measurements

Anthropometric measurements were performed by trained dietitians following standardized PREDIMED-Plus protocols.[21] Weight, height and waist circumference (WC), were measured using a calibrated scale, a stadiometer and an anthropometric tape, respectively. BMI was conventionally calculated as weight in kilograms divided by the height in square metres (kg/m^2). VAT was estimated using dual-energy X-ray absorptiometry (Lunar iDXA™, software version 6.0, Madison, WI, USA) connected with enCore™ software, which was assessed by trained operators according to standard procedures supplied by the manufacturer.

Non-invasive markers

TyG is a newly described marker reported as a useful screening tool for surrogated insulin resistance,[20,32,33] NAFLD,[34] and as an early predictor of MetS features.[35] This marker was calculated using biochemical data according to the following formula (Equation 1):[32,36]

$$\mathrm{TyG} = \mathrm{Ln}[\mathrm{triglyceride\,(mg/dl)}^{*}\ \mathrm{glucose\,(mg/dl)}/2] \qquad \text{Equation 1.}$$

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The hepatic steatosis index (HSI; Equation 2) was validated in a cohort of patients with NAFLD diagnosed by ultrasonography.[37]

$$\text{HSI} = 8 \times \text{ALT / AST ratio} + \text{BMI}(+2,\ \text{if diabetes};\ +2,\ \text{if female})$$ [37,38]

Equation 2.

HSI was also computed to estimate liver status. Another liver marker as an indicator of NAFLD is the fatty liver index (FLI), which was calculated as previously described (Equation 3)[39] by:

$$\frac{e^{0.953\times\log(\text{triglycerides})+0.139\times\text{BMI}+0.718\times\log(\text{GGT})+0.053\times\text{waist circumference}-15.745}}{1+e^{0.953\times\log(\text{triglycerides})+0.139\times\text{BMI}+0.718\times\log(\text{GGT})+0.053\times\text{waist circumference}-15.745}}\times 100$$

Equation 3.

Statistical analyses

We retrospectively estimated the sample size to find differences between groups with a precision of 0.40 and a standard deviation (SD) of 0.5, and $\alpha = 0.05$. The statistical power of the study was 90%. Continuous variables are presented as means ± SD and categorical variables as numbers (n) and percentages (%). One-way analysis of variance and Chi-square tests or Fisher's exact test for categorical variables were used to assess differences between groups, as appropriate. The analysis of covariance test after adjustment was used for the following potential confounders: age (years), physical activity (MET hours/week), energy intake (kcal/d), alcohol intake (g) and smoking status (never, former, current). Bonferroni correction was applied to assess differences in metabolic and liver parameters according to sex-specific VAT tertiles. VAT for men: T1 (1.29 to ⩽2.42), T2 (>2.42 to ⩽3.10), T3 (>3.10 to 5.45); VAT for women: T1 (0.77 to ⩽1.60), T2 (>1.60 to ⩽2.06), T3 (>2.06 to 3.59). Crude and multiple linear regression models adjusted by age (years), sex (male and female), physical activity (MET hours/week), energy intake (kcal/d), alcohol intake (g) and smoking status (never, former, current) were fitted to statistically analyse the association between NAFLD biomarkers and tertiles of TyG. Tests of linear trend were assessed assigning the median value of each tertile of TyG and then using it as a continuous variable and correlation was assessed using the Pearson's coefficient. The area under the receiver operating characteristics (ROC) curve (AUC) was performed to quantify the value of TyG as a predictor of VAT, considering as reference values the 50th percentile of VAT by sex. All tests were two sided, and cut-off level of significance was defined as 0.05. Statistical analyses were carried out with Stata 12.0 software (StataCorp LP, College Station, TX, USA).

Results

Study sample characteristics

Baseline characteristics of men and women according to VAT sex-specific tertiles are summarized in Table 1. As expected, BMI and WC increased across VAT tertiles. No significant differences were found in the frequency of diabetes, hypertension and smoking habits among tertiles in both sexes. Likewise, blood pressure [systolic blood pressure (SBP) and diastolic blood pressure (DBP)] measurements, energy intake, alcohol consumption, adherence to the Mediterranean diet score and physical activity did not differ statistically.

Crosstalk between VAT, TyG and NAFLD risk factors

Anthropometric, metabolic profile and liver status of participants are reported in Table 2. The adjusted analysis revealed that BMI and WC were significantly increased through VAT tertiles specific by sex. Moreover, insulin, TyG and HOMA-IR increased with VAT tertiles reaching statistical differences among them (all $p < 0.05$). Glucose and HbA1c did not show differences between tertiles. As concerns lipid markers, the T3 group presented significantly higher levels of VLDL-c [mean 32.4 mg/dl (95% CI 29.7–35.2)], triglycerides [mean 162.1 mg/dl (95% CI 148.3–175.9)] and triglyceride (TG)/HDL-c ratio [mean 3.8 mg/dl (95% CI 3.4–4.3)] than T1 participants, while no associations were found regarding total cholesterol, LDL-c and HDL-c serum levels. Participants in the highest VAT tertile showed significantly higher ALT levels, HSI and FLI scores as compared with subjects in the lowest tertile of VAT. No significant differences were found in AST and FGF-21 levels in VAT tertiles.

The association of TyG with variables related to liver health was explored (Table 3). Linear regression models were fitted considering NAFLD-related markers as dependent factors and TyG as

Table 1. Clinical and lifestyle characteristics of subjects with MetS according to tertiles of VAT by sex.

	Tertiles of visceral adipose tissue (kg)							
	Men ($n=133$)				Women ($n=121$)			
	T1 ($n=45$) (1.29 to ⩽2.42)	T2 ($n=44$) (>2.42 to ⩽3.10)	T3 ($n=44$) (>3.10 to 5.45)	*p* value*	T1 ($n=41$) (0.77 to ⩽1.60)	T2 ($n=40$) (>1.60 to ⩽2.06)	T3 ($n=40$) (>2.06 to 3.59)	*p* value*
Age (years)	63.9 ± 5.8	64.5 ± 5.8	64.6 ± 5.1	0.818	67.7 ± 3.5	67.0 ± 4.2	67.5 ± 4.4	0.751
BMI (kg/m^2)	30.0 ± 2.2	31.5 ± 2.2	34.0 ± 2.9	<0.001	30.6 ± 2.9	32.4 ± 3.6	34.4 ± 3.2	<0.001
WC (cm)	103.5 ± 5.8	109.5 ± 6.2	117.3 ± 7.6	<0.001	97.1 ± 6.5	102.8 ± 6.9	109.5 ± 7.2	<0.001
VAT (kg)	2.0 ± 0.3	2.8 ± 0.2	3.8 ± 0.5	<0.001	1.3 ± 0.2	1.8 ± 0.1	2.5 ± 0.4	<0.001
Diabetes, *n* (%)	19 (42.2)	16 (36.4)	17 (38.6)	0.849	10 (24.4)	16 (40.0)	19 (47.5)	0.089
Hypertension, n (%)	40 (88.9)	42 (95.5)	42 (95.5)	0.362	39 (95.1)	38 (95.0)	37 (92.5)	0.851
SBP (mmHg)	147.6 ± 18.5	142.3 ± 13.8	143.4 ± 14.7	0.250	145.8 ± 16.9	143.5 ± 15.8	142.0 ± 16.2	0.578
DBP (mmHg)	88.5 ± 9.9	88.6 ± 8.5	88.0 ± 6.7	0.941	85.1 ± 8.9	87.6 ± 9.9	86.7 ± 8.6	0.455
Smoking habits, *n* (%)				0.092				0.677
Never smoker	12 (26.7)	7 (15.9)	4 (9.1)		30 (73.2)	23 (57.5)	25 (62.5)	
Former smoker	23 (51.1)	26 (59.1)	34 (77.3)		8 (19.5)	13 (32.5)	11 (27.5)	
Current smoker	10 (22.2)	11 (25.0)	6 (13.6)		3 (7.3)	4 (10.0)	4 (10.0)	
Alcohol intake (g/d)	15.2 ± 14.3	18.5 ± 20.3	23.3 ± 20.6	0.119	2.4 ± 6.2	5.3 ± 8.3	2.6 ± 4.9	0.102
Energy intake (kcal/d)	2655.4 ± 580.2	2670.0 ± 439.7	2789.0 ± 550.2	0.428	2451.5 ± 549.5	2407.0 ± 511.9	2474.2 ± 413.4	0.827
Adherence to MedDiet (0–17 points)	9 ± 2.7	8.5 ± 2.2	8.8 ± 2.5	0.636	9.2 ± 2.5	9.0 ± 2.8	9.4 ± 2.5	0.767
Physical activity (MET hours/week)	59.0 ± 57.6	61.2 ± 49.6	46.9 ± 41.1	0.356	43.2 ± 33.6	39.4 ± 32.9	36.0 ± 26.5	0.588

*p value for differences between tertiles of visceral fat mass by sex was calculated by Chi-square, Fisher's exact test or ANOVA, as appropriate.
$p < 0.05$ is considered statistically significant. Data are expressed as mean ± SD.
ANOVA, analysis of variance; BMI, body mass index; DBP, diastolic blood pressure; MedDiet, Mediterranean diet; MET, metabolic equivalent; MetS, metabolic syndrome; SBP, systolic blood pressure; SD, standard deviation; WC, waist circumference; VAT, visceral adipose tissue.

Table 2. Anthropometric, body composition, metabolic profile and liver status in subjects with MetS according to VAT sex-specific tertiles.

	Tertiles of visceral adipose tissue (kg)			*p* value
	T1	T2	T3	
	(*n* = 86)	(*n* = 84)	(*n* = 84)	
Men	(1.29 to ⩽2.42)	(>2.42 to ⩽3.10)	(>3.10 to 5.45)	
Women	(0.77 to ⩽1.60)	(>1.60 to ⩽2.06)	(>2.06 to 3.59)	
Total	(0.77 to 2.42)	(>1.60 to 3.10)	(>2.06 to 5.45)	
Anthropometric and body composition				
BMI (kg/m^2)	30.3 (29.7–30.9)[a,b,c]	32.0 (31.4–32.6)[b,c]	34.1 (33.5–34.8)	<0.001
WC (cm)	101.0 (99.5–102.6)[a,b,c]	106.3 (104.8–107.8)[b,c]	113.1 (111.5–114.6)	<0.001
VAT (kg)	1.7 (1.6–1.8)[a,b,c]	2.3 (2.2–2.4)[b,c]	3.1 (3.0–3.2)	<0.001
Glucose profile				
Glucose (mg/dl)	115.1 (108.0–122.2)	118.2 (111.2–125.2)	123.6 (1116.6–130.6)	0.247
HbA1c (%)	6.0 (5.8–6.2)	6.2 (6.0–6.4)	6.2 (6.0–6.5)	0.281
TyG	8.8 (8.7–8.9)[a,b,c]	9.0 (8.9–9.1)	9.1 (9.0–9.2)	0.001
Insulin (mU/l)	10.2 (8.5–11.9)[a,b,c]	13.8 (12.1–15.4)	16.5 (14.9–18.2)	<0.001
HOMA-IR	2.9 (2.3–3.4)[a,b,c]	4.1 (3.5–4.6)	5.0 (4.5–5.5)	<0.001
Lipid profile				
Total cholesterol (mg/dl)	198.0 (190.2–205.8)	201.2 (193.4–209.0)	205.6 (197.7–213.5)	0.411
LDL-c (mg/dl)	125.5 (118.5–132.5)	125.8 (118.7–132.9)	129.1 (121.8–136.4)	0.747
HDL-c (mg/dl)	47.8 (45.6–49.9)	45.5 (43.3–47.6)	45.9 (43.7–48.1)	0.315
VLDL-c (mg/dl)	25.0 (22.2–27.7)[a,b,c]	30.2 (27.4–32.9)	32.4 (29.7–35.2)	<0.001
Triglycerides (mg/dl)	124.8 (111.0–138.5)[a,b,c]	150.9 (137.2–164.5)	162.1 (148.3–175.9)	<0.001
TG/HDL-c ratio	2.9 (2.4–3.3)[a,c]	3.6 (3.1–4.0)	3.8 (3.4–4.3)	0.008
Liver status				
ALT (U/l)	23.3 (19.1–27.4)[a,c]	29.2 (25.1–33.3)	32.0 (27.9–36.1)	0.013
AST (U/l)	21.8 (19.0–24.6)	24.2 (21.4–26.9)	25.0 (22.2–27.7)	0.268
GGT (U/l)	37.2 (28.7–45.6)	46.8 (38.5–55.2)	40.7 (32.2–49.2)	0.272
FGF-21 (pg/ml)*	378.5 (294.5–462.5)	484.7 (403.8–565.6)	430.5 (346.7–514.4)	0.207
FLI (arbitrary units)	66.6 (63.9–69.3)[a,b,c]	79.8 (77.1–82.5)[b,c]	86.9 (84.2–89.6)	<0.001
HSI (arbitrary units)	40.2 (39.4–41.1)[a,b,c]	43.2 (42.2–44.1)[b,c]	45.9 (45.1–46.8)	<0.001

*FGF-21 available in 211 patients.
$p < 0.05$ is considered statistically significant. Data are expressed as mean (95% CI). Variables were adjusted by age (years), physical activity (MET hours/week), energy intake (kcal/d), alcohol intake (g) and smoking status (never, former, current).
Data is stratified by VAT sex- specific tertiles.
[a,b]Significant differences between T1 vs T2.
[a,c]Significant differences between T1 vs T3.
[b,c]Significant differences between T2 vs T3.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FGF-21, fibroblast growth factor- 21; FLI, fatty liver index; GGT, gamma-glutamyl transferase; HbA1c, glycated haemoglobin A1c; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; HSI, hepatic steatosis index; LDL-c, low-density lipoprotein cholesterol; MET, Metabolic Equivalent Task; MetS, metabolic syndrome; TG/HDL ratio, triglycerides/high-density lipoprotein ratio; TyG, triglyceride glucose; VAT, visceral adipose tissue; VLDL-c, very-low-density lipoprotein cholesterol; WC, waist circumference.

Table 3. Multivariable linear regression analyses evaluating the association between TyG tertiles as independent variable and liver status as dependent variable.

	Tertiles of TyG			*p* for trend
	T1	T2	T3	
	(*n* = 109)	(*n* = 110)	(*n* = 107)	
	(7.3–8.7)	(>8.7–9.1)	(>9.1–10.7)	
	β estimates (95% CI)	β estimates (95% CI)	β estimates (95% CI)	
WC (cm)				
Crude	(0 Ref.)	1.32 (–1.06 to 3.71)	3.21 (0.81–5.61)	0.009
Multivariable adjusted	(0 Ref.)	1.81 (–0.37 to 3.99)	2.62 (0.41–4.84)	0.020
HOMA-IR				
Crude	(0 Ref.)	1.21 (0.43–1.99)	3.09 (2.31–3.88)	<0.001
Multivariable adjusted	(0 Ref.)	1.25 (0.48–2.01)	3.07 (2.28–3.86)	<0.001
ALT (U/l)				
Crude	(0 Ref.)	3.79 (–1.48 to 9.07)	8.14 (2.83–13.45)	0.003
Multivariable adjusted	(0 Ref.)	4.79 (–0.32 to 9.90)	7.43 (2.23–12.63)	0.005
AST (U/l)				
Crude	(0 Ref.)	1.84 (–1.51 to 5.18)	2.56 (–0.80 to 5.93)	0.137
Multivariable adjusted	(0 Ref.)	2.29 (–1.00 to 5.57)	1.98 (–1.36 to 5.33)	0.246
GGT (U/l)				
Crude	(0 Ref.)	3.06 (–7.39 to 13.51)	16.72 (6.17–27.26)	0.002
Multivariable adjusted	(0 Ref.)	4.07 (–6.21 to 14.34)	14.12 (3.64–24.61)	0.008
FGF-21 (pg/ml)*				
Crude	(0 Ref.)	92.45 (–2.17 to 187.07)	195.11 (100.49–289.73)	<0.001
Multivariable adjusted	(0 Ref.)	91.26 (–5.09 to 187.62)	190.69 (93.13–288.25)	<0.001
FLI (arbitrary units)				
Crude	(0 Ref.)	11.18 (7.46–14.90)	19.60 (15.84–23.36)	<0.001
Multivariable adjusted	(0 Ref.)	11.78 (8.18–15.39)	18.65 (14.97–22.33)	<0.001
HSI (arbitrary units)**				
Crude	(0 Ref.)	1.76 (0.52–3.00)	3.35 (2.11–4.60)	<0.001
Multivariable adjusted	(0 Ref.)	1.95 (0.75–3.16)	3.46 (2.23–4.68)	<0.001

*FGF-21 available in 278 patients.
**Adjusted for all variables except for sex.
$p < 0.05$ was considered statistically significant. Data are expressed as mean (95% CI). Models were adjusted by age (years), sex (male and female), physical activity (MET hours/week), energy intake (kcal/d), alcohol intake (g) and smoking status (never, former, current).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; FGF-21, fibroblast growth factor-21; FLI, fatty liver index; GGT, gamma-glutamyl transferase; HOMA-IR, homeostatic model assessment for insulin resistance; HSI, hepatic steatosis index; MET, Metabolic Equivalent Task; Ref., reference; TyG, triglyceride glucose; WC, waist circumference.

the independent variable (Table 3). A fully adjusted model revealed that individuals in the third TyG tertile (>9.1−10.7) were significantly associated with higher WC (β = 2.62; 95% CI 0.41–4.84, p for trend = 0.020), HOMA-IR (β = 3.07; 95% CI 2.28–3.86, p for trend < 0.001), ALT (β = 7.43; 95% CI 2.23–12.63, p for trend = 0.005), GGT (β = 14.12; 95% CI 3.64–24.61, p for trend = 0.008), FGF-21 levels (β = 190.69; 95% CI 93.13–288.25, p for trend < 0.001), FLI units (β = 18.65; 95% CI 14.97–22.33, p for trend < 0.001), HSI units (β = 3.46; 95% CI 2.23–4.68, p for trend < 0.001) than participants in the first TyG tertile. Furthermore, variables associated with glucose–insulin homeostasis were significantly correlated with VAT except for men in glucose levels (Figure 1). Glucose (men: $r = 0.093$, $p = 0.292$; women: $r = 0.264$, $p = 0.004$) [Figure 1(a)], triglycerides (men: $r = 0.291$, $p < 0.001$; women: $r = 0.220$, $p = 0.016$) [Figure 1(b)], HOMA-IR (men: $r = 0.345$, $p < 0.001$; women: $r = 0.500$, $p < 0.001$) [Figure 1(c)], and TyG (men: $r = 0.266$, $p = 0.002$; women: $r = 0.322$, $p < 0.001$) [Figure 1(d)].

Receiver operating characteristic (ROC) analyses for TyG to predict VAT

ROC curves were applied to assess the capacity of TyG to identify elevated VAT accumulation in both sexes (Figure 2). The AUCs of the TyG for prediction of VAT was 0.570 (95% CI 0.48–0.66) for men and 0.713 (95% CI 0.62–0.79) for women.

Discussion

In this translational study, VAT and TyG were associated with relevant liver and cardiometabolic risk factors linked to NAFLD and insulin resistance in subjects with MetS. Moreover, TyG could be a reliable indicator of visceral fat mass. Many metabolic abnormalities related to insulin resistance often occur in obese individuals with higher amount of VAT.[9,40] The link between altered VAT triggering with a disorder in glucose and insulin metabolism may appear to be a driving factor in T2DM and NAFLD.[4,5]

Interestingly, TyG and atherogenic lipid profiles (VLDL-c, triglycerides and TG/HDL-c ratio) were significantly increased across tertiles of sex-specific VAT independently of confounding factors. In line with our results, Lee and colleagues observed VAT and triglycerides being independent risk factors for hepatic steatosis.[41] VAT is the main source of free fatty acids (FFAs) and other biological compounds, which enter the portal circulation and contribute to hepatic fat accumulation,[40] insulin resistance[4,5] and glucose intolerance, promoting a decreased hepatic insulin sensitivity, increasing the risk of developing T2DM and NAFLD.[4] Moreover, a statically significant increase of non-invasive hepatic markers (ALT, FLI and HSI) in participants with higher fat-storage capacity in VAT was found. Previously, studies demonstrated that increased VAT was associated with higher ALT levels[42] or significant fibrosis in subjects diagnosed with NAFLD.[43] Based on these data, central adiposity plays a key role in NAFLD pathogenesis[10,14,44] promoting liver damage,[43] insulin resistance and disrupted lipid metabolism.[45]

Currently, NAFLD has become a public health problem with a negative impact over the individual's health, socioeconomic and healthcare system.[6,46] In this context, early screening is crucial in the NAFLD pathogenesis,[7] as it is an overlooked T2DM complication.[5] Liver biopsy is the gold standard for NAFLD diagnosis.[7] However, it has several limitations, such as sampling error, cost, medical complications and technical difficulties.[47] In this regard, several methodologies have been used in the detection and featuring of NAFLD shown to be relatively effective, inexpensive and useful in a primary healthcare setting.[19,47–49] TyG is a novel marker exhibiting accuracy for recognizing insulin resistance and diabetes-related manifestations.[20,50] Furthermore, this marker was found highly sensitive for detecting NAFLD.[34] Simental-Mendía *et al.* suggested that the best TyG level for diagnosis of insulin resistance was Ln 4.65, which showed the highest sensitivity (84.0%) and specificity (45.0%) values.[36] Interestingly, the multivariable regression analysis demonstrated that individuals with a higher TyG (>9.1) value were associated with higher levels of HOMA-IR ALT, GGT, FGF-21, FLI and HSI units compared with lower TyG values (≤8.7), after adjusting for potential confounders, which confirms the relationship with inflammation and T2DM. Evidence supports T2DM is an important risk factor for NAFLD,[5,6] which is characterized by a resistance of insulin action in targets tissues and a disruption of the beta cells in the pancreatic islets to secrete enough insulin to

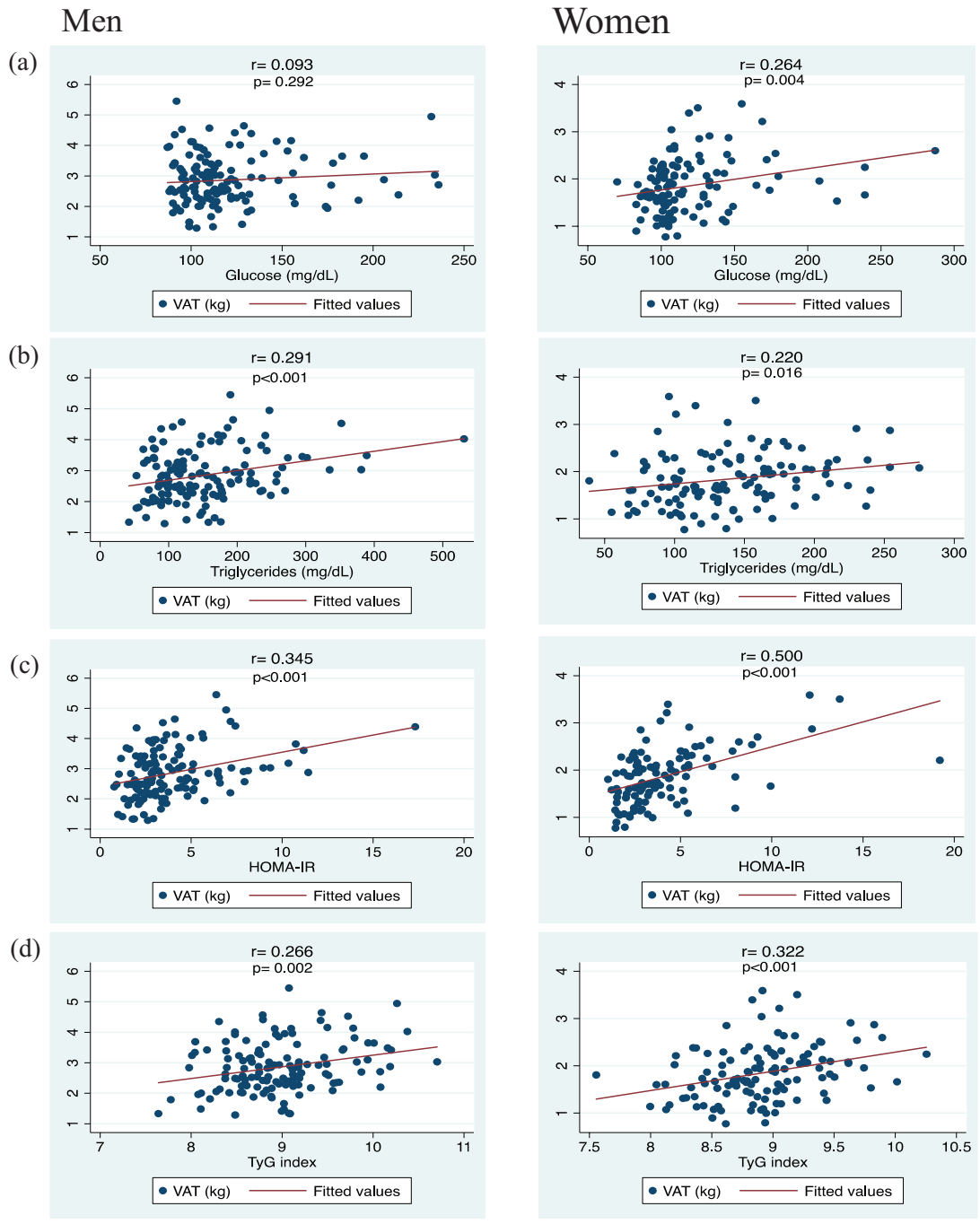

Figure 1. Correlations between VAT and parameters related to glucose and insulin homeostasis in subjects with MetS according to sex.
MetS, metabolic syndrome; VAT, visceral adipose tissue.

overcome this resistance.[24] The prevalence of NAFLD and non-alcoholic steatohepatitis (NASH) in individuals diagnosed with T2DM equates to over 60% increased risk of NAFLD pathogenesis and mortality.[6] These results suggested that insulin resistance is an important contributor to the development of NAFLD.[4] This finding is similar to results from Bonnet *et al.*, who reported that increased levels of ALT and GGT are strongly associated with hepatic insulin resistance and decreased hepatic insulin clearance.[51] Another liver marker is the FGF-21, primarily produced in

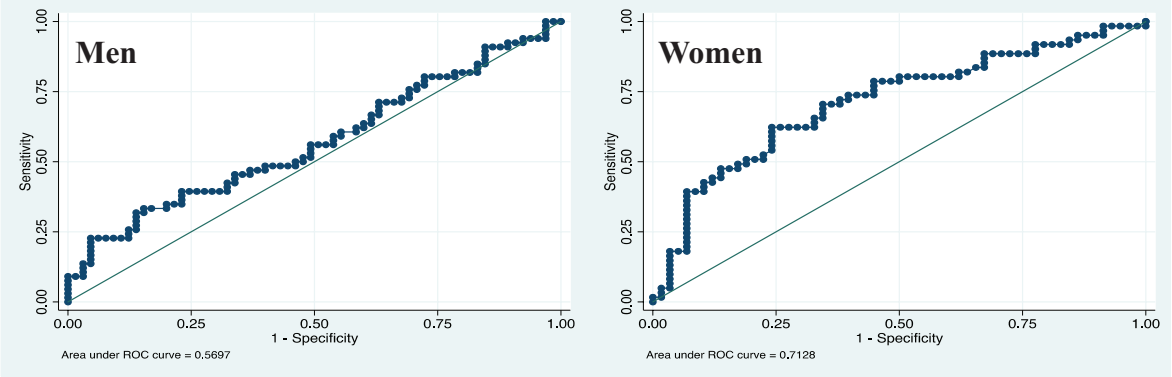

Figure 2. Receiver operating characteristics (ROC) curve analysis of predictive value of the TyG in subjects with MetS according to sex.
VAT cut-off men: ⩾2.777 kg; VAT cut-off women: ⩾1.748 kg.
MetS, metabolic syndrome; TyG, triglyceride glucose; VAT, visceral adipose tissue.

hepatocytes and implicated in the regulation of glucose–lipid metabolism, insulin sensitivity, inflammation and energy homeostasis.[52] Several clinical studies and reviews have documented that disrupted adipose tissue and excessive intrahepatic fat accumulation may trigger FGF-21 resistance.[52,53] Thus, Shen and colleagues[54] found that NAFLD patients showed significantly higher serum FGF-21 levels compared with subjects without NAFLD.[54] Furthermore, the present study showed that subjects with higher values of TyG had 3.46 more units of HSI compared with reference (lower values). Taken together, these results can be explained by insulin resistance being a major feature of NAFLD that works by increasing *de novo* lipogenesis and FFA flux to the liver through decreased inhibition of lipolysis,[4] promoting inflammation, oxidative stress[55] and hepatocyte injury.[56] Thus, individuals with pre-diabetes and T2DM represent an at-high-risk population where early diagnosis of NAFLD is crucial.[5]

DXA has been considered the gold standard for body composition measurements.[16] Nevertheless, this imaging technique for assessing adipose tissue distribution is expensive and not feasible for routine community screening. In our results, we observed a close relationship between insulin resistance and dysfunctional VAT. Interestingly, men had higher amounts of VAT than women. Meanwhile, women and men with ⩾VAT median had similar TyG values (data not shown). Moreover, the ROC curves indicate a moderate predictive ability of TyG to discriminate VAT in women (AUC = 0.713), but it was weak for men (AUC = 0.570).

The connection between body fat distribution and adipose-tissue biology with insulin resistance varies by sex, age and other factors.[11] In general, women have more total body fat mass and men present with higher abdominal/visceral fat mass.[11] However, decreased levels of oestrogen and adipose tissue redistribution by increased depots of VAT are characterized in postmenopausal women.[11,12] A disbalance of hormonal levels promotes insulin resistance and an atherogenic lipid profile, which increases the risk of CVD in older women.[11] Interestingly, some studies have suggested that obese women are more insulin sensitive than men despite a higher amount of VAT;[12] however, the mechanism is still unclear. Recently, the Netherlands Epidemiology of Obesity Study showed that in obese women, VAT was differently associated with cardiometabolic risk factors as compared with obese men.[57] However, this outcome was in contrast with Ferrara *et al.*, who reported that older obese men are more insulin resistant compared with older women, even adjusted for differences in abdominal fat distribution measured by DXA.[13] One possible explanation for our results could be that women exhibit a greater amount of FFA delivery derived from VAT lipolysis.[58] Moreover, Serra *et al.* showed that postmenopausal women (overweight or obese) diagnosed with MetS had lower adipose-tissue lipoprotein-lipase activity and limited capacity for lipid accumulation in subcutaneous abdominal adipose tissue, leading to higher levels of lipids, accumulation of VAT and insulin resistance.[59]

Our results reinforce that a VAT dysfunction and higher TyG values increase risk of developing

NAFLD and suggest a role for glucose intolerance. Moreover, the ROC analysis reflected that TyG could be a suitable predictor of VAT. Chronic diseases are the leading causes of death and disability worldwide. MetS comprises several clinical and metabolic risk factors that increase the risk of developing T2DM and other comorbidities.[1] Individuals with T2DM and NAFLD exhibit more severe insulin resistance and liver damage. Also, in T2DM the presence of fatty liver is associated with poor glycaemic control, resulting in the need for higher insulin doses.[5] Meanwhile, ageing and biological differences between men and women play an important role in body fat distribution and health status. Our findings suggest a strong association between excessive accumulation of VAT, insulin resistance, cardiometabolic risk factors and poor liver status in subjects with MetS. Moreover, the TyG, a novel marker of insulin resistance could be used as an easy and reliable marker for dysfunctional VAT, which could constitute a new proxy for healthcare professionals in the screening of individuals diagnosed with MetS. In this context, the improvement of knowledge of these inter-relationships in subjects with MetS should be useful in easily identifying individuals with a high risk of NAFLD, which may allow early intervention and prevention of NAFLD complications.

The strengths of this study are that VAT was objectively measured with a validated imaging technique. Also, the novelty of this study comes from the use of TyG as a suitable marker of VAT in subjects at high cardiovascular risk diagnosed with MetS. However, some limitations require consideration. First, there is the relatively small sample size. Despite this, the achieved statistical power for VAT and TyG variables was higher than 90%. Second, the cross-sectional design cannot imply a causal relationship. Third, there is lack of NAFLD diagnosis by liver biopsy or imaging techniques, but important to note that liver biopsy is not available or feasible in large epidemiological studies. On the other hand, we used validated non-invasive markers to estimate hepatic fat accumulation.[38]

Conclusion

VAT and the TyG were associated with liver and cardiometabolic risk factors linked to NAFLD in individuals with overweight/obesity and MetS. Moreover, we demonstrated that in addition to anthropometric measurements or the DXA approach, TyG could be a useful simple marker to identify dysfunctional VAT phenotype in patients with diabetic profiles and MetS manifestations.

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Conflict of interest statement

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