High Body Adiposity Drives Glucose Intolerance and Increases Cardiovascular Risk in Normoglycemic Subjects

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Objective: The objective of this study was to assess the utility of the 2-hour oral glucose tolerance test (OGTT) value to discriminate between different cardiometabolic profiles and examine the role of body composition in predicting the associated increased risk for glucose impairment, beta-cell dysfunction, and cardiovascular disease (CVD).

Methods: Subjects with normal fasting glucose completed a 2-hour OGTT and were categorized to the carbohydrate metabolism alterations (CMAs) or the control group based on a 2-hour glucose threshold of 7.8 mmol/L. Body composition, visceral adipose tissue, OGTT-based parameters, and cardiovascular risk factors (CVRFs) such as hypertension, dyslipidemia, obstructive sleep apnea, nonalcoholic fatty liver disease, and smoking status were measured.

Results: Subjects with CMAs exhibited a significantly higher 1-hour postload glucose level and a greater decline in beta-cell function and CVRF profiles. After multivariate adjustment, an excess of total body and visceral fat was associated with an increased risk of CMAs, beta-cell dysfunction, CVRFs, and lower whole-body insulin sensitivity.

Conclusions: These data support the etiopathogenic role of body and visceral fat in the development of glucose derangements and CVRFs early on in the metabolic dysregulation process. Thus, body composition analysis and OGTT assessment performed in individuals with normal fasting glucose enable a better identification of patients at risk of developing type 2 diabetes and CVD.

Introduction

Carbohydrate metabolism alterations (CMAs) have a close relationship with obesity, a fact that should be emphasized, as the prevalence of obesity is increasing dramatically worldwide (1). Half of individuals with type 2 diabetes mellitus (T2DM) have obesity and 90% are overweight (2). The current understanding of T2DM is based on a concept of a progressive failure of pancreatic beta-cell function with concomitant increased insulin resistance. Obesity is a pro-inflammatory state and plays a key role in T2DM progression, mainly by increased insulin resistance. In this context, excess visceral adiposity together with the accompanying low-grade chronic adipose tissue inflammation appears to contribute to a large extent to CMA risk with the subsequent development of insulin resistance and T2DM. Excess adiposity increases the risk of developing not only T2DM but also cardiovascular disease (CVD), nonalcoholic fatty liver disease (NAFLD), obstructive sleep apnea (OSA), and cancer, among others (3,4). This translates into increased health expenses (5) and leads to higher morbidity and reduced life expectancy (6).

Despite the elevated disease burden and the known public health consequences, targeted screening of the high-risk population remains an

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elusive goal in the clinical setting. Several obstacles in everyday clinical practice still hinder a precise identification of patients at risk. First, BMI is routinely used for diagnosis of obesity in clinical and public health practices. However, BMI reportedly underestimates obesity (7), and although an increased BMI is associated with T2DM at the population level, it does not adequately discriminate diabetes risk exerting a lower influence on the development of T2DM than body fat excess and distribution (8). Second, in addition to fasting blood glucose and glycated hemoglobin, the 2-hour oral glucose tolerance test (OGTT) represents the basis for screening and/or diagnosis of prediabetes, metabolic syndrome, and T2DM. However, despite the fact that 40% of subjects who develop T2DM have normal fasting glucose at baseline (9), there is still controversy about whether these patients should undergo an OGTT, as it might not be the simplest method to use. Therefore, it is clinically important to identify simple alternative approaches that improve clinical risk prediction in normoglycemic individuals and more accurately detect the presence of obesity-associated complications and beta-cell dysfunction heralding T2DM (10-13). In this context, body adiposity is a reasonable determinant, as it is a well-known risk factor for metabolic alterations, including glucose intolerance and insulin resistance, especially in patients with increased abdominal or visceral obesity. Therefore, in the present study performed in the routine clinical care setting, we carried out a comprehensive assessment of subjects with normal fasting glucose concentration who were unaware of having diabetes or glucose intolerance, complementing it with an OGTT-glucose response curve, total body and visceral adiposity analyses, and multiple cardiometabolic biomarkers. The information was gathered with the following aims: (1) to verify the utility of the 2-hour OGTT glucose value to discriminate between individuals with different cardiometabolic profiles, (2) to evaluate the ability of body adiposity and fat distribution to predict the risk of CMAs early on in the metabolic dysregulation process, and (3) to estimate the cardiovascular risk and proinflammatory differences when stratifying by total and visceral adiposity.

Methods

Study design and participants

Study participation was offered to subjects aged 18 to 70 years who attended the Department of Endocrinology and Nutrition of the University of Navarra Clinic from 2009 to 2014 for a check-up. The protocol included thorough anthropometric and body composition assessment, together with biochemical and hormonal determinations and an OGTT. Only those individuals with normal fasting glucose levels (≤ 5.5 mmol/L) were included in the present study. Subjects with T2DM or severe renal, liver, or thyroid dysfunction were excluded.

The experimental design was approved, from an ethical and scientific standpoint, by the Research Ethics Committee of the University of Navarra. Written informed consent was obtained from all individual participants included in the study. Before the study, participants were instructed to stop any medication known to affect glucose or lipid metabolism. On the day of the visit, each subject had a complete routine clinical assessment to evaluate the presence of cardiovascular, respiratory, renal, or endocrine disorders. All subjects underwent a 75-g OGTT and concomitant body composition measurements. In order to characterize the study sample, clinical characteristics, as well as cardiovascular risk factors (CVRFs) such as

hypertension, dyslipidemia, OSA, NAFLD, and smoking status, were studied. Patients were then categorized according to glucose tolerance status and body adiposity.

Anthropometric measurements

The anthropometric and body composition determinations, as well as the blood collection, were performed on a single day. All measurements were assessed using standardized methods, with participants dressed in light clothing and barefoot. Height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain Ltd., Crymych, UK), while body weight was measured with a calibrated electronic scale to the nearest 0.1 kg, with subjects wearing a swimming suit and cap, thereby allowing the calculation of BMI (kg/m^2). Blood pressure was measured after a 5-minute rest in the semisitting position with a sphygmomanometer. Blood pressure was determined at least three times at the right upper arm, and the mean was used in the analyses. The presence of hypertension was defined by a systolic blood pressure ≥ 140 mm Hg, a diastolic blood pressure ≥ 90 mm Hg, or the use of antihypertensives (14). NAFLD diagnosis was determined by ultrasonography and after other known liver diseases were excluded.

Body composition and physical activity

Body density was estimated by air-displacement plethysmography (Bod-Pod; Life Measurements, Concord, California). Body fat percentage (BF%) was calculated from body density by means of the Siri equation, as previously described (7). Data for estimation of fat mass (FM) by this plethysmographic method have been reported to agree closely with the traditional gold standard underwater weighing (15). It uses the pressure-volume relationship to estimate volume and density and has been shown to predict FM and fat-free mass more accurately than dual-energy x-ray absorptiometry and bioimpedance, using hydrodensitometry as reference method (15). The fat mass index (FMI) was calculated by dividing each subject's FM (in kilograms) by the square of their height (in meters) (16). In addition, the physical activity level (PAL) was estimated by a validated questionnaire, taking into account physical activity at home and at work and daily leisure time (17).

Abdominal and visceral adiposity

Visceral and abdominal adiposity was quantified by the use of the abdominal bioelectrical impedance analysis device ViScan (Tanita AB-140; Tanita Corp., Tokyo, Japan), which is designed to estimate visceral adiposity and trunk fat percentage and has been shown to closely correlate with computerized tomography measures (18). A wireless "electrode belt" is placed on the bare midriff of the subject in a supine position. The belt then uses dual-frequency bioimpedance (6.25 and 50 kHz) to measure trunk and visceral fat resistance and transmits the readings via infrared to the base unit. The ViScan abdominal body composition device provides a measure of the trunk fat percentage (including intra-abdominal and subcutaneous abdominal adipose tissue [range: 0%-75%]), as well as the amount of visceral fat (intra-abdominal adipose tissue) expressed in arbitrary units (a.u.) (range: 1-59 a.u.). As stated by the manufacturer, the intra-abdominal adipose tissue or visceral adipose tissue (VAT) area measured by computed tomography in centimeters squared corresponds to the visceral fat arbitrary units obtained by the ViScan multiplied by 10 (18). We categorized participants into quintiles according to body adiposity measures. This classification had the

advantage of a similar number of participants in each category, thus reducing the risk of having small and noninformative groups.

Biochemical measurements and metabolic studies

Blood samples were collected after a 10- to 12-hour overnight fast. Fasting blood samples were obtained for determination of the lipid and renal profiles and liver function tests, as well as for prothrombotic and inflammatory factors, homocysteine, and leptin. All participants underwent a 2-hour OGTT with an oral bolus containing 75 g of anhydrous glucose. Blood samples were obtained at 0, 30, 60, 90, and 120 minutes for the measurement of glucose and insulin concentrations. Patients were classified with regard to glucose tolerance on the basis of blood glucose levels, according to the American Diabetes Association diagnostic criteria for diabetes (2017) (19). For further analyses, subjects were also categorized into two groups, the CMA group or control group, based on a 2-hour OGTT glucose threshold of 7.8 mmol/L.

Consistent assay protocols were used for biochemical measurements over the study period. Plasma glucose and insulin were analyzed as previously described (20,21). Triglyceride concentrations were determined by enzymatic spectrophotometric methods (Roche, Basel, Switzerland). High-density lipoprotein (HDL) cholesterol was quantified by a colorimetric method in a Beckman Synchron CX analyzer (Beckman Instruments, Ltd., Bucks, UK). Low-density lipoprotein cholesterol was calculated by using the Friedewald formula. Uric acid and alanine aminotransferase were measured by enzymatic tests (Roche) in an automated analyzer (Roche/Hitachi Modular P800). Fibrinogen concentrations were determined according to the Clauss method of using a commercially available kit (Hemoliance; Instrumentation Laboratory, Barcelona, Spain). Measurement of the von Willebrand factor (vWF) antigen was performed by using a microlatex immunoassay (Diagnostica Stago, Inc., Parsippany, New Jersey). A standard curve was prepared with a universal reference (National Institute for Biological Standards and Control [NIBSC] 91/666), and the results were expressed as a percentage of the standard. Intra- and interassay coefficients of variation were 4.0% and 8.0%, respectively. High-sensitivity C-reactive protein (hsCRP) was measured by using the Tina-quant CRP (Latex) ultrasensitive assay (Roche). Homocysteine was determined by applying a fluorescence polarization immunoassay (Axis Biochemicals ASA, Oslo, Norway), using an IMx analyzer (Abbott Laboratories, Abbott Par, Illinois). Leptin was quantified by using a double-antibody radioimmunoassay method (Linco Research, Inc., St Charles, Missouri); intra- and interassay coefficients of variation were 5.0% and 4.5%, respectively, as previously described (22).

Assessment of insulin sensitivity and beta-cell function

Basal insulin resistance was calculated by the homeostasis model assessment of insulin resistance index (fasting insulin concentration $[I0] \times$ fasting glucose concentration $[G0])/405$) and the quantitative insulin sensitivity check index $(1/[(\log[I0] + \log[G0]]))$ (23). Whole-body insulin sensitivity was estimated by using the Matsuda index $(10,000/$square root of $[G0 \times I0] \times$ [mean glucose concentration $\times$ mean insulin concentration during the OGTT]) (23). Beta-cell function was estimated by the disposition index (DI), a measure of insulin secretion during the prevailing level of insulin action relative to the degree of insulin resistance $(\Delta I0 - 30/\Delta G0 - 30 \times 1/I0)$ (23).

Statistical analysis

Statistical analysis was performed using the Stata/SE version 12.0 (StataCorp, College Station, Texas). Participant characteristics were described using relative frequencies, means $\pm$ SD. The generalized linear model was used to assess the relationship between body adiposity and CMAs, CVRFs, and inflammatory markers in normal fasting glucose subjects. We estimated odds ratios and β-regression coefficients and their 95% CIs, using the lower tertile/quintile of body adiposity as the reference group. The absolute change in CMAs, CVRFs, and inflammatory markers according to body adiposity categories was analyzed using analysis of variance (ANOVA). Tests of linear trends were conducted by assigning medians of body adiposity for each category and treating this variable as continuous. Crude and multivariate logistic and linear regression models were fitted. As body fat distribution and visceral fat content can be affected by potential clinical confounders (24,25), multivariable analyses were adjusted for the following: gender, age, and physical activity. Statistical significance was defined as $P < 0.05$.

Results
Clinical and metabolic characteristics of the study sample

The demographics of the recruited subjects according to their glucose tolerance condition are shown in Table 1. A total of 493 Caucasian subjects (328 females/165 males) were studied. Patients' mean age and BMI were 40 ± 14 years and 34 ± 7.0, respectively. The prevalence of impaired glucose tolerance was 21.6%, while 2.7% fulfilled the criteria of T2DM.

Two-hour OGTT distinguishes different cardiometabolic profiles

Table 1 shows the descriptive characteristics of the 493 participants at baseline subdivided into two groups according to a 2-hour glucose concentration below (control group) or above (CMA group) 7.8 mmol/L. There was a significantly higher proportion of males in the CMA group, the members of which were significantly older and exhibited a higher BMI compared with the control group (Table 1).

Glucose response curve and beta-cell function data. The 60-minute glucose concentration in the CMA group was significantly higher compared with that of the control group. Plasma glucose and insulin concentrations at 120 minutes were also significantly higher in the CMA group. Hence, significant statistical differences were found in insulin sensitivity and beta-cell function indexes between groups, despite normal fasting glucose in both groups (Table 1). The DI was a significant 57% lower in the CMA group, indicating a significant decrease in beta-cell response relative to the degree of insulin resistance.

Body adiposity analysis. Although no statistically significant differences in FM or trunk fat percentages between the CMA group and control group were observed, CMA participants exhibited

TABLE 1 Characteristics of participants according to their glucose tolerance condition

	Control group (FG < 7.8 mmol/L)	CMA group (FG ≥ 7.8 mmol/L)	P value[a]
N	371	122	
Gender, n (% women)	261 (70.3)	67 (54.9)	**0.002**
Age, y	38 ± 13	45 ± 15	**< 0.001**
BMI, kg/m^2	33.5 ± 7.0	36.1 ± 8.5	**< 0.001**
Waist circumference, cm			
Male	114.6 ± 17.2	120.9 ± 17.0	**0.013**
Female	100.3 ± 15.1	106.3 ± 18.4	**0.003**
Body composition			
FMI, kg/m^2	15.1 ± 5.7	16.9 ± 6.8	**0.004**
BF, %	43.9 ± 8.7	45.6 ± 8.5	0.072
FM, kg	41.6 ± 16.0	46.1 ± 17.8	**0.009**
Trunk fat, %	53.2 ± 53.7	50.7 ± 41.8	0.639
Visceral fat content, a.u.	17.9 ± 15.1	21.9 ± 26.4	**0.04**
PAL	1.6 ± 0.12	1.5 ± 0.12	0.203
SBP, mm Hg	120 ± 17	127 ± 18	**< 0.001**
DPB, mm Hg	75 ± 11	79 ± 13	**0.002**
CVRFs (yes/no), n (%)			
Hypertension	64 (17.2)	39 (32.0)	**< 0.001**
Dyslipidemia	222 (59.8)	78 (63.9)	0.421
Smoking	76 (20.5)	32 (26.2)	0.183
CVD	3 (0.81)	1 (0.82)	0.991
OSA	66 (17.8)	42 (34.4)	**< 0.001**
NAFLD	71 (50.7)	51 (73.9)	**0.001**
Fasting glucose, mmol/L	5.0 ± 0.3	5.0 ± 0.3	0.332
Glucose: 1-h OGTT, mmol/L	7.6 ± 1.8	10.2 ± 1.9	**< 0.001**
Glucose: 2-h OGTT, mmol/L	5.9 ± 1.0	9.5 ± 1.5	**< 0.001**
Insulin, pmol/L	73.9 ± 64.5	96.9 ± 78.9	**0.002**
Insulin: 2-h OGTT, pmol/L	580.4 ± 371.6	1,069.8 ± 535.6	**< 0.001**
HOMA-IR	2.3 ± 2.1	3.0 ± 2.3	**0.002**
QUICKI	0.36 ± 0.05	0.34 ± 0.04	**0.005**
ISI	5.2 ± 3.5	3.5 ± 2.4	**< 0.001**
DI	6.1 ± 7.3	2.6 ± 1.9	**< 0.001**
TG, mmol/L	1.1 ± 0.6	1.5 ± 0.7	**< 0.001**
LDL cholesterol, mmol/L	2.8 ± 1.1	2.8 ± 1.2	0.509
HDL cholesterol, mmol/L	1.4 ± 0.3	1.3 ± 0.3	**< 0.001**
TG/HDL ratio	0.7 ± 0.6	1.2 ± 0.9	**< 0.001**
Uric acid, μmol/L	297.4 ± 77.3	345.0 ± 83.3	**< 0.001**
Creatinine, μmol/L	69.8 ± 15.0	71.6 ± 18.6	0.362
Leptin, μg/L	34.6 ± 23.3	33.7 ± 20.5	0.717
Fibrinogen, g/L	3.8 ± 0.7	4.0 ± 0.9	0.470
vWF antigen, U/mL	1.2 ± 0.5	1.3 ± 0.5	0.394
Homocysteine, μmol/mL	10.1 ± 4.0	10.8 ± 3.2	0.246
hsCRP, nmol/L	6.2 ± 6.6	7.8 ± 7.6	0.228
WBC count, 10^9 cells/L	6.4 ± 1.7	7.0 ± 2.0	**< 0.001**
γ-GT, U/L	20.1 ± 18.7	27.4 ± 29.1	**0.002**
ALT, U/L	19.0 ± 12.8	23.9 ± 16.6	**0.001**

Data presented as means ± SD.
[a]Student t tests used for continuous variables and χ^2 tests used for categorical variables for comparison between normal and altered glucose tolerance groups. All P values are two-sided; bold values represent $P < 0.05$.
γ-GT, gamma-glutamyl transferase; ALT, alanine aminotransferase; a.u., arbitrary units; BF, body fat; CMA, carbohydrate metabolism alteration; CVD, cardiovascular disease; CVRF, cardiovascular risk factor; DI, disposition index; FG, fasting glucose; FM, fat mass, FMI, fat mass index; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; OGTT, oral glucose tolerance test; OSA, obstructive sleep apnea; PAL, physical activity level; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TG, triglyceride; vWF, von Willebrand factor; WBC, white blood cells.
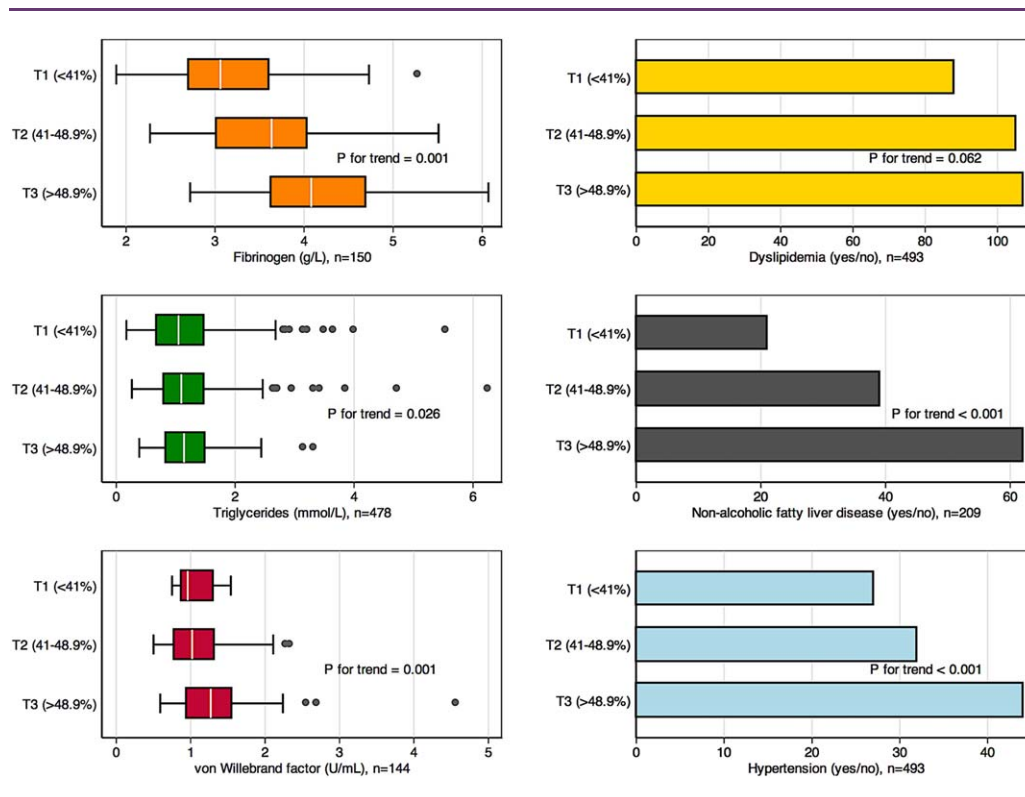
Figure 1 Median and prevalence estimates for participants' cardiovascular risk factors and proinflammatory variables according to tertiles of body fat percentage. *P* values were calculated using multivariate logistic and linear regression models, accordingly. [Color figure can be viewed at wileyonlinelibrary.com]

significantly increased BMI, waist circumference, and FM and visceral fat content (Table 1).

CVRFs. Participants in the control group exhibited a healthier metabolic profile, as indicated by significantly lower BMI, systolic blood pressure, diastolic blood pressure, uric acid, triglycerides/HDL ratio and gamma-glutamyl transferase and alanine aminotransferase compared with those in the CMA group (Table 1). No significant differences in PAL or leptin were noted. With regard to CVRFs, the prevalence of hypertension, OSA, and NAFLD was significantly higher in the CMA group (Table 1). Fasting triglyceride concentrations were significantly higher and HDL cholesterol was significantly lower in the CMA group (Table 1).

Inflammatory variables. Although prothrombotic factors such as fibrinogen and vWF were higher in the CMA group, the comparisons did not reach statistical significance. Among systemic inflammation markers, leukocytes and uric acid levels were significantly higher in subjects with CMAs compared with controls, with hsCRP and homocysteine concentrations being similar between groups.

Body adiposity and fat distribution as predictors of CMA risk

When the sample was stratified by FM categories, the quintile with the highest FMI and BF% (Q5) was independently associated with a tripled risk of having altered glucose tolerance compared with the reference category (Q1) (Table 2). BF% exhibited the highest

increase in the glucose level per unit of adiposity increase compared with FMI, FM (kg), VAT, and trunk fat (Table 3). As illustrated in Figure 1, after multivariate logistic and linear regression models, the worse the BF%, the worse the inflammatory profile, and a rise in the prevalence of CVRFs could be seen (Figure 1).

Cardiovascular risk and proinflammatory differences when stratifying by total and visceral adiposity

Tables 4 and 5 show in more detail the increment in the risk of presenting CVRFs, raised prothrombotic factors, and inflammatory parameters according to tertiles of BF% and VAT. Those in the highest tertile of VAT showed a 65% higher risk of suffering from NAFLD and a 64% higher risk of hypertension ($P < 0.001$) compared with those in the highest tertile of BF% (Tables 4 and 5).

Discussion

We aimed to examine the ability of body composition and fat distribution to predict the risk of glucose impairment, beta-cell dysfunction, and CVD in normoglycemic subjects. We herein demonstrate that the OGTT differentiates the metabolic profile of patients who have normal fasting glycemia. Furthermore, an excess in body and visceral fat content was associated with a decline in beta-cell function and whole-body insulin sensitivity and worse cardiovascular risk profiles. These data extend previous cross-sectional studies

TABLE 2 ORs and 95% CIs of altered glucose tolerance condition according to body composition

	No. of cases	Crude	Multivariate adjusted*	P for trend[a]
FMI, kg/m²				**0.011**
Q1 ($<$ 10.8)	15	1 (ref.)	1 (ref.)	
Q2 (10.8-13.2)	21	1.53 (0.73, 3.18)	1.45 (0.67, 3.10)	
Q3 (13.3-15.9)	33	2.80 (1.40, 5.59)	2.65 (1.28, 5.49)	
Q4 (16.0-19.5)	21	1.53 (0.73, 3.18)	1.60 (0.72, 3.54)	
Q5 ($>$ 19.5)	32	2.72 (1.36, 5.43)	2.87 (1.35, 6.12)	
Body fat, %				**0.009**
Q1 ($<$ 36.7)	18	1 (ref.)	1 (ref.)	
Q2 (36.7-42.6)	28	1.92 (0.98, 3.77)	2.60 (1.26, 5.34)	
Q3 (42.7-47.1)	23	1.41 (0.71, 2.82)	1.99 (0.91, 4.37)	
Q4 (47.2-51.4)	24	1.51 (0.76, 3.01)	2.46 (1.12, 5.43)	
Q5 ($>$ 51.4)	29	1.96 (1.00, 3.83)	3.27 (1.46, 7.31)	
Body fat, kg				0.190
Q1 ($<$ 29.4)	17	1 (ref.)	1 (ref.)	
Q2 (29.4-36.2)	20	1.22 (0.60, 2.50)	1.15 (0.54, 2.42)	
Q3 (36.3-43.2)	26	1.72 (0.86, 3.42)	1.49 (0.72, 3.06)	
Q4 (43.3-54.7)	35	2.68 (1.38, 5.22)	2.58 (1.27, 5.22)	
Q5 ($>$ 54.7)	24	1.56 (0.78, 3.14)	1.38 (0.64, 2.98)	
Trunk fat, %				0.192
Q1 ($<$ 40.2)	19	1 (ref.)	1 (ref.)	
Q2 (40.2-44.8)	26	1.54 (0.79, 3.01)	1.71 (0.85, 3.42)	
Q3 (44.9-49.1)	30	1.88 (0.97, 3.64)	2.22 (1.09, 4.48)	
Q4 (49.2-53.4)	22	1.19 (0.60, 2.36)	1.44 (0.69, 2.99)	
Q5 ($>$ 53.4)	25	1.50 (0.76, 2.95)	1.79 (0.87, 3.67)	
Visceral fat content, a.u.				0.433
Q1 ($<$ 11)	14	1 (ref.)	1 (ref.)	
Q2 (11-14)	23	1.68 (0.81, 3.49)	1.51 (0.71, 3.20)	
Q3 (14.5-17.5)	26	2.19 (1.06, 4.51)	1.76 (0.84, 3.71)	
Q4 (18-23.5)	28	2.43 (1.19, 4.97)	1.42 (0.64, 3.18)	
Q5 ($>$ 23.5)	31	3.09 (1.51, 6.29)	1.60 (0.63, 4.03)	

[a]Adjusted for gender, age, and physical activity. All P values are two-sided; bold values represent $P < 0.05$.
a.u., arbitrary units; FMI, fat mass index; OR, odds ratio; ref., reference.

examining the utility of OGTT-derived data and body adiposity studies to identify those at the highest risk of developing T2DM and CVD (10-12,26-32).

Metabolic profile according to 2-hour glucose values

In the present study, almost one in four young adults exhibited some degree of carbohydrate disturbance, despite normal basal (fasting) glycemia, which is a proportion substantially higher than that in previous reports (32); this is possibly related to the higher BMI of our patients. When dividing the group by glucose tolerance condition, early blood glucose disturbances during the OGTT were found.

Sixty-minute glucose. The 60-minute glucose in the CMA group was significantly higher compared with that of the controls. This finding is clinically relevant, given that even in the early stages of glucose homeostasis, disturbances in the tolerance of the early postload glucose value can be observed and represent an early

manifestation of metabolic dysfunction (26). Indeed, a 1-hour postload glucose of more than 8.6 mmol/L (155 mg/dl) has recently been identified as a better predictor of incident T2DM and associated complications than fasting or 2-hour glucose levels (26,30,31,33). Hence, the 1-hour postload glucose level has been postulated as an early marker of carbohydrate disturbances and has been proposed as a more suitable screening tool for risk assessment than the currently used 2-hour postload level.

Beta-cell function and insulin sensitivity indices. Similarly, in the CMA group, we found a significant decline in beta-cell function, in spite of normal fasting glucose, measured by the DI. The DI is the product of measures of insulin sensitivity and first-phase insulin secretion, and it has been shown to predict conversion to diabetes (13,27). Our study demonstrates that early beta-cell damage (57% drop) is associated with adiposity, which is developed prior to the appearance of impaired fasting glucose, a fact that would have been unperceived if an OGTT had not been performed. The insulin sensitivity index, a reliable index of whole-body insulin sensitivity, which

TABLE 3 Estimates (β-regression coefficients and 95% CIs) for participants' glucose tolerance condition according to their body composition characteristics

	Absolute glucose tolerance condition, mean ± SD	Crude	Multivariate adjusted[a]	P for trend[a]
FMI, kg/m^2				< 0.001
Q1 (< 10.8)	6.1 ± 1.9	0 (ref.)	0 (ref.)	
Q2 (10.8-13.2)	6.5 ± 1.9	0.4 (0.1, 0.9)	0.3 (−0.2, 0.8)	
Q3 (13.3-15.9)	6.9 ± 1.8	0.8 (0.3, 1.3)	0.6 (0.2, 1.1)	
Q4 (16.0-19.5)	7.1 ± 2.2	1.0 (0.4, 1.6)	1.0 (0.4, 1.5)	
Q5 (> 19.5)	7.2 ± 1.7	1.1 (0.6, 1.6)	1.1 (0.6, 1.6)	
Body fat, %				< 0.001
Q1 (< 36.7)	6.3 ± 1.9	0 (ref.)	0 (ref.)	
Q2 (36.7-42.6)	6.8 ± 1.9	0.5 (0.0, 1.1)	0.8 (0.3, 1.4)	
Q3 (42.7-47.1)	6.6 ± 1.9	0.3 (0.2, 0.9)	0.7 (0.2, 1.2)	
Q4 (47.2-51.4)	7.0 ± 1.9	0.7 (0.2, 1.3)	1.2 (0.6, 1.7)	
Q5 (> 51.4)	7.2 ± 1.9	0.9 (0.4, 1.5)	1.3 (0.8, 2.0)	
Body fat, kg				0.001
Q1 (< 29.4)	6.2 ± 1.9	0 (ref.)	0 (ref.)	
Q2 (29.4-36.2)	6.4 ± 1.7	0.2 (−0.3, 0.7)	0.1 (−0.4, 0.6)	
Q3 (36.3-43.2)	6.9 ± 2.0	0.8 (0.2, 1.3)	0.6 (0.1, 1.1)	
Q4 (43.3-54.7)	7.4 ± 2.3	1.2 (0.6, 1.8)	1.0 (0.5, 1.6)	
Q5 (> 54.7)	7.0 ± 1.4	0.8 (0.3, 1.3)	0.6 (0.1, 1.1)	
Trunk fat, %				0.001
Q1 (< 40.2)	6.3 ± 2.0	0 (ref.)	0 (ref.)	
Q2 (40.2-44.8)	6.7 ± 1.9	0.5 (−0.1, 1.0)	0.5 (0.0, 1.0)	
Q3 (44.9-49.1)	7.0 ± 1.8	0.7 (0.2, 1.3)	0.8 (0.3, 1.3)	
Q4 (49.2-53.4)	6.8 ± 1.8	0.5 (0.0, 1.1)	0.6 (0.1, 1.2)	
Q5 (> 53.4)	6.8 ± 2.0	0.8 (0.3, 1.4)	0.9 (0.4, 1.5)	
Visceral fat content, a.u.				0.005
Q1 (< 11)	5.8 ± 1.6	0 (ref.)	0 (ref.)	
Q2 (11-14)	6.6 ± 1.6	0.7 (0.3, 1.2)	0.6 (0.2, 1.0)	
Q3 (14.5-17.5)	6.9 ± 2.2	1.1 (0.6, 1.6)	0.9 (0.4, 1.4)	
Q4 (18-23.5)	7.3 ± 2.0	1.5 (1.0, 2.0)	1.0 (0.5, 1.6)	
Q5 (> 23.5)	7.3 ± 1.9	1.5 (1.0, 2.0)	1.0 (0.3, 1.7)	

[a]Adjusted for gender, age, and physical activity. All *P* values are two-sided; bold values represent *P*< 0.05.
a.u., arbitrary units; FMI, fat mass index; ref., reference.

correlates well with the rate of whole-body glucose disposal, was also significantly decreased in the CMA group (23). Altogether, these results reflect a decrease in insulin sensitivity and beta-cell pancreatic reserve, a fact that would not have been perceived if the OGTT had not been performed. In patients with obesity, this leads to the concept of "metabolically healthy obesity" to define patients without major CVRFs (34). However, in the literature, there is (paradoxically) a lack of a clear-cut definition about what constitutes metabolically healthy obesity (35). Thus, different definitions allow a diverse degree of unhealthy derangements, with no universally accepted criteria to define the metabolically healthy obesity phenotype. In routine clinical practice, a simple metabolic assessment is performed that does not include an OGTT on a regular basis, thereby possibly resulting in a misdiagnosis of some of the not-so-evident metabolic alterations. In this study, the CMA group could have been categorized as having metabolically healthy obesity,

possibly leading to an underestimation of cardiometabolic risk if the OGTT had not been performed. Thus, these patients would not have benefited from receiving specific treatments aimed at preventing the future development of T2DM.

Inflammatory and thrombotic state. Proinflammatory and pro-thrombotic factors were also more evident in the CMA group, thus increasing the cardiometabolic risk of these patients. In our study, among systemic inflammatory markers, leukocytes and uric acid levels were significantly increased in patients with CMAs. These results are congruent with other findings showing that hsCRP and proinflammatory cytokine levels are elevated in both impaired glucose tolerance and T2DM and are thus able to predict the progression to T2DM (36,37). These findings support the notion that prediabetes may represent a true intermediate phenotype between metabolically healthy obesity and diabetes.

TABLE 4 Estimates (β-regression coefficients, ORs, and 95% CIs) for participant CVRFs and inflammatory parameters according to their BF%

	T1 (<41%)	T2 (41%-48.9%)	T3 (>48.9%)	P for trend
Fibrinogen (n = 150)				<0.001
Absolute value, mean ± SD	3.2 ± 0.8	3.6 ± 0.7	4.2 ± 0.7	
Crude	0 (ref.)	0.3 (0.1, 0.7)	0.9 (0.5, 1.3)	
Multivariate adjusted	0 (ref.)	0.2 (−0.2, 0.7)	0.8 (0.3, 1.3)	
vWF (n = 144)				<0.001
Absolute value, mean ± SD	1.0 ± 0.2	1.1 ± 0.4	1.3 ± 0.6	
Crude	0 (ref.)	0.6 (−1.1, 2.4)	2.6 (1.0, 4.2)	
Multivariate adjusted	0 (ref.)	0.8 (−1.1, 2.8)	3.6 (1.4, 5.7)	
Homocysteine (n = 151)				0.078
Absolute value, mean ± SD	10.4 ± 2.8	10.5 ± 3.9	10.3 ± 3.9	
Crude	0 (ref.)	0.07 (−1.6, 1.7)	−0.1 (−1.5, 1.3)	
Multivariate adjusted	0 (ref.)	0.5 (−1.2, 2.2)	1.3 (−0.3, 2.9)	
hsCRP (n = 176)				<0.001
Absolute value, mean ± SD	2.8 ± 3.1	4.6 ± 3.0	9.5 ± 8.5	
Crude	0 (ref.)	1.7 (0.4, 3.0)	6.6 (4.5, 8.85)	
Multivariate adjusted	0 (ref.)	1.9 (0.47, 3.4)	7.0 (4.4, 9.7)	
Uric acid (n = 461)				<0.001
Absolute value, mean ± SD	321.2 ± 83.3	297.4 ± 83.3	315.2 ± 77.3	
Crude	0 (ref.)	−23.8 (−41.6, −5.9)	−0.0 (−23.8, 17.8)	
Multivariate adjusted	0 (ref.)	17.8 (5.9, 35.7)	53.5 (35.7, 71.4)	
LDL cholesterol (n = 458)				0.462
Absolute value, mean ± SD	2.7 ± 1.2	2.8 ± 1.2	2.9 ± 1.1	
Crude	0 (ref.)	0.15 (−0.1, 0.4)	0.2 (−0.02, 0.5)	
Multivariate adjusted	0 (ref.)	0.1 (−0.1, 0.3)	0.1 (−0.1, 0.3)	
HDL cholesterol (n = 460)				0.125
Absolute value, mean ± SD	1.4 ± 0.4	1.4 ± 0.4	4.4 ± 0.4	
Crude	0 (ref.)	0.1 (0.0, 0.2)	0.0 (0.0, 0.1)	
Multivariate adjusted	0 (ref.)	0.0 (−0.1, 0.1)	−0.1 (−0.2, 0.0)	
Triglycerides (n = 478)				0.022
Absolute value, mean ± SD	1.2 ± 0.8	1.2 ± 0.7	1.2 ± 0.5	
Crude	0 (ref.)	0.03 (−0.1, 0.2)	−0.0 (−0.2, 0.1)	
Multivariate adjusted	0 (ref.)	0.2 (0.0, 0.4)	0.2 (0.0, 0.3)	
Dyslipidemia (yes/no; n = 493)				0.065
No. of cases	88	105	107	
Crude OR	1 (ref.)	1.5 (1.0, 2.4)	1.7 (1.1, 2.6)	
Multivariate adjusted OR	1 (ref.)	1.6 (1.0, 2.6)	1.6 (1.0, 2.8)	
NAFLD (yes/no; n = 209)				<0.001
No. of cases	21	39	62	
Crude OR	1 (ref.)	2.5 (1.2, 5.3)	2.8 (1.4, 5.6)	
Multivariate adjusted OR	1 (ref.)	6.0 (2.3, 16.0)	9.9 (3.6, 27.2)	
Hypertension (yes/no; n = 493)				<0.001
No. of cases	27	32	44	
Crude OR	1 (ref.)	1.2 (0.7, 2.2)	1.9 (1.1, 3.2)	
Multivariate adjusted OR	1 (ref.)	1.9 (1.0, 3.7)	3.9 (1.9, 8.0)	

Data presented as means ± SD. Multivariate adjusted for gender, age, and physical activity. All P values are two-sided; bold values represent P < 0.05.
BF%, body fat percentage; CVRF, cardiovascular risk factor; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; ref., reference; vWF, von Willebrand factor.

TABLE 5 Estimates (β-regression coefficients, ORs and 95% CIs) for participant CVRFs according to their VAT

	T1 (<13.5 a.u)	T2 (13.5-19 a.u)	T3 (>19 a.u)	*P* for trend
vWF				0.439
Absolute value, mean ± SD	1.1 ± 0.5	1.2 ± 0.4	1.3 ± 0.6	
Crude	0 (ref.)	0.05 (−0.2, 0.4)	0.1 (−0.2, 0.4)	
Multivariate adjusted	0 (ref.)	0.09 (−0.2, 0.4)	0.2 (−0.2, 0.6)	
Homocysteine				0.444
Absolute value, mean ± SD	9.94 ± 4.52	8.82 ± 2.81	11.19 ± 3.82	
Crude	0 (ref.)	−1.12 (−3.74, 1.49)	1.25 (−1.36, 3.86)	
Multivariate adjusted	0 (ref.)	−1.05 (−3.46, 1.36)	0.21 (−2.66, 3.08)	
hsCRP				0.766
Absolute value, mean ± SD	7.2 ± 10.3	7.0 ± 6.4	6.8 ± 8.6	
Crude	0 (ref.)	−0.2 (−4.4, 4.0)	−0.47 (−4.7, 3.7)	
Multivariate adjusted	0 (ref.)	−0.1 (−4.6, 4.4)	0.48 (3.8, 4.8)	
Uric acid (*n* = 461)				**< 0.001**
Absolute value, mean ± SD	249.8 ± 53.5	309.3 ± 77.3	368.8 ± 65.4	
Crude	0 (ref.)	59.5 (41.6, 71.4)	118.9 (107.1, 130.9)	
Multivariate adjusted	0 (ref.)	45.2 (30.9, 60.0)	60.7 (40.4, 81.5)	
LDL cholesterol (*n* = 458)				0.366
Absolute value, mean ± SD	2.6 ± 1.1	2.9 ± 1.2	2.9 ± 1.0	
Crude	0 (ref.)	0.3 (0.0, 0.5)	0.2 (0.02, 0.5)	
Multivariate adjusted	0 (ref.)	0.2 (0.0, 0.4)	−0.1 (−0.4, 0.1)	
HDL cholesterol (*n* = 460)				**< 0.001**
Absolute value, mean ± SD	1.6 ± 0.4	1.4 ± 0.4	1.2 ± 0.3	
Crude	0 (ref.)	−0.2 (−0.3, −0.1)	−0.4 (−0.4, −0.3)	
Multivariate adjusted	0 (ref.)	−0.1 (−0.2, −0.0)	−0.2 (−0.3, −0.1)	
Triglycerides (*n* = 478)				**0.023**
Absolute value, mean ± SD	1.0 ± 0.6	1.2 ± 0.6	1.5 ± 0.7	
Crude	0 (ref.)	0.3 (0.1, 0.4)	0.5 (0.4, 0.6)	
Multivariate adjusted	0 (ref.)	0.2 (0.0, 0.3)	0.2 (0.0, 0.4)	
Dyslipidemia (yes/no; *n* = 493)				0.269
No. of cases	89	108	103	
Crude OR	1 (ref.)	2.0 (1.3, 3.1)	1.6 (1.0, 2.5)	
Multivariate adjusted OR	1 (ref.)	1.99 (1.2, 3.0)	1.4 (0.7, 2.9)	
NAFLD (yes/no; *n* = 209)				**< 0.001**
No. of cases	11	43	68	
Crude OR	1 (ref.)	5.3 (2.3, 11.9)	12.7 (5.5, 29.5)	
Multivariate adjusted OR	1 (ref.)	6.5 (2.6, 15.9)	15.2 (5.3, 43.6)	
Hypertension (yes/no; *n* = 493)				**< 0.001**
No. of cases	14	26	63	
Crude OR	1 (ref.)	2.2 (1.1, 4.0)	7.2 (3.8, 13.5)	
Multivariate adjusted OR	1 (ref.)	1.9 (1.0, 3.9)	6.1 (2.6, 14.4)	

Data presented as means ± SD. Multivariate adjusted for gender, age, and physical activity. All *P* values are two-sided; bold values represent *P* < 0.05.
CVRF, cardiovascular risk factor; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; ref., reference; VAT, visceral adipose tissue; vWF, von Willebrand factor.

Correlations between body composition and metabolic and CVRFs

Glucose metabolism. On the other hand, despite the fact that excess adiposity underlies the increased risk of CMAs observed in obesity, the mechanisms behind the transition from functional to dysfunctional adiposity are not well understood yet. Moreover, large studies analyzing the relation between BF% and/or VAT and CMAs in normoglycemic subjects are scarce. It has previously been shown that subjects with normal glucose tolerance but excessive FM or VAT present with complications such as atherogenic dyslipidemia, hyperinsulinemia, and hypertension, leading to the development of CVD and NAFLD (38,39).

Our group has previously reported the importance of BF% as a better predictor of T2DM than BMI, especially in male subjects with

BMI < 25 and over the age of 40 years (7,21). Along these lines, FMI has been shown to be independently and positively associated with the presence of metabolic syndrome, and consequently, we have included it in the present study. However, scarce information is available about the role of FMI, BF%, and VAT on the metabolic risk profile, specifically in subjects with normal glucose tolerance. Therefore, we analyzed the degree of association and the influence of body composition and visceral adiposity on the risk of developing CMA, several CVRFs, and a proinflammatory environment in subjects with normal fasting glucose.

Patients in the highest quintile (Q5) of BF% or FMI had a significantly higher risk of having CMAs after multivariate adjustment. In addition, a dysfunctional adiposity phenotype in any of its variants (excess FMI, FM, BF%, VAT, or trunk fat percentage) independently predicted the presence of an increased risk of having CMAs. Therefore, the present study provides evidence that body composition, especially BF% or FMI, should be measured when estimating the risk of prediabetes and T2DM, even in normoglycemic subjects, independently of age, gender, and PAL. This endorses previous data supporting the discriminatory ability of adipose tissue distribution for the diabetes risk assessment (32).

Inflammatory and thrombotic state. Along the same lines, excess adiposity was associated not only with glucose intolerance and beta-cell dysfunction but also with an increase in inflammatory and prothrombotic parameters such as hsCRP, uric acid, fibrinogen, and vWF. On the basis of these results, many key inflammatory markers have been consistently associated with both obesity and the risk of adverse outcomes in obesity-associated diseases (40). A meta-analysis of 51 independent cross-sectional studies supported a positive association between body composition and CRP as a marker of systemic inflammation (41). Similar associations have also been reported for fibrinogen and other prothrombotic parameters (42) and uric acid (43). Increases in a range of inflammatory markers have also been reproducibly associated with an increased risk of several obesity-associated diseases, including CVD and T2DM.

CVRFs. Our study also shows the predictive value of the rise in the concentrations of each of these factors per unit of increase in BF%. Along these lines, a gradual increase in the risk of atherogenic dyslipidemia, NAFLD, and hypertension with the worsening in body adiposity was consistently observed, which involved a high morbidity in these young patients with normal glucose tolerance status. Our data also suggest that VAT is a better predictor of the presence of atherogenic dyslipidemia, hyperuricemia, hypertension, and fatty liver than BF% (Tables 4 and 5). These results are supported by previously published studies comparing the role of VAT with total FM in the development of atherogenic dyslipidemia (44). However, in the present study, a larger increase in the risk of presenting NAFLD and hypertension compared with whole-body adiposity was observed in comparison to what has previously been reported (29).

Strengths and limitations

One strength of this investigation was the comprehensive assessment in a large cohort of normoglycemic patients in a routine clinical care setting of the OGTT-glucose response curve, body and visceral adiposity, and multiple biomarkers of the cardiometabolic profile. Another strength was the comparison of the effect of body adiposity dysfunction on the increased risk of presenting glucose impairment

and CVRFs in normoglycemic individuals. To the best of our knowledge, no previous study has performed body composition studies with glucose tolerance testing and beta-cell function analyses in order to examine the ability of body and visceral adiposity to predict glucose impairment in normoglycemic patients. A final strength of this study was an adjustment of the analyses by using important confounders, including age, gender, and PAL.

Although our study has several strengths, we also acknowledge some limitations. First, the study was cross-sectional, and hence, cause-effect relations among the degree of obesity and glucose impairment and cardiometabolic parameters could not be ascertained. Secondly, the present work was conducted in Caucasian subjects and in patients attending a specialized endocrinology clinic, and it therefore needs to be extended to other populations and settings to determine its validity. In addition, the OGTT-glucose response concentrations were determined by means of a single OGTT.

Conclusion

The prevalence of CMAs after the 2-hour OGTT was 24% in our cohort of patients with normal fasting glucose, highlighting the importance of performing an OGTT in light of the fact that static normal fasting glycemia does not rule out CMAs in this population. BF% was associated not only with an increased risk of CMAs but also with important cardiometabolic risk factors and inflammatory parameters, independently of age, gender, and PAL. Furthermore, VAT was shown to be a better predictor than total body adiposity for the increased risk of atherogenic dyslipidemia, hyperuricemia, hypertension, and NAFLD. It is concluded that the inclusion of body composition measurements along with an OGTT in everyday clinical practice in populations with obesity and normal fasting glucose is a useful clinical tool to identify patients at risk, who may benefit from intensive lifestyle modification and therapeutic interventions.**O**

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