Increased Cardiometabolic Risk Factors and Inflammation in Adipose Tissue in Obese Subjects Classified as Metabolically Healthy

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OBJECTIVE

It has been suggested that individuals with the condition known as metabolically healthy obesity (MHO) may not have the same increased risk for the development of metabolic abnormalities as their non–metabolically healthy counterparts. However, the validity of this concept has recently been challenged, since it may not translate into lower morbidity and mortality. The aim of the current study was to compare the cardiometabolic/inflammatory profile and the prevalence of impaired glucose tolerance (IGT) and type 2 diabetes (T2D) in patients categorized as having MHO or metabolically abnormal obesity (MAO).

RESEARCH DESIGN AND METHODS

We performed a cross-sectional analysis to compare the cardiometabolic/inflammatory profile of 222 MHO and 222 MAO patients (62% women) matched by age, including 255 lean subjects as reference (cohort 1). In a second cohort, we analyzed the adipokine profile and the expression of genes involved in inflammation and extracellular matrix remodeling in visceral adipose tissue (VAT; *n* = 82) and liver (*n* = 55).

RESULTS

The cardiometabolic and inflammatory profiles (CRP, fibrinogen, uric acid, leukocyte count, and hepatic enzymes) were similarly increased in MHO and MAO in both cohorts. Moreover, above 30% of patients classified as MHO according to fasting plasma glucose exhibited IGT or T2D. The profile of classic (leptin, adiponectin, resistin) as well as novel (serum amyloid A and matrix metallopeptidase 9) adipokines was almost identical in MHO and MAO groups in cohort 2. Expression of genes involved in inflammation and tissue remodeling in VAT and liver showed a similar alteration pattern in MHO and MAO individuals.

CONCLUSIONS

The current study provides evidence for the existence of a comparable adverse cardiometabolic profile in MHO and MAO patients; thus the MHO concept should be applied with caution. A better identification of the obesity phenotypes and a more precise diagnosis are needed for improving the management of obese individuals.

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Excess adiposity favors the clustering of cardiometabolic alterations such as type 2 diabetes (T2D), hypertension, and dyslipidemia, leading to an increase in morbidity (1,2) and reduced life expectancy (3). The risk of developing obesity-related derangements is proportional to the degree of adiposity (1,4) and, in particular, to the accumulation of fat in the visceral region (2). However, a proportion of obese individuals might not be at increased risk for the development of metabolic abnormalities, and therefore, their clinical condition has been termed metabolically healthy obesity (MHO) (4,5). In contrast, obese patients exhibiting insulin resistance, increased blood pressure, and dyslipidemia are considered as having metabolically abnormal obesity (MAO) (6). The lack of consensus criteria to define MHO does not allow the accurate estimation of the prevalence of the MHO and MAO phenotypes, making the comparison between different studies difficult (4,7). In this sense, the reported prevalence of MHO varies widely, ranging from 3 to 57% of obese patients, depending on the method used to define this condition (5,8–10).

Several mechanisms have been reported to explain the apparently less deleterious metabolic profile of MHO subjects. Among them, a lower inflammatory profile (7), higher lipolytic activity (11), increased physical activity, lower uric acid (12), or reduced liver fat evidenced by lower liver enzyme concentrations (13) have been put forward. These factors might differentiate metabolically unhealthy from metabolically healthy obese individuals.

Most of the studies regarding MHO subjects use fasting plasma glucose (FPG) or other indicators of insulin resistance such as HOMA, different indices obtained after an oral glucose tolerance test (OGTT), or hyperinsulinemic-euglycemic clamp as classification criteria (4,5), but to our knowledge, there are no studies analyzing the prevalence of impaired glucose tolerance (IGT) or T2D according to the OGTT in MHO patients based on their FPG levels applied as a surrogate marker of insulin resistance. Importantly, identification of prediabetes and T2D based on FPG or OGTT yields highly discordant results in obese patients, in particular, exerting a relevant impact on the diagnosis and management of these conditions (14). Therefore, diagnosis of MHO based on FPG (as surrogate marker of altered glucose metabolism among the other cardiometabolic risk factor criteria) may be misclassifying individuals who actually have IGT or even diabetes.

Recently, several studies have questioned the apparently healthy metabolic profile of MHO, showing that it may not translate into lower morbidity and mortality (15–17). Since the differentiation between the diverse obese phenotypes may have important therapeutic implications, an adequate definition for the stratification of obese individuals and a correct diagnosis are of paramount importance for the personalized management of the obese patient. Therefore, the aim of the current study was to compare the cardiometabolic profile, the actual prevalence of impaired glucose homeostasis, and the systemic inflammatory profile as well as the expression of genes related to inflammation and matrix remodeling in visceral adipose tissue (VAT) and liver between obese patients defined as having MHO or MAO.

RESEARCH DESIGN AND METHODS

Study Design

We conducted a cross-sectional analysis of 222 MHO and 222 MAO patients (168 men and 276 women) matched for age, with similar socioeconomic characteristics, including patients visiting the Department of Endocrinology and Nutrition and the Department of Surgery of the Clínica Universidad de Navarra (Pamplona, Spain) for weight loss treatment (cohort 1). Obesity was defined as a BMI $\geq$30 kg/m^2. The study included 78 men and 177 women as reference group subjects classified as lean by BMI (18.5–24.9 kg/m^2) from the hospital and university staff undergoing an annual routine health checkup. The final sample included 699 Caucasian subjects (453 females/246 males) aged 19–73 years.

In order to confirm the findings of cohort 1 and further gain insight into the actual effect at the tissue level, gene expression in VAT and liver together with the adipokine profile were analyzed in a group of 82 subjects (16 males and 66 females) recruited from lean patients undergoing Nissen fundoplication for hiatus hernia repair and from patients undergoing Roux-en-Y gastric bypass for morbid obesity (cohort 2). In addition, an intraoperative liver biopsy was performed in the obese patients during bariatric surgery (n = 55).

All patients were weight stable ($\pm$2 kg) for the previous 3 months. Using previously accepted criteria, MAO was defined as having at least two of the following cardiometabolic abnormalities: glucose concentrations $\geq$100 mg/dL or antidiabetes medication use; systolic blood pressure (SBP) $\geq$130 mmHg, diastolic blood pressure (DBP) $\geq$85 mmHg, or antihypertensive medication use; triglyceride concentrations $\geq$150 mg/dL; and HDL cholesterol levels $<$40 mg/dL for men and $<$50 mg/dL for women or lipid-lowering medication use similar to previously reported studies (4,10). Patients with signs of infection were excluded. The experimental design was approved, from an ethical and scientific standpoint, by the hospital's ethics committee responsible for research, and informed consent was obtained from all subjects.

Anthropometric Measurements

The anthropometric and body composition determinations as well as the blood extraction were performed on a single day. Height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain Ltd., Crymych, U.K.), while body weight was measured with a calibrated electronic scale to the nearest 0.1 kg with subjects wearing a swimming suit and cap. Waist circumference was measured at the midpoint between the iliac crest and the rib cage on the midaxillary line. Hip circumference was measured at the maximum protuberance of the buttocks, and the waist-to-hip ratio (WHR) was calculated. Blood pressure was measured after a 5-min 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.

Body Composition

Body density was estimated by air displacement plethysmography (BOD POD, Life Measurement, Concord, CA) as previously described (1,18). Percentage of body fat (BF%) was estimated from body density using the Siri equation.

Laboratory Procedures

Blood samples were collected after an overnight fast in the morning in order to avoid potential confounding influences due to hormonal rhythmicity. Plasma glucose and insulin were analyzed as previously

described (18). Indirect measures of insulin resistance and insulin sensitivity were calculated by using HOMA and QUICKI, respectively. In obese patients, plasma glucose concentrations after a 75-g OGTT were performed. Normoglycemia (NG) was defined as having a glucose level below 7.8 mmol/L 2 h after the OGTT. IGT was defined as exhibiting a glucose concentration between 7.8 and 11.0 mmol/L 2 h after the OGTT. T2D was defined as having glycemia ≥11.1 mmol/L 2 h after the OGTT, following the criteria of the American Diabetes Association (19). Total cholesterol and triglyceride concentrations were determined by enzymatic spectrophotometric methods (Roche, Basel, Switzerland). HDL cholesterol was quantified by a colorimetric method in a Beckman Synchron CX analyzer (Beckman Coulter, Bucks, U.K.). LDL cholesterol was calculated by the Friedewald formula.

Uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and γ-glutamyltransferase (γ-GT) were measured by enzymatic tests (Roche) in an automated analyzer (Roche/Hitachi Modular P800). Measurement of von Willebrand factor antigen was performed by a microlatex immunoassay (Diagnostica Stago, Parsippany, NJ). Homocysteine was determined applying a fluorescence polarization immunoassay (Axis Biochemicals ASA, Oslo, Norway) using an IMX analyzer (Abbott, Abbott Park, IL). hs-CRP was measured using the Tina-Quant CRP (Latex) ultrasensitive assay (Roche). Fibrinogen concentrations were determined according to the Clauss method using a commercially available kit (Hemoliance; Instrumentation Laboratory, Barcelona, Spain). White blood cell count was measured using an automated cell counter (Beckman Coulter, Fullerton, CA). Leptin (Linco, St. Charles, MO), adiponectin (R&D Systems, Minneapolis, MN), resistin (R&D Systems), serum amyloid A (SAA; BioSource, Camarillo, CA), vascular endothelial growth factor (VEGF; R&D Systems), and matrix metallopeptidase 9 (MMP9; R&D Systems) were quantified by immunoassays.

Gene Expression by Real-Time PCR

Omental adipose tissue was obtained from patients undergoing either Roux-en-Y gastric bypass or Nissen fundoplication, while an intraoperative liver biopsy was obtained in the obese patients only from cohort 2. The samples were immediately frozen in liquid nitrogen and stored at −80°C. RNA isolation was performed as previously described (20). Transcript levels were quantified by real-time PCR (7300 Real-Time PCR System, Applied Biosystems, Foster City, CA). Primers and probes (Sigma-Aldrich, Madrid, Spain) were designed using the software Primer Express 2.0 (Applied Biosystems). Primers used to amplify the cDNA are shown in Supplementary Table 1. The cDNA was amplified at the following conditions: 95°C for 10 min, followed by 45 cycles of 15 s at 95°C and 1 min at 59°C, using the TaqMan Universal PCR Master Mix (Applied Biosystems). The primer and probe concentrations for gene amplification were 300 and 200 nmol/L, respectively. All results were normalized to the levels of *18S* rRNA (Applied Biosystems), and relative quantification was calculated using the ΔΔCt formula (20,21). Relative mRNA expression was expressed as fold expression over the calibrator sample (average of gene expression corresponding to the lean group in VAT and MHO group in liver). All samples were run in triplicate, and the average values were calculated.

Statistical Analysis

Data are presented as mean ± SD unless otherwise specified. Differences in quantitative variables between groups were analyzed by one-way ANOVA followed by Tukey post hoc tests, Kruskal-Wallis followed by Mann-Whitney *U* tests, or Student *t* tests as appropriate. Differences in qualitative variables were analyzed by χ^2 analysis. The calculations were performed using the SPSS version 15.0.1 (SPSS, Chicago, IL). A *P* value lower than 0.05 was considered statistically significant.

RESULTS

Cardiometabolic Risk Factors in MHO

The anthropometric, biochemical, and hormonal characteristics of the subjects from cohort 1 are shown in Table 1. No differences in age between groups from both sexes were observed, since age was a matching criterion. No statistically significant differences were found in body weight, BMI, BF%, waist circumference, or WHR between male MHO and MAO, while BMI, waist circumference, and WHR were significantly increased in female MAO as compared with MHO. Blood pressure was significantly increased only in female MAO in comparison with MHO, even though it was a classification factor. As expected, glucose homeostasis was slightly altered in MHO as evidenced by increased insulin concentrations and reduced QUICKI with normal glycemia, while it was greatly impaired in MAO as shown by all the glucose metabolism variables measured. Triglyceride concentrations were slightly elevated in MHO, reaching statistical significance only in women, while being further increased in the MAO groups. Male MHO patients exhibited significantly increased total and LDL cholesterol together with reduced HDL cholesterol concentrations, while MAO showed alterations only in HDL. In women, no changes in total and LDL cholesterol were observed, with HDL levels being reduced in MHO and further decreased in the MAO group.

Circulating concentrations of uric acid were similarly increased in both obese groups in men, being elevated in MHO and further increased in MAO in women as well as in the sample as a whole (Table 1 and Supplementary Fig. 1). Homocysteine concentrations were significantly increased in both obese groups in the whole sample, with no differences between them, being only significantly increased in women from the MAO group when the analysis was performed by sex. Von Willebrand factor levels were elevated only in women from the MAO group. Markers of inflammation such as CRP, fibrinogen, and leukocyte number were similarly increased in both groups of obese patients, with no differences between them (Table 1 and Supplementary Fig. 1). Markers of liver function, including ALT, AST, alkaline phosphatase, and γ-GT, were similarly augmented in MHO and MAO groups from both sexes, exhibiting no differences between them, with the exception of AST concentrations in women, which were unaltered. Circulating levels of leptin were similarly and dramatically increased in both obese groups. Similar results were obtained after adjusting for age and BF%.

Prevalence of IGT in MHO

OGTTs were performed in the MHO and MAO patients from cohort 1 to determine the actual prevalence of IGT and T2D. In men, 67.8, 27.1, and 5.1% of

Table 1—Anthropometric and metabolic characteristics of subjects included in cohort 1

	Male				Female			
	Lean	MHO	MAO	*P* value	Lean	MHO	MAO	*P* value
n	78	84	84		177	138	138	
Age, years	45.2 ± 13.8	44.4 ± 9.9	47.6 ± 8.4	0.147	47.0 ± 10.0	47.2 ± 10.2	49.3 ± 10.5	0.100
Body weight, kg	71 ± 8	121 ± 28*	121 ± 23*	<0.001	59 ± 6	102 ± 19*	105 ± 19*	<0.001
BMI, kg/m^2	23.3 ± 1.6	39.4 ± 9.2*	40.2 ± 7.7*	<0.001	22.7 ± 1.7	39.7 ± 7.0*	41.2 ± 7.4*†	<0.001
BF%	21.4 ± 7.5	41.2 ± 8.0*	42.1 ± 7.0*	<0.001	33.3 ± 5.9	52.3 ± 6.1*	53.5 ± 5.3*	<0.001
Waist circumference, cm	87 ± 6	124 ± 18*	126 ± 16*	<0.001	79 ± 7	113 ± 13*	119 ± 14*†	<0.001
WHR	0.92 ± 0.05	1.02 ± 0.07*	1.04 ± 0.07*	<0.001	0.83 ± 0.07	0.90 ± 0.07*	0.95 ± 0.07*†	<0.001
SBP, mmHg	117 ± 14	123 ± 13*	127 ± 12*	<0.001	108 ± 15	119 ± 13*	126 ± 14*†	<0.001
DBP, mmHg	72 ± 8	78 ± 10*	81 ± 9*	<0.001	68 ± 8	75 ± 7*	78 ± 8*†	<0.001
Glucose, mg/dL	98 ± 18	94 ± 10	113 ± 29*†	<0.001	88 ± 8	92 ± 10	104 ± 21*†	<0.001
2-h OGTT glucose, mg/dL	—	129 ± 35	153 ± 54	0.007	—	129 ± 36	148 ± 48	0.001
Insulin, μU/mL	6.5 ± 4.3	14.6 ± 8.8*	21.5 ± 17.6*†	<0.001	5.1 ± 3.0	10.6 ± 9.3*	15.9 ± 13.3*†	<0.001
2-h OGTT insulin, μU/mL	—	115 ± 64	127 ± 71	0.380	—	88 ± 59	118 ± 64	0.001
HOMA	1.7 ± 1.4	3.4 ± 2.2	6.2 ± 6.0*†	<0.001	1.1 ± 0.7	2.4 ± 2.2*	4.1 ± 3.6*†	<0.001
QUICKI	0.37 ± 0.05	0.33 ± 0.03*	0.31 ± 0.03*†	<0.001	0.39 ± 0.04	0.35 ± 0.04*	0.33 ± 0.04*†	<0.001
Triglycerides, mg/dL	94 ± 51	110 ± 37	152 ± 133*†	<0.001	73 ± 27	92 ± 31*	121 ± 53*†	<0.001
Total cholesterol, mg/dL	182 ± 38	199 ± 41*	193 ± 43	0.024	197 ± 40	197 ± 38	198 ± 35	0.970
LDL cholesterol, mg/dL	108 ± 33	127 ± 37*	115 ± 37	0.004	112 ± 36	117 ± 32	120 ± 32	0.069
HDL cholesterol, mg/dL	55 ± 14	51 ± 11*	48 ± 8*	<0.001	71 ± 17	61 ± 14*	53 ± 13*†	<0.001
Uric acid, mg/dL	5.5 ± 1.2	6.4 ± 1.2*	6.6 ± 1.3*	<0.001	3.8 ± 0.9	4.8 ± 1.1*	5.4 ± 1.2*†	<0.001
Homocysteine, μmol/L	10.9 ± 5.6	10.6 ± 3.1	11.1 ± 4.1	0.729	7.1 ± 1.9	8.6 ± 3.0	9.4 ± 3.9*	0.002
von Willebrand factor, %	115 ± 41	124 ± 50	135 ± 54	0.396	109 ± 34	125 ± 60	144 ± 60*†	0.005
CRP, mg/L	1.6 ± 1.5	6.0 ± 8.6*	6.4 ± 7.5*	<0.001	1.0 ± 1.0	8.7 ± 10.0*	8.8 ± 9.8*	<0.001
Fibrinogen, mg/dL	299 ± 44	351 ± 85	369 ± 78	0.049	319 ± 68	382 ± 85*	383 ± 716*	<0.001
White blood cell, 10^6 cells/mL	5.9 ± 1.5	6.7 ± 1.9*	7.3 ± 1.7*	<0.001	5.7 ± 1.6	6.7 ± 1.8*	7.5 ± 4.6*	<0.001
ALT, units/L	19 ± 26	31 ± 17*	33 ± 16*	<0.001	15 ± 11	17 ± 21	20 ± 9*	0.019
AST, units/L	14 ± 4	18 ± 7*	19 ± 7*	<0.001	15 ± 9	13 ± 6	14 ± 5	0.136
Alkaline phosphatase, units/L	61 ± 28	76 ± 37*	73 ± 30*	0.007	69 ± 34	85 ± 39*	88 ± 34*	<0.001
γ-GT, units/L	20 ± 16	38 ± 48*	46 ± 50*	<0.001	13 ± 10	19 ± 32*	22 ± 17*	0.002
Creatinine, mg/dL	0.94 ± 0.15	0.92 ± 0.15	0.91 ± 0.14	0.401	0.75 ± 0.13	0.73 ± 0.12	0.76 ± 0.14	0.209
Leptin, ng/mL	4.4 ± 2.5	30.1 ± 21.4*	28.1 ± 15.1*	<0.001	12.8 ± 9.0	51.5 ± 25.9*	57.2 ± 25.1*	<0.001

Data are mean ± SD unless otherwise indicated. Differences between groups were analyzed by one-way ANOVA followed by Tukey post hoc tests or Student *t* tests as appropriate. CRP concentrations were logarithmically transformed for statistical analysis. *$P < 0.05$ versus lean. †$P < 0.05$ versus MHO.

MHO and 51.0, 25.5, and 23.5% of MAO were classified as having NG, IGT, and T2D, respectively ($P = 0.017$). In women, 68.4, 24.5, and 7.1% of MHO and 45.5, 44.6, and 9.9% of MAO were classified as having NG, IGT, and T2D, respectively ($P = 0.005$). Our data identify that 32.2 and 31.6% of male and female MHO patients presented an altered glucose homeostasis, while this was the case for 49.0 and 54.5% of male and female MAO subjects (Fig. 1).

Altered Adipokine Profile in MHO

We explored a second cohort (cohort 2) of obese patients undergoing bariatric surgery in whom the cardiometabolic as well as the adipokine profile were analyzed using a group of lean patients as reference. In this case, the groups were not matched by age, with patients included in the MAO group being significantly older than those of the MHO group (Table 2). No differences in sex distribution were found. BMI was significantly higher in the MAO group, while BF%, waist circumference, and WHR were similarly increased in both obese groups. As expected, since they were entailed in the classification criteria, blood pressure and markers of glucose and lipid metabolism were increased in the MAO group. Uric acid, proinflammatory factors, and ALT concentrations were similarly increased in both groups, confirming the results obtained in cohort 1 (Table 2 and Supplementary Fig. 2).

The adipokine profile of both obese groups was almost superimposable. Adiponectin concentrations were significantly reduced in the MHO and slightly further reduced in the MAO group, exhibiting no differences between them (Fig. 2*A*). Resistin levels were marginally increased in both obese groups, but the rise did not reach statistical significance (Fig. 2*B*). Leptin concentrations were dramatically increased in both obese groups, showing no differences between them (Table 2 and Fig. 2*C*), confirming the findings of cohort 1. The acute-phase reactant SAA was greatly overproduced in the MHO group, being further increased in the MAO group but without exhibiting statistically significant differences between both obese groups (Fig. 2*D*). Although the circulating concentrations of the angiogenic adipokine VEGF were 40% increased in the MHO group in comparison with the lean group, the differences did not reach

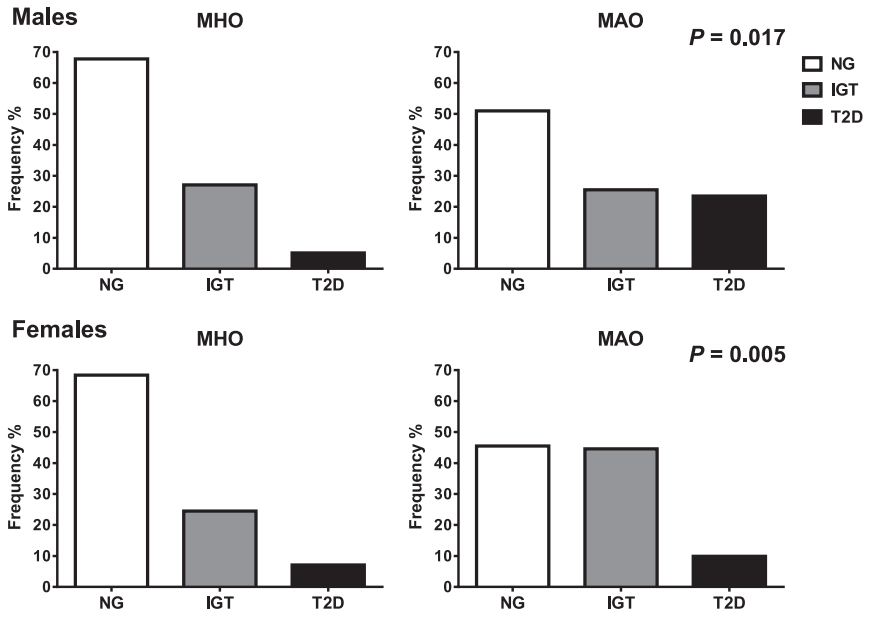

Figure 1—Frequency distribution of individuals with NG, IGT, and T2D in MHO (left) and MAO (right) patients segregated by sex (males top, females bottom). Differences in the prevalence of NG, IGT, and T2D in MHO and MAO groups in males and females were analyzed by χ^2 analysis.

statistical significance. Levels of VEGF were significantly increased in the MAO group compared with the lean group but not the MHO group (Fig. 2*E*). Circulating concentrations of MMP9, involved in the matrix remodeling that takes place during adipose tissue expansion, were dramatically increased in both obese groups, being even slightly higher in the MHO group (Fig. 2*F*).

Gene Expression of Inflammatory and Matrix Remodeling Genes Is Similarly Increased in Adipose Tissue of MHO and MAO Groups

To explore whether changes in inflammation and matrix remodeling in adipose tissue and liver underlie the apparently benign metabolic status of MHO as opposed to MAO patients, gene expression of major genes involved in these processes was analyzed. The mRNA expression levels of secreted phosphoprotein 1 (*SPP1*), also known as osteopontin (*OPN*), tumor necrosis factor (*TNF*), and Toll-like receptor 4 (*TLR4*) were similarly increased, while the levels of the anti-inflammatory adipokine secreted frizzled-related protein 5 (*SFRP5*) were similarly decreased in both obese groups in comparison with lean individuals (Fig. 2*G*). Analogously, the expression levels of tenascin C (*TNC*) and *MMP9*, two genes involved in matrix remodeling, were likewise upregulated in both obese groups, as compared with the lean individuals (Fig. 2*G*). No significant changes in the hepatic expression of *TNF*, *SFRP5*, and *TNC* between MHO and MAO patients were observed (Fig. 2*H*).

CONCLUSIONS

In the current study, we provide evidence that one-third of obese patients considered to be metabolically healthy following the commonly accepted definition exhibit impaired glucose intolerance or even diabetes. Moreover, most of the cardiometabolic risk factors and circulating adipokines analyzed, as well as the expression of genes in VAT and liver, were similarly modified in the MHO subjects as compared with the MAO patients. Our data reinforce the notion that the clinical concept of the MHO patient using the current definition should be used with caution and show, for the first time, that the apparently benign metabolic profile of MHO does not withstand a more functional and in-depth analysis.

Most studies define MHO by applying FPG concentrations as a marker of insulin resistance (4,5). However, since the OGTT better reflects the functional condition, providing additional prognostic information and enabling the detection of individuals with IGT who exhibit an increased risk for future adverse cardiovascular events and death (22), we aimed to determine the actual prevalence of IGT and T2D using an OGTT challenge in MHO classified according to FPG. Messier et al. (23) found significant differences in the identification of MHO subjects, applying diverse insulin resistance assessment methods. However, they focused on the common characteristics of MHO more than on the impact of the different method of classification, and in addition, when insulin sensitivity was assessed, it was used as the method of classification (23). In the current study, we found that more than 30% of obese subjects classified as MHO according to the FPG exhibited either IGT or T2D. In agreement with our findings, it has been shown that MHO in individuals is less common than previously thought when the presence of IGT or T2D is actually assessed according to the OGTT (24). Thus, our findings provide evidence that one out of three obese patients considered to be metabolically healthy are actually at high risk of developing T2D or have already done so.

We found no significant differences in adiponectinemia between MHO and MAO groups. Some authors have found higher adiponectin concentrations in MHO as compared with MAO, which has been related to reduced inflammation and improved metabolic function (25,26). The similarly reduced levels observed in the current study have been reported previously (7,27,28) and together with the comparable concentrations of leptin and resistin suggest that

Table 2—Anthropometric and metabolic characteristics of subjects included in cohort 2

	Lean	MHO	MAO	*P* value
n	27	24	31	
Sex, male/female	6/21	3/21	7/24	0.588
Age, years	40.1 ± 15.6	34.7 ± 11.6	47.5 ± 12.1†	0.002
Body weight, kg	60 ± 13	114 ± 15*	122 ± 21*	<0.001
BMI, kg/m^2	21.3 ± 2.8	42.0 ± 4.1*	45.7 ± 7.1*†	<0.001
BF%	25.3 ± 5.8	52.0 ± 4.3*	53.9 ± 5.8*	<0.001
Waist circumference, cm	71 ± 9	118 ± 12*	126 ± 13*	<0.001
WHR	0.77 ± 0.07	0.92 ± 0.10*	0.94 ± 0.08*	<0.001
SBP, mmHg	103 ± 7	118 ± 14*	134 ± 15*†	<0.001
DBP, mmHg	64 ± 6	75 ± 8*	83 ± 8*†	<0.001
Glucose, mg/dL	88 ± 15	88 ± 11	112 ± 27*†	<0.001
Insulin, μU/mL	6.8 ± 2.9	16.4 ± 11.8	21.0 ± 16.9*	0.013
HOMA	1.5 ± 0.8	3.7 ± 2.8	5.4 ± 4.0*	0.003
QUICKI	0.37 ± 0.04	0.33 ± 0.04*	0.31 ± 0.02*†	<0.001
Triglycerides, mg/dL	67 ± 24	89 ± 38	132 ± 69*†	<0.001
Total cholesterol, mg/dL	181 ± 30	180 ± 42	194 ± 34	0.329
LDL cholesterol, mg/dL	103 ± 25	110 ± 36	119 ± 28	0.260
HDL cholesterol, mg/dL	64 ± 12	53 ± 12	49 ± 16*	0.017
Uric acid, mg/dL	4.2 ± 0.7	5.6 ± 1.3*	5.6 ± 1.1*	<0.001
Homocysteine, μmol/L	6.8 ± 1.5	8.6 ± 2.1	9.1 ± 3.3*	0.043
von Willebrand factor, %	56 ± 25	122 ± 48*	136 ± 59*	<0.001
CRP, mg/L	1.0 ± 0.8	11.1 ± 8.3*	7.9 ± 5.5*	<0.001
Fibrinogen, mg/dL	215 ± 69	400 ± 75*	358 ± 84*	<0.001
ALT, units/L	7 ± 3	21 ± 9*	22 ± 10*	<0.001
AST, units/L	13 ± 4	15 ± 5	16 ± 14	0.532
Alkaline phosphatase, units/L	93 ± 30	90 ± 29	100 ± 29	0.517
γ-GT, units/L	11 ± 6	18 ± 11	25 ± 17*	0.012
Creatinine, mg/dL	0.80 ± 0.07	0.79 ± 0.16	0.77 ± 0.12	0.767
Leptin, ng/mL	8.1 ± 4.6	55.4 ± 17.9*	58.0 ± 28.4*	<0.001

Data are presented as mean ± SD. Differences between groups were analyzed by ANOVA followed by Tukey post hoc tests. Differences in sex distribution were analyzed by χ^2 analysis. *$P < 0.05$ versus lean. †$P < 0.05$ versus MHO.

MHO and MAO show a very similarly altered adipokine profile. To our knowledge, this is the only study analyzing the circulating concentrations of SAA, VEGF, and MMP9 in MHO subjects. The acute-phase reactant SAA has been related to adipose tissue and systemic inflammation and could be a link between obesity and metabolic disease (29). On the other hand, MMP9 is a matrix remodeling protein involved in the expansion of adipose tissue that takes place in obesity (30). Our data show that SAA and MMP9 are similarly increased in MHO and MAO groups, suggesting that they are related to obesity and that both obese phenotypes are not so metabolically different. Levels of the angiogenic factor VEGF were significantly increased in the MAO group, showing no differences with the MHO group. Although it is possible that angiogenesis plays a role in the metabolic differences between MHO and MAO, our data do not support this notion.

Inflammation in adipose tissue has been proposed as a key factor explaining the metabolic alterations associated with obesity (31,32). In the current study, we found similarly changed expression of several genes involved in the inflammatory response such us *OPN*, *TNF*, *TLR4*, and *SFRP5*, pointing to a comparable inflammatory response in the VAT of patients from the MHO and MAO groups. In the study of Barbarroja et al. (31), the expression of *TNF* was very close to that of the current study, but they found increased expression of other genes such as *IL-1β* and *IL-6* in patients from the MAO group. A recent study also showed increased activation of the NLRP3 inflammasome in VAT from MAO patients (32). In agreement with our findings, no differences in the expression of inflammatory genes in peripheral blood mononuclear cells between MHO and MAO groups were found in a recent study (33). In the same line, we found that *TNC* and *MMP9*, genes involved in matrix remodeling (21,30), were similarly upregulated in both obese groups, suggesting that VAT remodeling, as suggested by the expression of genes involved in this process, is analogously contributing to adipose expansion in both obesity phenotypes. To analyze the potential contribution of subcutaneous adipose tissue and, in particular, deep abdominal subcutaneous adipose tissue will be of interest in future studies. Finally, we analyzed the expression of *TNF*, *SFRP5*, and *TNC* in the liver from MHO and MAO obese groups, finding no differences in their levels. To our knowledge, no previous comparable studies have been performed analyzing the hepatic expression of inflammatory genes in MHO. Our gene expression data suggest that VAT and liver from MHO and MAO patients exhibit a similar profile, thereby highlighting that individuals coined as MHO exhibit comparable dysfunctional characteristics that contrast with the term *healthy*.

Data from the current study show that circulating concentrations of proinflammatory factors are similarly increased in the MHO and the MAO groups. No differences in CRP levels were found between the MHO and MAO groups in the two cohorts analyzed, even though subjects included in the MHO group of cohort 2 were younger than those from the MAO group. Furthermore, fibrinogen concentrations in both cohorts and the leukocyte number, analyzed only in cohort 1, were elevated in both obese groups. Our results regarding inflammation are similar to those reported in previous studies (12,13,26,27,32,34) but contrast with others (8,24,26,35,36). The younger age of the MHO subjects in most of the latter studies may explain this discrepancy.

Uric acid concentrations, which have been also proposed as a potential differential factor in MHO subjects (12), were similarly increased in the MHO and MAO groups from both cohorts, although

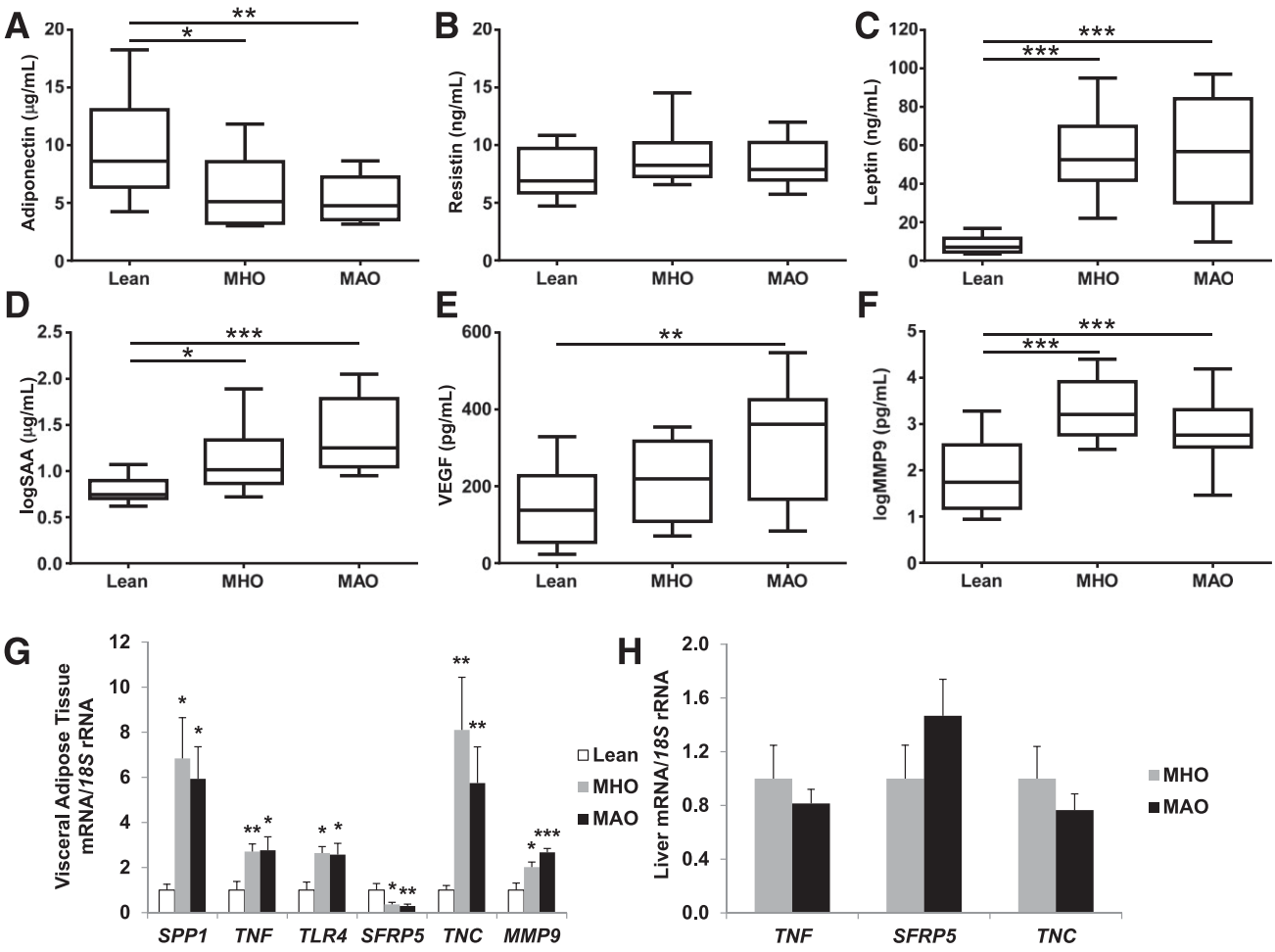

Figure 2—*A–F*: Adipokine concentrations of lean, MHO, and MAO subjects from cohort 2. Box represents interquartile range and median inside, with whiskers showing from minimum to maximum. Differences between groups were analyzed by one-way ANOVA followed by Tukey post hoc tests or Kruskal-Wallis followed by Mann-Whitney *U* as appropriate. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Comparison of mRNA expression levels of genes of interest among lean, MHO, and MAO subjects in VAT (*G*) or among MHO and MAO subjects in liver (*H*) from cohort 2. Bars represent the mean ± SEM of the ratio to *18S* rRNA. The expression in the lean (VAT) or MHO (liver) group was assumed to be 1. Differences between groups were analyzed by one-way ANOVA followed by Tukey post hoc tests or Kruskal-Wallis followed by Mann-Whitney *U* (VAT) or Student *t* tests (liver). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 versus lean.

they were further elevated in the MAO group of cohort 1. These data are similar to those recently obtained by Peppa et al. (37). Since uric acid is positively associated with the development of T2D (38), our data further support the notion that the cardiometabolic risk of MHO subjects is less benign than previously thought.

Increased hepatic enzymes have also been proposed as metabolic factors explaining the differences between MHO and MAO patients (13). In the current study, we consistently found that circulating concentrations of ALT and γ-GT were similarly increased in both obese groups without statistical differences between them. Our findings agree with previously published studies (25,27,28) but contrast with others (12,13,32). Other authors find similarly increased ALT levels in MHO and MAO groups, with γ-GT concentrations being further increased in the MAO group (37). Importantly, it has been reported that these hepatic enzymes are associated with insulin resistance and nonalcoholic fatty liver disease, leading to an increased risk for T2D development (39). Our data suggest a similar liver fat content and hepatic insulin resistance in the MHO and MAO groups.

There are several limitations to the current study. First, given its cross-sectional nature, it is not possible to determine a causal relationship among circulating cardiometabolic factors and the development of cardiovascular events or the incidence of T2D. Second, because the study included only Caucasian subjects, the results may not be extrapolated to other ethnic populations.

Whether MHO patients present a harmless metabolic profile is a matter of debate (40). Some authors consider the appearance of metabolic abnormalities and other comorbidities associated with obesity to be only a matter of time; i.e., it is merely a question of evolution of the disease as evidenced by studies showing that MHO subjects exhibit increased risk of developing diabetes, hypertension, or metabolic syndrome in the long term (41,42). In this sense, a growing number of studies have questioned the apparently healthy metabolic condition of MHO, showing that these obese patients have increased morbidity and mortality as compared

with lean or overweight subjects (15–17,43–45). Therefore, it is mandatory to clearly and more precisely define stratification of obese individuals according to the actual as well as future risk and perform better and more functional diagnosis for the adequate management of the obese patients. In this sense, the analysis of the potential contribution of adipose tissue expandability determining the cardiometabolic risk of the obese patient may be of major interest. Our data clearly show that MHO patients need to be classified properly and that further research on a more accurate definition of MHO, getting insight into the broad spectrum of possibilities expanding from metabolic health to alteration and their actual translation into increased risk, represents a potential future area of investigation.

In summary, the current study provides evidence for the existence of a similarly adverse cardiometabolic profile in MHO and MAO patients. Moreover, one-third of the obese subjects classified as MHO according to the FPG exhibit IGT or T2D. Finally, expression of genes involved in inflammation and tissue remodeling in VAT and liver show comparable changes in MHO and MAO individuals. A better definition of the obesity subphenotypes and a precise diagnosis that more accurately identifies the actual metabolic state together with the function and expansion capacity of adipose tissue, without incurring the contradiction of applying the term *healthy* when actually metabolic derangements are already present both at the circulating and tissue level, are needed to improve the management of obese patients.

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Author Contributions. J.G.-A. designed the study, collected and analyzed data, wrote the first draft of the manuscript, contributed to discussion, and reviewed the manuscript. V.C. and A.R. collected and analyzed data, contributed to discussion, and reviewed the manuscript. P.A., R.M., V.V., C.S., and J.S. enrolled patients, collected data, contributed to discussion, and reviewed the manuscript. B.R., P.I., N.V., S.R., M.A.M., and M.J.G. collected data, contributed to discussion, and reviewed the manuscript. G.F. designed the study, enrolled patients, collected and analyzed data, wrote the first draft of the manuscript, contributed to discussion, and reviewed the manuscript. J.G.-A. and G.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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