



Universidad Autónoma  
de Madrid

**Biblos-e Archivo**  
Repositorio Institucional UAM

**Repositorio Institucional de la Universidad Autónoma de Madrid**

<https://repositorio.uam.es>

Esta es la **versión de autor** del artículo publicado en:  
This is an **author produced version** of a paper published in:

GeroScience (2021): 07 May

**DOI:** <https://doi.org/10.1007/s11357-021-00370-w>

**Copyright:** © American Aging Association 2021

El acceso a la versión del editor puede requerir la suscripción del recurso  
Access to the published version may require subscription

## **TITLE PAGE**

**Title:** Metabolic syndrome and Growth Differentiation Factor 15 in older adults

### **Authors' names and affiliations:**

Adrián Carballo-Casla,<sup>1,2</sup> Esther García-Esquinas,<sup>1,2</sup> Antonio Buño-Soto,<sup>3</sup> Ellen A. Struijk,<sup>1,2</sup>

Esther López-García,<sup>1,2,4</sup> Fernando Rodríguez-Artalejo,<sup>1,2,4</sup> Rosario Ortolá<sup>1,2</sup>

<sup>1</sup> Department of Preventive Medicine and Public Health, Universidad Autónoma de Madrid/Idipaz. Calle del Arzobispo Morcillo 4, 28029 Madrid, Spain.

<sup>2</sup> CIBER of Epidemiology and Public Health (CIBERESP), Avenida de Monforte de Lemos 3-5, 28029 Madrid, Spain.

<sup>3</sup> Department of Laboratory Medicine, La Paz University Hospital-IdiPaz, Paseo de la Castellana 261, 28046 Madrid, Spain.

<sup>4</sup> IMDEA Food Institute, CEI UAM+CSIC. Carretera de Canto Blanco 8, 28049 Madrid, Spain.

### **Corresponding authors:**

Adrián Carballo-Casla, MSc, or Rosario Ortolá, MD

Department of Preventive Medicine and Public Health. School of Medicine, Universidad Autónoma de Madrid. Calle del Arzobispo Morcillo 4, 28029 Madrid, SPAIN.

Telephone: (+34) 914975441; Fax: (+34) 914975353

E-mail: [adrian.carballo@uam.es](mailto:adrian.carballo@uam.es), or [ortolarosario@gmail.com](mailto:ortolarosario@gmail.com)

## ABSTRACT

**Background:** Growth Differentiation Factor 15 (GDF-15) is a cytokine produced in response to tissue injury and inflammatory states that may capture distinct pathways between the risk factors aggregated within metabolic syndrome (MS) and the development of diabetes and cardiovascular disease.

**Objective:** To study the association of MS and its components with GDF-15 among older adults, examining the roles of body fat distribution, glucose metabolism, and inflammation.

**Methods:** Data were taken from the Seniors-ENRICA-2 study in Spain, which included 1938 non-institutionalized individuals aged  $\geq 65$  years free of diabetes and cardiovascular disease. MS was defined as the presence of  $\geq 3$  of the following components: high waist circumference, elevated fasting blood glucose levels, raised blood pressure, increased triglyceride levels; and low serum high-density lipoprotein (HDL) cholesterol. Statistical analyses were performed with linear regression models and adjusted for potential sociodemographic and lifestyle confounders.

**Results:** MS was associated with higher GDF-15 levels (fully adjusted mean increase [95% confidence interval] = 9.34% [5.16,13.7]). The MS components showing the strongest associations were high waist circumference (6.74% [2.97,10.6]), elevated glucose levels (4.91% [0.77,9.23]), and low HDL-cholesterol (8.13% [3.51,13.0]). High waist-to-hip ratio (7.07% [2.63,11.7]), urine albumin (12.1% [2.57,22.5]), and C-reactive protein (10.4% [3.89,17.3]) were also associated with increased GDF-15.

**Conclusion:** MS was associated with higher GDF-15 levels in older adults. Abdominal obesity, hyperglycemia -possibly linked to microvascular disease, as inferred from elevated urine albumin-, low HDL-cholesterol, and inflammation were the main drivers of this association.

## INTRODUCTION

Metabolic syndrome (MS) is a cluster of five cardiometabolic risk factors comprising abdominal obesity and abnormal blood pressure, blood glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol. MS has been advocated as a simple clinical tool for predicting type 2 diabetes and cardiovascular disease, as it increases the risk for the former fivefold and doubles the risk for the latter [1–3]. Several pathophysiological links between the metabolic risk factors aggregated within MS and the development of diabetes and cardiovascular disease have been proposed [2–4]. First, insulin resistance is the primary cause of hyperglycemia, whose most relevant clinical outcome is microvascular disease, manifested as neuropathy and nephropathy. Insulin resistance may also be linked with hypertension through increased renal reabsorption of sodium leading to an expansion of intravascular volume, further accelerating the development of heart failure and atherosclerosis. Second, high levels of triglycerides and reduced HDL cholesterol, jointly with elevated low-density lipoproteins, seem to be the main cause of atherosclerotic cardiovascular disease. Finally, adipose tissue is associated with: 1) insulin resistance via the supply of ectopic fat to muscle and pancreas; 2) hypertension through activation of the renin-angiotensin-aldosterone and sympathetic nervous systems; and 3) insulin resistance, hypertension, and atherosclerosis via the production of inflammatory adipokines and other bioactive peptides [2–5].

In this regard, Growth Differentiation Factor 15 (GDF-15) is a cytokine produced in response to tissue injury and inflammatory states by cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells, where it plays a tissue-protective role through up- and downregulation of several signaling pathways [6,7]. Higher serum GDF-15 concentrations may be a clinically relevant biomarker within the context of MS, as they have been associated with several MS components [6,8,9] and with the development and progression of cardiovascular and

diabetes-related conditions, specifically cardiac hypertrophy, heart failure, atherosclerosis, endothelial dysfunction, insulin resistance, diabetes, and chronic kidney disease, as well as with cardiovascular and all-cause mortality [6,7]. Nevertheless, little is known about the association of MS with GDF-15 in older adults and its main drivers [8,10], and it is uncertain if they are related to alterations in glucose metabolism, inflammation, or both. Moreover, these assessments might render different results than in younger populations [8,11], as both MS prevalence and GDF-15 levels are consistently higher in the elderly than in younger subjects [7,12–14]. Furthermore, despite being an adipokine, it is unclear whether GDF-15 is associated with abdominal fat -that clustered within MS-, gluteofemoral fat, or overall adiposity [7,8].

We hence aimed to 1) assess the association of MS and its components with GDF-15 in community-dwelling older adults and 2) delve into these relationships by examining the associations of ancillary adiposity measures (body mass index, hip circumference, and waist-to-hip ratio) and auxiliary metabolic biomarkers (Homeostatic Model Assessment for Insulin Resistance [HOMA-IR], glycated hemoglobin, urine albumin, and high-sensitivity C-reactive protein [hs-CRP]) with GDF-15.

## **METHODS**

### **Study design, setting, and participants**

Our data came from the baseline wave of the Seniors-ENRICA-2, a cohort study on cardiovascular health, nutrition, and physical functioning in older adults in Spain (ClinicalTrials.gov Identifier: NCT01133093) [15,16]. Subjects were recruited between December 2015 and June 2017 by sex- and district-stratified random sampling of the community-

dwelling, 65-years and older national healthcare cardholders living in the city of Madrid (Spain) or four surrounding large towns: Getafe, Torrejón, Alcorcón, and Alcalá de Henares.

The study methods were analogous to those of the Seniors-ENRICA-1 cohort, which have been detailed elsewhere [17]. In brief, a comprehensive set of physical examinations, blood, and urine tests were collected during two home visits by trained personnel, whereas data on socio-demographic, lifestyle, and morbidity variables were gathered through a telephone interview [17]. All subjects gave written informed consent, and the Clinical Research Ethics Committee of the “La Paz” University Hospital in Madrid approved the research protocol.

### **Variables, data sources, and measurements**

#### GDF-15

Fasting blood samples were obtained from every subject at the first home visit in rapid serum tubes with a thrombin-based clot activator and polymer gel (Becton Dickinson). Tubes were centrifuged at 3000 rpm for 10 minutes and serum was aliquoted and stored at -80°C in the Department of Preventive Medicine and Public Health at Universidad Autónoma de Madrid. Serum GDF-15 was quantified at the Department of Laboratory Medicine of “La Paz” University Hospital by an electrochemiluminescence Elecsys® immunoassay method using a cobas® 6000 analyzer (Roche Diagnostics). The inter-assay coefficient of variation was 5.4% for a mean concentration of 7343 pg/mL and 7.7% for 1428 pg/mL.

#### Metabolic syndrome

Waist circumference was measured by trained staff with a flexible, inelastic, belt-type tape at the midpoint between the lowermost rib and the iliac crest at the end of a normal expiration [18].

Blood pressure was determined three times under standardized conditions with validated automatic sphygmomanometers (Mobil-O-Graph® 24h PWA, I.E.M., Stolberg, Germany), and the mean of the 2<sup>nd</sup> and 3<sup>rd</sup> assessments was used in the analyses [19]. 12-hour fasting serum glucose, triglycerides, and HDL-cholesterol were measured with colorimetric enzymatic methods using Atellica® solution (Siemens Healthineers).

MS was defined as the presence of  $\geq 3$  of the following 5 components: a waist circumference  $\geq 102$  cm in men and  $\geq 88$  cm in women; fasting blood glucose  $\geq 100$  mg/dl or drug treatment of hyperglycemia; a systolic blood pressure  $\geq 130$ , a diastolic blood pressure  $\geq 85$  mm Hg, or being on antihypertensive drug treatment; serum triglycerides  $\geq 150$  mg/dl; and serum HDL-cholesterol  $< 40$  mg/dl in men or  $< 50$  mg/dl in women [1]. Since it has been argued that MS is a pre-morbid condition rather than a clinical diagnosis [2], we excluded individuals with self-reported cardiovascular disease (myocardial infarction, stroke, or heart failure) or established diabetes (blood glucose  $\geq 126$  mg/dL, HbA1c  $\geq 6.5$  %, being treated with antidiabetic drugs, or self-reported diagnosis of diabetes mellitus).

#### Ancillary adiposity measures

To further investigate the association between body fat and GDF-15, we used data on three additional adiposity measures. First, body mass index (BMI) was calculated as weight (in kg) divided by squared height (in m). Weight and height measurements were conducted under standardized conditions using electronic scales and portable extendable stadiometers, respectively [18]. Normal weight was considered as BMI  $< 25$ , overweight as BMI 25–29.9, and obesity as BMI  $\geq 30$  kg/m<sup>2</sup>. Hip circumference was measured by trained staff with a flexible, non-distensible, belt-type tape on the maximum circumference over the femoral trochanters; for

analyses, hip circumference was divided into sex-specific tertiles (cutoffs at 98 and 104 cm in men, and 99.6 and 107 cm in women). The waist-to-hip ratio was computed as waist circumference (in cm) divided by hip circumference (in cm). Cutoff values of  $\geq 0.90$  in men and  $\geq 0.85$  in women were used, for they have been associated with a substantially increased risk of metabolic complications [20].

#### Ancillary metabolic biomarkers

To explore in more depth the relationship between glucose metabolism and GDF-15 levels, we first measured 12-h fasting serum insulin through a chemiluminescent immunoassay using Atellica® solution (Siemens Healthineers) and calculated the HOMA-IR as blood glucose (in mg/dl) multiplied by serum insulin (in mU/l) and further divided by 405 [21]. We used cutoff values of  $\geq 2.25$  in men and  $\geq 2.38$  in women, as they have demonstrated a 70% specificity in MS classification in older adults [22]. Second, we determined glycated hemoglobin (HbA<sub>1c</sub>) using high-performance liquid chromatography with Arkray Adams™ A1c HA-8180 (Menarini). The threshold level for prediabetes was set at  $\geq 5.7$  % [23]. We finally measured albumin excretion in spot urine, as an early predictor of progressive renal function loss in prediabetes and diabetes, through the immunoturbidimetry technique using Atellica® solution (Siemens Healthineers). Urinary albumin excretions  $<10$ , 10-20, and  $\geq 20$  mg/l were considered normal, high normal, and microalbuminuria, respectively [24].

As a marker of inflammation, we also determined hs-CRP levels using the abovementioned immunoturbidimetry technique. Cutoff points were set at 1.0 mg/l and 3.0 mg/dl, according to relative risk categories of cardiovascular disease [25].

#### Potential confounders



We used data on several self-reported potential confounders of the association between MS and GDF-15, specifically sex, age, educational level (primary or less, secondary, or university), smoking status (never, former, or current), and alcohol consumption (never, former, moderate [ $\leq 10$  g/day in women and  $\leq 20$  g/day in men], or heavy). Physical activity time (min/day) was assessed with an ActiGraph GT9X (ActiGraph Inc) accelerometer. Intensity thresholds were set at  $<45$  miligravitational units (mg) for sedentary behavior,  $\geq 45$  and  $<100$  mg for light physical activity, and  $\geq 100$  mg for moderate-to-vigorous physical activity [16]. When not available, we used self-reported data on sedentary behavior and physical activity instead [26,27]. Dietary information, including energy intake (kcal/day), was obtained from a validated diet history [28]. Diet quality was assessed with the Mediterranean Diet Adherence Screener (MEDAS), with higher scores indicating better adherence to the Mediterranean diet [29].

## **Statistical Methods**

### Study size

From the 3273 individuals recruited in the study (51% of those invited), we excluded 758 with inadequate data (719 subjects had incomplete information on MS or ancillary adiposity measures, 684 on GDF-15, and 488 on potential confounders; note that one individual may lack data in more than one variable). We also excluded 577 individuals with established diabetes or known cardiovascular disease. Hence, the main analytical sample comprised 1938 individuals. For the analyses regarding ancillary metabolic biomarkers, we excluded another 1085 participants without data on HOMA-IR, glycated hemoglobin, urine albumin, or hs-CRP. Thus, this secondary analytical sample included 853 subjects (Supplemental Figure 1).

### Statistical methods

Differences in characteristics of study participants across the categories of MS and its components were evaluated with Pearson's chi-squared tests for discrete variables and Wilcoxon rank-sum tests for continuous variables.

Main analyses were conducted with linear regression models where the outcome was log-transformed GDF-15, as a continuous variable, and exposures were MS or its components, as dichotomous variables. We calculated GDF-15 mean percentage differences and their 95% confidence interval (CI) between the subjects with and without MS and each of its components. This was done by taking 1 from the exponentiated  $\beta$  coefficients in the regression models and multiplying the result by 100. Dose-response relationships were assessed by restricted cubic spline regression. To control for potential confounding, two *a priori* hierarchical models were used: 1) adjusted for sociodemographic characteristics (age, sex, and educational level), and 2) additionally adjusted for lifestyle variables (tobacco smoking, alcohol consumption, diet quality, energy intake, light physical activity, moderate-to-vigorous physical activity, and sedentary behavior).

The associations of ancillary adiposity measures and metabolic biomarkers with GDF-15 were examined alike, except that we used tests for trend to check for dose-response relationships instead, modeling the median values per category as a single continuous variable.

Statistical significance was set at a two-sided p-value <0.05. Analyses were performed with Stata® (StataCorp LLC), version 14.

#### Missing data, interactions, and sensitivity analyses

Firstly, to investigate how incomplete data may have affected our findings, we compared the characteristics between participants who were and were not included in the analyses because of missing values on any variable of interest. Secondly, we tested whether the association of MS with GDF-15 differed in men and women, or subjects  $\leq 70$  and  $>70$  years, as both MS prevalence and GDF-15 levels appear to steadily increase with age [7,12–14]. To do so, we used Wald tests that compared models with and without interaction terms, defined as the product of sex or the age subgroup by the dichotomous MS variable. Since no statistically significant interactions were found, results are presented for the total sample. Finally, to provide further insight on the dose-response relationship between MS and GDF-15 at higher levels of its components -particularly fasting glucose-, we replicated the analyses without excluding individuals with established diabetes or known cardiovascular disease.

## **RESULTS**

### **Descriptive data**

The prevalence of MS [95% CI] was 26.9% [25.0,28.9]. Subjects with MS were more likely to be women, slightly older, more sedentary, did less light and moderate-to-vigorous physical activity, and had a higher energy intake. Table 1 also shows a detailed distribution of each MS component by sociodemographic and lifestyle variables.

Compared to participants included in the analyses, those with incomplete data had higher GDF-15 levels (1389 vs 1124 pg/ml). They also were more likely to have MS (41.7 vs 26.9%), older (72.8 vs 71.4 years), less educated (68.1% vs 62.2% had primary or lower studies), more sedentary (1066 vs 783 min/day), did less moderate-to-vigorous physical activity (31.4 vs 61.2 min/day), and had lower diet quality (6.91 vs 7.13 MEDAS scores).

## Main results

The geometric GDF-15 means [95% CI] were 1227 [1183,1272] and 1088 pg/ml [1065,1111] for participants with and without MS, respectively (mean percentage difference = 12.7% [8.17,17.5]) (Table 1, Table 2). This association remained when adjusting for sociodemographic (model 1 = 11.4% [7.12,15.8]) and lifestyle variables (model 2 = 9.34% [5.16,13.7]), and it was dose-dependent (Table 2, Figure 1). The components of MS that contributed the most to this finding were high waist circumference (model 2 mean percentage difference = 6.74% [2.97,10.6]), high glucose levels (4.91% [0.77,9.23]), and low HDL-cholesterol (8.13% [3.51,13.0]). Conversely, the associations of high blood pressure and triglycerides with GDF-15 were modest and did not reach statistical significance (1.49% [-2.55,5.69] and 2.54% [-2.04,7.34], respectively) (Table 2, Figure 1).

Consistent with the findings for waist circumference alone, a high waist-to-hip ratio was associated with 7.07% [2.63,11.7] higher levels of GDF-15, even though there was little to no association for hip circumference alone (0.77% [-3.50,5.23]) and BMI  $\geq 30$  (2.38% [-2.47,7.48]) (Table 3).

Regarding ancillary metabolic biomarkers, glycated hemoglobin  $\geq 5.7\%$  did not show an association with GDF-15 levels (-0.05% [-4.94,5.09]). Still, some trend was found for high HOMA-IR (3.13% [-1.91,8.43]), while participants with high urine albumin and hs-CRP concentrations did have higher GDF-15 levels (12.1% [2.57,22.5] and 10.4% [3.89,17.3], respectively) (Table 4).

## Other analyses

When including in the analyses the 577 participants with established diabetes or known cardiovascular disease, the strength of the association between MS and GDF-15 substantially increased (model 2 mean percentage difference = 24.3% [19.7,29.1]). So was the case for all the MS components, and even the associations with high blood pressure and triglycerides reached statistical significance. (Supplemental Table 1). Contrary to the analyses restricted to premorbid subjects, BMI  $\geq 30$  was significantly associated with increased GDF-15 levels (Supplemental Table 2), as were both glycated hemoglobin  $\geq 5.7\%$  and high HOMA-IR (Supplemental Table 3).

## **DISCUSSION**

### **Key results**

In this study of older adults in Spain, MS was consistently associated with higher GDF-15 levels. The MS components that showed the strongest associations were high waist circumference, elevated glucose levels, and low serum HDL-cholesterol. Ancillary adiposity measures as the waist-to-hip ratio and auxiliary metabolic biomarkers as urine albumin and hs-CRP were also associated with increased GDF-15.

### **Interpretation**

Our main results are in line with the few studies that have directly examined the association between MS and GDF-15. Specifically, in two cross-sectional studies (mean ages 80 and 59 years), higher GDF-15 levels were associated with MS ( $\approx 2.6$  greater odds and  $p < 0.001$ , respectively) [10,30], whereas a small case-control study of obese subjects (mean age 34 years) found that patients with MS had  $\approx 120\%$  higher GDF-15 concentrations than otherwise healthy controls [11].

Contrary to other investigations conducted in younger populations [31,32], we could hardly demonstrate a trend between BMI  $\geq 30$  and higher GDF-15, which only became apparent when including those subjects with diabetes or cardiovascular disease. It has been argued that the current BMI classification may not be appropriate in older adults, as overweight and even moderate obesity (BMI 24-33) do not seem to be associated with increased mortality in this age subgroup [33,34]. Anyway, since total body weight is primarily composed of both fat and muscle mass and only the former may be linked with higher GDF-15 concentrations, associations with BMI could be weaker than with other adiposity measures [8,35]. In this regard, the association between high waist circumference, waist-to-hip ratio, and GDF-15 found by us and others [8,31] -and the lack of it for hip circumference- may suggest that it is abdominal fat that mediates the corresponding association of obesity with GDF-15. Despite being a cytokine produced in response to adipose-tissue-driven inflammation (note that increased hs-CRP has been linked to both obesity and GDF-15 [30,36]), abdominal fat leads to a higher release of free fatty acids into circulation than gluteofemoral fat, which may be deposited in other tissues and organs or re-esterified into triglycerides in the liver [3,37]. On one hand, GDF-15 could then be reflecting the mitochondrial dysfunction associated with ectopic fat accumulation in muscle [12]. On the other hand, both elevated triglyceride levels and reduced serum HDL-cholesterol -as part of atherogenic dyslipidemia- play a role in the initiation and development of atherosclerosis [3], which may, in turn, lead to a rise in GDF-15 levels, as suggested by the strong association of the latter with cardiovascular disease [6,7]. We and others have indeed found a link between serum HDL-cholesterol, triglycerides, and GDF-15 [8,30,35], though in our study the second was not statistically significant after adjustment for lifestyle variables. As for elevated blood pressure, its contribution to atherogenesis may exert some degree of tissue damage at a systemic level [3], though there is little -if any- evidence linking hypertension with GDF-15 concentrations [30,35].

Another pillar of MS are alterations in glucose metabolism. As the primary cause of hyperglycemia in patients with MS, insulin resistance has traditionally been associated with higher levels of GDF-15 [31,35,38]. In our study, the magnitude of the estimates for HOMA-IR was rather smaller than for fasting glucose levels -both in main and sensitivity analyses-, waist circumference, and hs-CRP. Since obesity increases insulin requirements, imposes metabolic stress on pancreatic beta cells, and promotes cellular exhaustion via pro-inflammatory signals [5], part of the association between hyperglycemia and GDF-15 might not be mediated by insulin resistance. Moreover, since insulin resistance habitually precedes the rise in blood glucose levels (at the expense of compensatory hyperinsulinemia), it may be of particular relevance during the initial phases of MS [3]. However, in more advanced stages, such as those more likely represented in a prevalence study like ours, blood glucose may already be elevated, and hence the association of glycemia with GDF-15 might predominate over that with insulin resistance.

A further discrepancy with other epidemiologic studies is that HbA1c was not associated with higher GDF-15 [35], contrary to fasting glucose levels. Any explanation must be conjectural, as subjects with altered single time-point glucose measurements likely have altered average glycemia as well [39]. However, HbA1c levels may rise with age beyond the expected elevations in fasting glucose, and the specificity of HbA1c-based diagnostic criteria for prediabetes might decrease with increasing age [40]. Accordingly, in our main analytical sample, the Pearson's correlation coefficient between fasting glucose and HbA1c was low ( $r=0.39$ ), yet doubled when including those subjects with established diabetes, in line with the observed substantial increase in the association of HbA1c with GDF-15 (Supplemental Table 3).

Finally, microalbuminuria, being the primary clinical outcome of hyperglycemia [3,41], also was associated with elevated GDF-15 levels, in line with the robust evidence on the secretion of

GDF-15 in response to early endothelial and microvascular damage [8,42] and on its role as a risk marker of diabetic nephropathy [8,43].

### **Generalizability**

On one hand, the prevalence of MS found in our study might be lower than that in other settings and countries. For instance, 42.3% of the Spanish population  $\geq 65$  years [13] and 50.4% of adults  $\geq 60$  years in the United States [14] have MS, compared to 36.3% of our participants -note that all figures comprise individuals with diabetes and cardiovascular disease-. Nevertheless, 1) Subjects included in our analyses met 0 to 5 MS criteria, while their ranges of waist circumference, fasting glucose, blood pressure, triglyceride, and serum HDL-cholesterol levels were also broad (Figure 1); 2) The association of MS with GDF-15 was stronger when including in the analyses those subjects with diabetes and cardiovascular disease, who more frequently complied with  $\geq 4$  MS criteria and generally had higher levels of its components; and 3) The association between MS and GDF-15 showed a clear dose-response relationship with the number of MS criteria. So was the case for waist circumference, though some saturation seemed to arise at higher triglyceride and HDL-cholesterol levels (Figure 1).

On the other hand, GDF-15 levels are reported to steadily increase with age [12,30,44] and our study comprised people  $\geq 65$  years. The mean serum GDF-15 concentration found in subjects without MS (1088 pg/ml) was consistent with that of other studies of healthy community-dwelling older adults [44,45], but  $\approx 60\%$  higher than that observed in subjects 17-71 years [8,46]. Also, the strength of the association between MS and GDF-15 appeared to increase somewhat with increasing age (model 2 mean percentage difference = 11.8% [5.73,18.3] for  $>70$  years vs 7.38% [1.46,13.6] for  $\leq 70$  years;  $p$  for interaction=0.23), in line with the stronger association of



obesity with GDF-15 in middle-aged subjects compared to children [32,47], and perhaps reflecting a cumulative exposure to tissue injury and inflammatory states [8,44].

## **Limitations**

Some study limitations should be acknowledged. First, because we used cross-sectional data, we cannot assure that MS always preceded the rise in GDF-15 levels. We cannot rule out that GDF-15 also plays a role in the regulation of food intake and in carbohydrate and lipid metabolism [8,9], which might be in turn associated with MS itself. Second, anthropometric techniques can be helpful for the assessment of body fat distribution, but they might not be used to make inferences on subcutaneous and visceral fat -note that the former may exceed the latter by twofold or threefold even in subjects with abdominal obesity- [3,48].

Moreover, despite MS and GDF-15 being assessed with standardized procedures and analytical techniques, some measurement error is unavoidable, though this would usually bias study results towards the null [49]. Also, the self-reported nature of some covariates may not allow to rule out residual confounding, even after adjusting the models for several lifestyle and sociodemographic variables -notably age, the presumed single strongest predictor of GDF-15 levels [12,30,35]-. Finally, some imprecision may have arisen due to the limited sample size, especially in the analyses regarding ancillary metabolic biomarkers (n=853).

## **Conclusions**

MS was associated with higher GDF-15 levels in older adults, highlighting its potential role as a biomarker for the development of diabetes and cardiovascular disease. The main drivers of this association were abdominal obesity, hyperglycemia -possibly linked to microvascular disease-,

low HDL-cholesterol, and inflammation. Nonetheless, these findings should be confirmed by longitudinal studies using, wherever possible, imaging techniques for the assessment of body fat.

## **ACKNOWLEDGMENTS**

This work was supported by Instituto de Salud Carlos III, State Secretary of R+D+I and FEDER/FSE (FIS grants 18/287, 19/319); grant 2020/017 from the National Plan on Drug Addiction (Ministry of Health), and the MITOFUN project grant from the Fundación Francisco Soria Melguizo. Adrián Carballo-Casla has an FPI contract from the Universidad Autónoma de Madrid. Reagents for measuring Growth Differentiation Factor 15 have been provided by Roche Diagnostics International through a Research Agreement with the FUAM (Fundación de la Universidad Autónoma de Madrid). The funding agencies had no role in study design, data collection, and analysis, interpretation of results, manuscript preparation, or the decision to submit this manuscript for publication. We also wish to thank Beatriz Martín-Moreno for her fine handling of the biological samples and laboratory determinations.

This is a preprint of an article published in GeroScience. The final authenticated version is available online at: <https://doi.org/10.1007/s11357-021-00370-w>

## REFERENCES

- [1] Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International atherosclerosis society; And international association for the study of obesity. *Circulation* 2009;120:1640–5. <https://doi.org/10.1161/CIRCULATIONAHA.109.192644>.
- [2] Simmons RK, Alberti KGMM, Gale EAM, Colagiuri S, Tuomilehto J, Qiao Q, et al. The metabolic syndrome: Useful concept or clinical tool? Report of a WHO expert consultation. *Diabetologia* 2010;53:600–5. <https://doi.org/10.1007/s00125-009-1620-4>.
- [3] Grundy SM. Metabolic syndrome update. *Trends Cardiovasc Med* 2016;26:364–73. <https://doi.org/10.1016/j.tcm.2015.10.004>.
- [4] Xu H, Li X, Adams H, Kubena K, Guo S. Etiology of metabolic syndrome and dietary intervention. *Int J Mol Sci* 2019;20. <https://doi.org/10.3390/ijms20010128>.
- [5] Kolb H, Mandrup-Poulsen T. The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. *Diabetologia* 2010;53:10–20. <https://doi.org/10.1007/s00125-009-1573-7>.
- [6] Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem* 2017;63:140–51. <https://doi.org/10.1373/clinchem.2016.255174>.

- [7] Adela R, Banerjee SK. GDF-15 as a target and biomarker for diabetes and cardiovascular diseases: A translational prospective. *J Diabetes Res* 2015;2015.  
<https://doi.org/10.1155/2015/490842>.
- [8] Desmedt S, Desmedt V, De Vos L, Delanghe JR, Speeckaert R, Speeckaert MM. Growth differentiation factor 15: A novel biomarker with high clinical potential. *Crit Rev Clin Lab Sci* 2019;56:333–50. <https://doi.org/10.1080/10408363.2019.1615034>.
- [9] Cheung CL, Tan KCB, Au PCM, Li GHY, Cheung BM. Evaluation of GDF15 as a therapeutic target of cardiometabolic diseases in human: A Mendelian randomization study. *EBioMedicine* 2019;41:85–90. <https://doi.org/10.1016/j.ebiom.2019.02.021>.
- [10] Echouffo Tcheugui JB, Daya NR, Matsushita K, Ndumele CE, Hoogeveen RC, Al Rifai MS, et al. Abstract P275: Growth Differentiation Factor (GDF)-15 and Metabolic Outcomes: The ARIC Study. *Circulation* 2020;141.  
[https://doi.org/10.1161/circ.141.suppl\\_1.p275](https://doi.org/10.1161/circ.141.suppl_1.p275).
- [11] Shariat A, Farhangi MA, Zeinalian R. Association between serum levels of vascular endothelial growth factor, macrophage inhibitory cytokine and markers of oxidative stress, with the metabolic syndrome and its components in obese individuals. *Nutr Clin Metab* 2018;32:95–101. <https://doi.org/10.1016/j.nupar.2018.02.003>.
- [12] Fujita Y, Taniguchi Y, Shinkai S, Tanaka M, Ito M. Secreted growth differentiation factor15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. *Geriatr Gerontol Int* 2016;16:17–29. <https://doi.org/10.1111/ggi.12724>.

- [13] Guallar-Castillón P, Pérez RF, López García E, León-Muñoz LM, Aguilera MT, Graciani A, et al. Magnitude and Management of Metabolic Syndrome in Spain in 2008-2010: The ENRICA Study. *Rev Española Cardiol (English Ed)* 2014;67:367–73.  
<https://doi.org/10.1016/j.rec.2013.08.014>.
- [14] Hirode G, Wong RJ. Trends in the Prevalence of Metabolic Syndrome in the United States, 2011-2016. *JAMA - J Am Med Assoc* 2020;323:2526–8.  
<https://doi.org/10.1001/jama.2020.4501>.
- [15] Ortolá R, García-Esquinas E, Cabanas-Sánchez V, Migueles JH, Martínez-Gómez D, Rodríguez-Artalejo F. Association of Physical Activity, Sedentary Behavior, and Sleep With Unhealthy Aging: Consistent Results for Device-Measured and Self-reported Behaviors Using Isotemporal Substitution Models. *Journals Gerontol Ser A* 2020;76:85–94. <https://doi.org/10.1093/gerona/glaa177>.
- [16] Cabanas-Sánchez V, Esteban-Cornejo I, Migueles JH, Banegas JR, Graciani A, Rodríguez-Artalejo F, et al. Twenty four-hour activity cycle in older adults using wrist-worn accelerometers: The seniors-ENRICA-2 study. *Scand J Med Sci Sport* 2020;30:700–8. <https://doi.org/10.1111/sms.13612>.
- [17] Rodríguez-Artalejo F, Graciani A, Guallar-Castillón P, León-Muñoz LM, Zuluaga MC, López-García E, et al. Rationale and Methods of the Study on Nutrition and Cardiovascular Risk in Spain (ENRICA). *Rev Española Cardiol* 2011;64:876–82.  
<https://doi.org/10.1016/j.rec.2011.05.023>.

- [18] Gutiérrez-Fisac JL, Guallar-Castillón P, León-Muñoz LM, Graciani A, Banegas JR, Rodríguez-Artalejo F. Prevalence of general and abdominal obesity in the adult population of Spain, 2008-2010: the ENRICA study. *Obes Rev* 2012;13:388–92.  
<https://doi.org/10.1111/j.1467-789X.2011.00964.x>.
- [19] Banegas JR, Graciani A, De La Cruz-Troca JJ, León-Muñoz LM, Guallar-Castillón P, Coca A, et al. Achievement of cardiometabolic goals in aware hypertensive patients in Spain: A nationwide population-based study. *Hypertension* 2012;60:898–905.  
<https://doi.org/10.1161/HYPERTENSIONAHA.112.193078>.
- [20] Waist Circumference and Waist-Hip Ratio. Report of a World Health Organization Expert Consultation. Geneva: 2008.
- [21] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and  $\beta$ -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9.  
<https://doi.org/10.1007/BF00280883>.
- [22] Gayoso-Diz P, Otero-González A, Rodríguez-Alvarez MX, Gude F, García F, De Francisco A, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: Effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord* 2013;13:47. <https://doi.org/10.1186/1472-6823-13-47>.
- [23] Vijan S. In the clinic. Type 2 diabetes. *Ann Intern Med* 2015;162:ITC1–16.  
<https://doi.org/10.7326/AITC201503030>.

- [24] De Jong PE, Curhan GC. Screening, Monitoring, and Treatment of Albuminuria: Public Health Perspectives. *J Am Soc Nephrol* 2006;17:2120–6.  
<https://doi.org/10.1681/ASN.2006010097>.
- [25] Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. *Circulation* 2003;107:499–511. <https://doi.org/10.1161/01.CIR.0000052939.59093.45>.
- [26] Pols MA, Peeters PH, Ocké MC, Slimani N, Bueno-de-Mesquita HB, Collette HJ. Estimation of reproducibility and relative validity of the questions included in the EPIC Physical Activity Questionnaire. *Int J Epidemiol* 1997;26:181–9.  
[https://doi.org/10.1093/ije/26.suppl\\_1.s181](https://doi.org/10.1093/ije/26.suppl_1.s181).
- [27] Martínez-González MA, López-Fontana C, Varo JJ, Sánchez-Villegas A, Martinez JA. Validation of the Spanish version of the physical activity questionnaire used in the Nurses' Health Study and the Health Professionals' Follow-up Study. *Public Health Nutr* 2005;8:920–7. <https://doi.org/10.1079/phn2005745>.
- [28] Guallar-Castillón P, Sagardui-Villamor J, Balboa-Castillo T, Sala-Vila A, Ariza Astolfi MJ, Sarrión Pelous MD, et al. Validity and Reproducibility of a Spanish Dietary History. *PLoS One* 2014;9:e86074. <https://doi.org/10.1371/journal.pone.0086074>.
- [29] Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A Short Screener Is Valid for Assessing Mediterranean Diet Adherence among Older

Spanish Men and Women. *J Nutr* 2011;141:1140–5.

<https://doi.org/10.3945/jn.110.135566>.

- [30] Ho JE, Mahajan A, Chen MH, Larson MG, McCabe EL, Ghorbani A, et al. Clinical and genetic correlates of growth differentiation factor 15 in the community. *Clin Chem* 2012;58:1582–91. <https://doi.org/10.1373/clinchem.2012.190322>.
- [31] Kempf T, Guba-Quint A, Torgerson J, Magnone MC, Haefliger C, Bobadilla M, et al. Growth differentiation factor 15 predicts future insulin resistance and impaired glucose control in obese nondiabetic individuals: Results from the XENDOS trial. *Eur J Endocrinol* 2012;167:671–8. <https://doi.org/10.1530/EJE-12-0466>.
- [32] Dostálová I, Roubíček T, Bártlová M, Mráz M, Lacinová Z, Haluzíková D, et al. Increased serum concentrations of macrophage inhibitory cytokine-1 in patients with obesity and type 2 diabetes mellitus: The influence of very low calorie diet. *Eur J Endocrinol* 2009;161:397–404. <https://doi.org/10.1530/EJE-09-0417>.
- [33] Winter JE, MacInnis RJ, Wattanapenpaiboon N, Nowson CA. BMI and all-cause mortality in older adults: A meta-analysis. *Am J Clin Nutr* 2014;99:875–90. <https://doi.org/10.3945/ajcn.113.068122>.
- [34] Javed AA, Aljied R, Allison DJ, Anderson LN, Ma J, Raina P. Body mass index and all-cause mortality in older adults: A scoping review of observational studies. *Obes Rev* 2020;21. <https://doi.org/10.1111/obr.13035>.
- [35] Vila G, Riedl M, Anderwald C, Resl M, Handisurya A, Clodi M, et al. The relationship between insulin resistance and the cardiovascular biomarker growth differentiation factor-



- 15 in obese patients. Clin Chem 2011;57:309–16.  
<https://doi.org/10.1373/clinchem.2010.153726>.
- [36] Khera A, Vega GL, Das SR, Ayers C, McGuire DK, Grundy SM, et al. Sex Differences in the Relationship between C-Reactive Protein and Body Fat. J Clin Endocrinol Metab 2009;94:3251–8. <https://doi.org/10.1210/jc.2008-2406>.
- [37] Boden G. Obesity, insulin resistance and free fatty acids. Curr Opin Endocrinol Diabetes Obes 2011;18:139–43. <https://doi.org/10.1097/MED.0b013e3283444b09>.
- [38] Karczewska-Kupczewska M, Kowalska I, Nikolajuk A, Adamska A, Otziomek E, Gorska M, et al. Hyperinsulinemia acutely increases serum macrophage inhibitory cytokine-1 concentration in anorexia nervosa and obesity. Clin Endocrinol (Oxf) 2012;76:46–50. <https://doi.org/10.1111/j.1365-2265.2011.04139.x>.
- [39] Hall H, Perelman D, Breschi A, Limcaoco P, Kellogg R, McLaughlin T, et al. Glucotypes reveal new patterns of glucose dysregulation. PLoS Biol 2018;16. <https://doi.org/10.1371/journal.pbio.2005143>.
- [40] Dubowitz N, Xue W, Long Q, Ownby JG, Olson DE, Barb D, et al. Aging is associated with increased HbA1c levels, independently of glucose levels and insulin resistance, and also with decreased HbA1c diagnostic specificity. Diabet Med 2014;31:927–35. <https://doi.org/10.1111/dme.12459>.
- [41] Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage - The Steno hypothesis. Diabetologia 1989;32:219–26. <https://doi.org/10.1007/BF00285287>.

- [42] Kahli A, Guenancia C, Zeller M, Grosjean S, Stamboul K, Rochette L, et al. Growth Differentiation Factor-15 (GDF-15) levels are associated with cardiac and renal injury in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass. *PLoS One* 2014;9:e105759. <https://doi.org/10.1371/journal.pone.0105759>.
- [43] Hellemons ME, Mazagova M, Gansevoort RT, Henning RH, De Zeeuw D, Bakker SJL, et al. Growth-Differentiation Factor 15 Predicts Worsening of Albuminuria in Patients With Type 2 Diabetes. *Diabetes Care* 2012;35:2340–6. <https://doi.org/10.2337/dc12-0180>.
- [44] Eggers KM, Kempf T, Wallentin L, Wollert KC, Lind L. Change in Growth Differentiation Factor 15 Concentrations over Time Independently Predicts Mortality in Community-Dwelling Elderly Individuals. *Clin Chem* 2013;59:1091–8. <https://doi.org/10.1373/clinchem.2012.201210>.
- [45] Doerstling S, Hedberg P, Öhrvik J, Leppert J, Henriksen E. Growth differentiation factor 15 in a community-based sample: age-dependent reference limits and prognostic impact. *Ups J Med Sci* 2018;123:86–93. <https://doi.org/10.1080/03009734.2018.1460427>.
- [46] Brown DA, Ward RL, Buckhaults P, Liu T, Romans KE, Hawkins NJ, et al. MIC-1 Serum Level and Genotype: Associations with Progress and Prognosis of Colorectal Carcinoma 1. *Clin Cancer Res* 2003;9:2642–50.
- [47] Yuca SA, Cimbek EA, Şen Y, Güvenç O, Vatansev H, Buğrul F, et al. The Relationship between Metabolic Parameters, Cardiac Parameters and MIC-1/GDF15 in Obese Children. *Exp Clin Endocrinol Diabetes* 2017;125:86–90. <https://doi.org/10.1055/s-0042-114220>.

- [48] Grundy SM, Neeland IJ, Turer AT, Vega GL. Waist circumference as measure of abdominal fat compartments. *J Obes* 2013;2013. <https://doi.org/10.1155/2013/454285>.
- [49] MacMahon S, Peto R, Collins R, Godwin J, MacMahon S, Cutler J, et al. Blood pressure, stroke, and coronary heart disease. Part 1, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* 1990;335:765–74. [https://doi.org/10.1016/0140-6736\(90\)90878-9](https://doi.org/10.1016/0140-6736(90)90878-9).

**Table 1.** Characteristics of 1938 older adults free of diabetes and cardiovascular disease,<sup>a</sup> by metabolic syndrome status and its components.

	Components of metabolic syndrome											
	Metabolic syndrome		Waist circumference		Fasting glucose		Blood pressure		Triglycerides		HDL-cholesterol	
	No	Yes <sup>b</sup>	Normal	High <sup>c</sup>	Normal	High <sup>d</sup>	Normal	High <sup>e</sup>	Normal	High <sup>f</sup>	Normal	Low <sup>g</sup>
<b>n</b>	1417	521	1035	903	1480	458	440	1498	1612	326	1557	381
GDF-15 (pg/ml), geometric mean [geometric SD factor]	1088 [1.50]	1227 [1.53]*	1094 [1.53]	1159 [1.50]*	1105 [1.50]	1188 [1.54]*	1082 [1.50]	1136 [1.52]*	1115 [1.51]	1166 [1.51]	1096 [1.50]	1243 [1.55]*
Sex, %												
Men	47.6	40.7*	57.8	31.9*	42.8	55.2*	41.1	47.1*	45.4	47.2	47.2	39.6*
Women	52.4	59.3	42.2	68.1	57.2	44.8	58.9	52.9	54.6	52.8	52.8	60.4
Age (years)	71.3 [4.22]	71.9 [4.48]*	71.2 [4.11]	71.7 [4.49]*	71.3 [4.27]	71.8 [4.36]*	70.8 [4.22]	71.6 [4.30]*	71.5 [4.25]	71.2 [4.50]	71.3 [4.25]	71.8 [4.44]
Educational level, %												
Primary or less	61.2	64.9	57.2	67.9*	61.6	64.0	60.2	62.8	62.0	62.9	61.8	63.5
Secondary	19.5	18.8	22.2	15.9	19.1	20.1	19.8	19.2	18.9	21.5	19.9	16.8
University	19.3	16.3	20.6	16.2	19.3	15.9	20	18.1	19.1	15.6	18.2	19.7
Tobacco smoking, %												
Never	54.3	52.6	52.1	55.9	55.8	47.6*	53.2	54.1	54.5	50.9*	53.8	54.3*
Former	36.6	37.4	37.3	36.2	34.6	43.9	37.3	36.6	37.0	35.6	37.7	33.1
Current	9.1	10.0	10.6	7.9	9.6	8.5	9.6	9.3	8.5	13.5	8.5	12.6
Alcohol consumption, %												
Never	17.2	20.9	15.6	21.3*	19.0	15.7*	20	17.7	17.8	20.2	17.1	22.6*
Former	6.35	5.76	6.76	5.54	6.55	5.02	7.95	5.67	6.33	5.52	5.33	9.71
Moderate <sup>h</sup>	53.0	53.6	53.7	52.5	53.3	52.6	50.7	53.9	53.7	50.3	51.9	58.3
Heavy	23.4	19.8	24.0	20.7	21.1	26.6	21.4	22.8	22.1	23.9	25.6	9.4
Diet quality (MEDAS)	7.15 [1.67]	7.09 [1.78]	7.12 [1.67]	7.15 [1.74]	7.12 [1.68]	7.19 [1.75]	7.07 [1.63]	7.15 [1.72]	7.17 [1.69]	6.98 [1.74]*	7.18 [1.70]	6.94 [1.68]*
Energy intake (kcal/day)	1936 [337]	1985 [366]*	1950 [342]	1947 [350]	1924 [333]	2029 [374]*	1915 [337]	1959 [348]*	1939 [345]	1996 [346]*	1954 [346]	1927 [345]
Light physical activity (min/day)	159 [52.2]	145 [52.8]*	156 [52.1]	153 [53.5]	157 [52.3]	148 [53.7]*	162 [51.5]	153 [53.0]*	157 [52.6]	144 [52.5]*	158 [52.1]	141 [53.2]*
Moderate-to-vigorous PA (min/day)	65.5 [39.4]	50.3 [34.6]*	67.1 [39.5]	54.8 [36.9]*	61.8 [38.1]	59.9 [40.8]	67.1 [41.2]	59.7 [37.9]*	63.2 [39.4]	52.6 [34.4]*	64.8 [39.8]	47.6 [30.5]*
Sedentary behavior (min/day)	769 [138]	822 [158]*	774 [152]	794 [137]*	776 [140]	806 [161]*	772 [147]	787 [145]*	775 [139]	823 [170]*	775 [142]	818 [155]*

MEDAS = Mediterranean Diet Adherence Screener. PA = Physical activity. Values are means [standard deviations] unless otherwise indicated. \*P value <0.05 for differences in means (Wilcoxon rank-sum) or proportions (Pearson's chi-squared) across the categories of metabolic syndrome and its components.

<sup>a</sup> Participants who had a blood glucose level  $\geq 126$  mg/dL, had HbA<sub>1c</sub> levels  $\geq 6.5$  %, were treated with antidiabetic drugs, or had a diagnosis of diabetes mellitus or cardiovascular disease (myocardial infarction, stroke, or heart failure).

<sup>b</sup>  $\geq 3$  components of metabolic syndrome.

<sup>c</sup> Waist circumference  $\geq 102$  cm in men and  $\geq 88$  cm in women. <sup>d</sup> Fasting glucose levels  $\geq 100$  mg/dL. <sup>e</sup> Systolic blood pressure  $\geq 130$  mm Hg, or diastolic blood pressure  $\geq 85$  mm Hg, or treatment with antihypertensive medication. <sup>f</sup> Triglyceride levels  $\geq 150$  mg/dL. <sup>g</sup> Serum HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women.

<sup>h</sup> Moderate drinking:  $\leq 10$  g/day in women and  $\leq 20$  g/day in men.

**Table 2.** Association of metabolic syndrome and its components with GDF-15 in 1938 older adults free of diabetes and cardiovascular disease.<sup>a</sup>

Mean percentage difference in GDF-15 [95% confidence interval]				
	n	Crude	Model 1 <sup>b</sup>	Model 2 <sup>c</sup>
<b>Metabolic syndrome</b>				
No	1417	0 (reference)	0 (reference)	0 (reference)
Yes <sup>d</sup>	521	12.7 [8.17,17.5]***	11.4 [7.12,15.8]***	9.34 [5.16,13.7]***
Components of metabolic syndrome				
<b>Waist circumference</b>				
Normal	1035	0 (reference)	0 (reference)	0 (reference)
High <sup>e</sup>	903	5.94 [2.10,9.93]**	7.40 [3.63,11.3]***	6.74 [2.97,10.6]***
<b>Fasting glucose levels</b>				
Normal	1480	0 (reference)	0 (reference)	0 (reference)
High <sup>f</sup>	458	7.57 [3.00,12.3]***	4.40 [0.21,8.76]*	4.91 [0.77,9.23]*
<b>Blood pressure</b>				
Normal	440	0 (reference)	0 (reference)	0 (reference)
High <sup>g</sup>	1498	4.99 [0.46,9.72]*	1.64 [-2.48,5.93]	1.49 [-2.55,5.69]
<b>Triglyceride levels</b>				
Normal	1612	0 (reference)	0 (reference)	0 (reference)
High <sup>h</sup>	326	4.54 [-0.50,9.83]	5.24 [0.50,10.2]*	2.54 [-2.04,7.34]
<b>Serum HDL cholesterol</b>				
Normal	1557	0 (reference)	0 (reference)	0 (reference)
Low <sup>i</sup>	381	13.4 [8.26,18.7]***	12.6 [7.81,17.5]***	8.13 [3.51,13.0]***

\*p<0.05. \*\*p<0.01. \*\*\*p<0.001.

<sup>a</sup> Participants who had a blood glucose level  $\geq 126$  mg/dL, had HbA<sub>1c</sub> levels  $\geq 6.5$  %, were treated with antidiabetic drugs, or had a diagnosis of diabetes mellitus or cardiovascular disease (myocardial infarction, stroke, or heart failure).

<sup>b</sup> Model 1: Linear regression model adjusted for sex, age, and educational level (primary or less, secondary, or university).

<sup>c</sup> Model 2: As Model 1 and further adjusted for smoking status (never, former, or current), alcohol consumption (never, former, moderate [ $\leq 10$  g/day in women and  $\leq 20$  g/day in men], or heavy), diet quality (Mediterranean Diet Adherence Screener), energy intake (kcal/day), light physical activity (min/day), moderate-to-vigorous physical activity (min/day), and sedentary behavior (min/day).

<sup>d</sup>  $\geq 3$  components of metabolic syndrome.

<sup>e</sup> Waist circumference  $\geq 102$  cm in men and  $\geq 88$  cm in women. <sup>f</sup> Fasting glucose levels  $\geq 100$  mg/dl. <sup>g</sup> Systolic blood pressure  $\geq 130$  mm Hg, or diastolic blood pressure  $\geq 85$  mm Hg, or treatment with antihypertensive medication. <sup>h</sup> Triglyceride levels  $\geq 150$  mg/dl. <sup>i</sup> Serum HDL cholesterol  $< 40$  mg/dl in men and  $< 50$  mg/dl in women.

**Table 3.** Association of ancillary adiposity measures with GDF-15 in 1938 older adults free of diabetes and cardiovascular disease.<sup>a</sup>

		Mean percentage difference in GDF-15 [95% confidence interval]		
	n	Crude	Model 1 <sup>b</sup>	Model 2 <sup>c</sup>
<b>Body mass index (kg/m<sup>2</sup>)</b>				
Categories				
<25	559	0 (reference)	0 (reference)	0 (reference)
25 to 30	932	-2.39 [-6.55,1.94]	-3.08 [-6.95,0.96]	-2.27 [-6.13,1.74]
≥30	447	3.30 [-1.89,8.77]	2.90 [-1.96,8.01]	2.38 [-2.47,7.48]
p for trend	1938	0.220	0.249	0.353
<b>Hip circumference</b>				
Tertiles <sup>d</sup>				
1 (lower)	694	0 (reference)	0 (reference)	0 (reference)
2	681	-1.17 [-5.41,3.26]	-1.25 [-5.21,2.88]	-0.56 [-4.49,3.53]
3 (higher)	563	1.77 [-2.82,6.58]	1.55 [-2.74,6.02]	0.77 [-3.50,5.23]
p for trend	1938	0.448	0.477	0.724
<b>Waist-to-hip ratio</b>				
Categories				
Normal	462	0 (reference)	0 (reference)	0 (reference)
High <sup>e</sup>	1476	11.9 [7.16,16.8]***	8.18 [3.65,12.9]***	7.07 [2.63,11.7]**
p for trend	1938	<0.001	<0.001	0.002

\*p<0.05. \*\*p<0.01. \*\*\*p<0.001.

<sup>a</sup> Participants who had blood glucose levels ≥ 126 mg/dL, had HbA<sub>1c</sub> levels ≥6.5 %, were treated with antidiabetic drugs, or had a diagnosis of diabetes mellitus or cardiovascular disease (myocardial infarction, stroke, or heart failure).

<sup>b</sup> Model 1: Linear regression model adjusted for sex, age, and educational level (primary or less, secondary, or university).

<sup>c</sup> Model 2: As Model 1 and further adjusted for smoking status (never, former, or current), alcohol consumption (never, former, moderate [≤10 g/day in women and ≤20 g/day in men], or heavy), diet quality (Mediterranean Diet Adherence Screener), energy intake (kcal/day), light physical activity (min/day), moderate-to-vigorous physical activity (min/day), and sedentary behavior (min/day).

<sup>d</sup> Hip circumference tertiles: tertile 1, ≤ 98 cm in men and ≤ 99.6 cm in women; tertile 2, 98 to 104 cm in men and 99.6 to 107 cm in women; tertile 3, >104 cm in men and >107 cm in women.

<sup>e</sup> Waist-to-hip ratio ≥0.90 in men and ≥0.85 in women.

**Table 4.** Association of ancillary metabolic biomarkers with GDF-15 in 853 older adults free of diabetes and cardiovascular disease.<sup>a</sup>

		Mean percentage difference in GDF-15 [95% confidence interval]		
	n	Crude	Model 1 <sup>b</sup>	Model 2 <sup>c</sup>
<b>HOMA-IR</b>				
Categories				
Normal	478	0 (reference)	0 (reference)	0 (reference)
High <sup>d</sup>	375	4.85 [-0.50,10.5]	3.53 [-1.50,8.82]	3.13 [-1.91,8.43]
p for trend	853	0.076	0.172	0.227
<b>Glycated hemoglobin (%)</b>				
Categories				
<5.7	512	0 (reference)	0 (reference)	0 (reference)
5.7 to 6.4	341	2.34 [-2.96,7.93]	0.57 [-4.41,5.80]	-0.05 [-4.94,5.09]
p for trend	853	0.393	0.826	0.984
<b>Urine albumin (mg/l)</b>				
Categories				
<10	649	0 (reference)	0 (reference)	0 (reference)
10 to 20	132	7.56 [0.086,15.6]*	4.76 [-2.20,12.2]	3.42 [-3.40,10.7]
≥20	72	18.1 [7.56,29.7]***	13.6 [3.87,24.2]**	12.1 [2.57,22.5]*
p for trend	853	<0.001	0.003	0.010
<b>High-sensitivity C-reactive protein (mg/l)</b>				
Categories				
<1	391	0 (reference)	0 (reference)	0 (reference)
1 to 3	248	-1.02 [-6.91,5.24]	-0.53 [-6.14,5.42]	-0.71 [-6.24,5.15]
>3	214	11.0 [4.09,18.4]**	12.6 [5.96,19.7]***	10.4 [3.89,17.3]**
p for trend	853	<0.001	<0.001	<0.001

\*p<0.05. \*\*p<0.01. \*\*\*p<0.001.

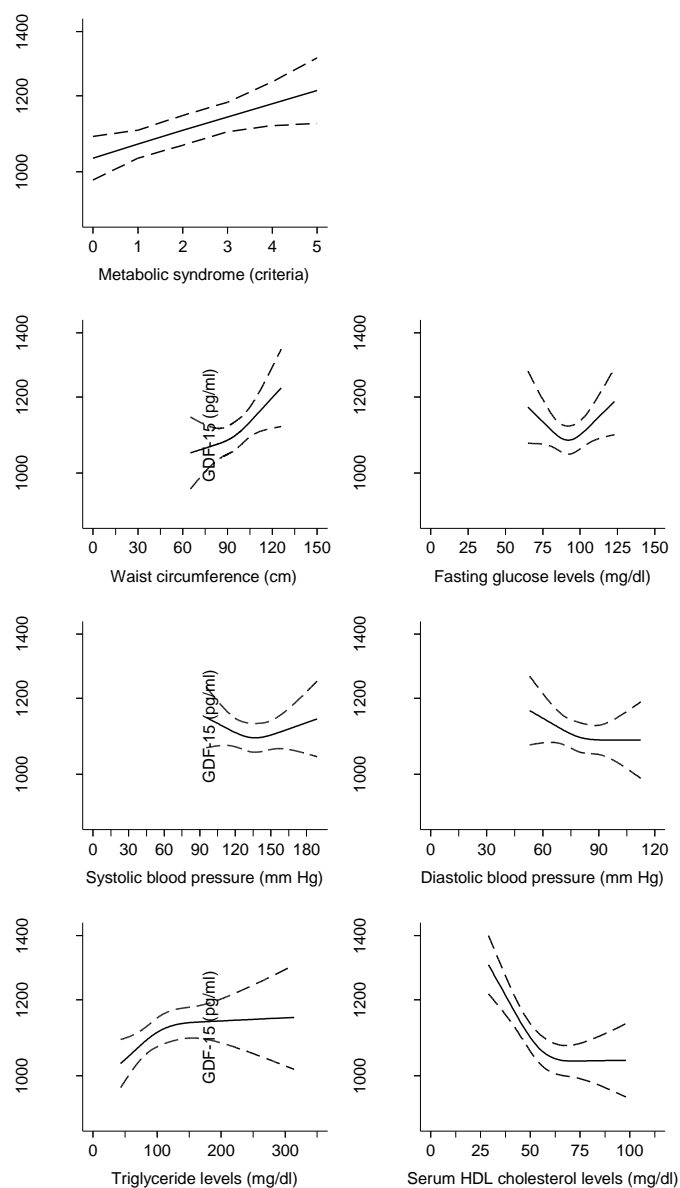
<sup>a</sup> Participants who had a blood glucose level ≥ 126 mg/dL, had HbA<sub>1c</sub> levels ≥6.5 %, were treated with antidiabetic drugs, or had a diagnosis of diabetes mellitus or cardiovascular disease (myocardial infarction, stroke, or heart failure).

<sup>b</sup> Model 1: Linear regression model adjusted for sex, age, and educational level (primary or less, secondary, or university).

<sup>c</sup> Model 2: As Model 1 and further adjusted for smoking status (never, former, or current), alcohol consumption (never, former, moderate [≤10 g/day in women and ≤20 g/day in men], or heavy), diet quality (Mediterranean Diet Adherence Screener), energy intake (kcal/day), light physical activity (min/day), moderate-to-vigorous physical activity (min/day), and sedentary behavior (min/day).

<sup>d</sup> HOMA-IR ≥ 2.25 in men and ≥ 2.38 in women.

**Figure 1.** Association of metabolic syndrome and its components with GDF-15 in 1938 older adults free of diabetes and cardiovascular disease.<sup>a</sup>



<sup>a</sup> Participants who had a blood glucose level  $\geq 126$  mg/dL, had HbA<sub>1c</sub> levels  $\geq 6.5$  %, were treated with antidiabetic drugs, or had a diagnosis of diabetes mellitus or cardiovascular disease (myocardial infarction, stroke, or heart failure).

Plotted values are geometric means (95% confidence intervals) obtained from a linear regression model adjusted as Model 2 in Table 2: sex, age, educational level (primary or less, secondary, or university), smoking status (never, former, or current), alcohol consumption (never, former, moderate [ $\leq 10$  g/day in women and  $\leq 20$  g/day in men], or heavy), diet quality (Mediterranean Diet Adherence Screener), energy intake (kcal/day), light physical activity (min/day), moderate-to-vigorous physical activity (min/day), and sedentary behavior (min/day).



The restricted cubic spline knots are located at 1-2-3 components for metabolic syndrome, 81-95-109 cm for waist circumference, 79-91-107 mg/dl for fasting glucose levels, 112-133-158 mm Hg for systolic blood pressure, 67-80-94 mm Hg for diastolic blood pressure, 64-99-169 mg/dl for triglyceride levels, and 40-54-74 mg/dl for serum HDL-cholesterol levels.

**Supplemental Table 1.** Association of metabolic syndrome and its components with GDF-15 in 2515 older adults.

		Mean percentage difference [95% confidence interval]		
	n	Crude	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>
Metabolic syndrome				
No	1601	0 (reference)	0 (reference)	0 (reference)
Yes <sup>c</sup>	914	28.7 [23.8,33.8]***	27.6 [23.0,32.4]***	24.3 [19.7,29.1]***
Components of metabolic syndrome				
Waist circumference				
Normal	1248	0 (reference)	0 (reference)	0 (reference)
High <sup>d</sup>	1267	10.1 [5.92,14.4]***	12.7 [8.57,17.1]***	10.4 [6.25,14.7]***
Fasting glucose levels				
Normal	1549	0 (reference)	0 (reference)	0 (reference)
High <sup>e</sup>	966	36.0 [30.9,41.2]***	31.8 [27.0,36.7]***	30.1 [25.5,34.9]***
Blood pressure				
Normal	515	0 (reference)	0 (reference)	0 (reference)
High <sup>f</sup>	2000	9.38 [4.26,14.7]***	6.25 [1.52,11.2]**	5.61 [1.01,10.4]*
Triglyceride levels				
Normal	2021	0 (reference)	0 (reference)	0 (reference)
High <sup>g</sup>	494	14.6 [9.14,20.3]***	15.8 [10.6,21.2]***	12.3 [7.30,17.5]***
Serum HDL cholesterol				
Normal	1906	0 (reference)	0 (reference)	0 (reference)
Low <sup>h</sup>	609	25.4 [20.0,31.1]***	25.0 [19.9,30.4]***	19.2 [14.2,24.4]***

\*p<0.05. \*\*p<0.01. \*\*\*p<0.001.

<sup>a</sup> Model 1: Linear regression model adjusted for sex, age, and educational level (primary or less, secondary, or university).

<sup>b</sup> Model 2: As Model 1 and further adjusted for smoking status (never, former, or current), alcohol consumption (never, former, moderate [ $\leq 10$  g/day in women and  $\leq 20$  g/day in men], or heavy), diet quality (Mediterranean Diet Adherence Screener), energy intake (kcal/day), light physical activity (min/day), moderate-to-vigorous physical activity (min/day), and sedentary behavior (min/day).

<sup>c</sup>  $\geq 3$  components of metabolic syndrome.

<sup>d</sup> Waist circumference  $\geq 102$  cm in men and  $\geq 88$  cm in women. <sup>e</sup> Fasting glucose levels  $\geq 100$  mg/dl or treatment with antidiabetic drugs. <sup>f</sup> Systolic blood pressure  $\geq 130$  mm Hg, or diastolic blood pressure  $\geq 85$  mm Hg, or treatment with antihypertensive medication. <sup>g</sup> Triglyceride levels  $\geq 150$  mg/dl. <sup>h</sup> Serum HDL cholesterol  $< 40$  mg/dl in men and  $< 50$  mg/dl in women.

**Supplemental Table 2.** Association of ancillary adiposity measures with GDF-15 in 2515 older adults.

Mean percentage difference in GDF-15 [95% confidence interval]				
	n	Crude	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>
<b>Body mass index (kg/m<sup>2</sup>)</b>				
Categories				
<25	672	0 (reference)	0 (reference)	0 (reference)
25 to 30	1184	-0.36 [-4.92,4.41]	-0.91 [-5.22,3.60]	-0.76 [-5.00,3.67]
≥30	659	9.31 [3.65,15.3]**	9.55 [4.16,15.2]***	6.99 [1.66,12.6]**
p for trend	2515	<0.001	<0.001	0.007
<b>Hip circumference</b>				
Tertiles <sup>c</sup>				
1 (lower)	884	0 (reference)	0 (reference)	0 (reference)
2	850	-2.70 [-7.13,1.95]	-2.25 [-6.48,2.16]	-1.92 [-6.08,2.43]
3 (higher)	781	2.32 [-2.45,7.31]	2.25 [-2.26,6.98]	-0.13 [-4.58,4.53]
p for trend	2515	0.314	0.304	0.981
<b>Waist-to-hip ratio</b>				
Categories				
Normal	518	0 (reference)	0 (reference)	0 (reference)
High <sup>d</sup>	1997	19.9 [14.3,25.7]***	15.8 [10.5,21.4]***	13.0 [7.85,18.4]***
p for trend	2515	<0.001	<0.001	<0.001

\*p<0.05. \*\*p<0.01. \*\*\*p<0.001.

<sup>a</sup> Model 1: Linear regression model adjusted for sex, age, and educational level (primary or less, secondary, or university).

<sup>b</sup> Model 2: As Model 1 and further adjusted for smoking status (never, former, or current), alcohol consumption (never, former, moderate [ $\leq 10$  g/day in women and  $\leq 20$  g/day in men], or heavy), diet quality (Mediterranean Diet Adherence Screener), energy intake (kcal/day), light physical activity (min/day), moderate-to-vigorous physical activity (min/day), and sedentary behavior (min/day).

<sup>c</sup> Hip circumference tertiles: tertile 1,  $\leq 98$  cm in men and  $\leq 99.6$  cm in women; tertile 2, 98 to 104 cm in men and 99.4 to 107 cm in women; tertile 3,  $>104$  cm in men and  $>107$  cm in women.

<sup>d</sup> Waist-to-hip ratio  $\geq 0.90$  in men and  $\geq 0.85$  in women.

**Supplemental Table 3.** Association of ancillary metabolic biomarkers with GDF-15 in 1080 older adults.

		Mean percentage difference in GDF-15 [95% confidence interval]		
	n	Crude	Model 1 <sup>a</sup>	Model 2 <sup>b</sup>
<b>HOMA-IR</b>				
Categories				
Normal	532	0 (reference)	0 (reference)	0 (reference)
High <sup>c</sup>	548	12.0 [5.97,18.4]***	10.9 [5.22,17.0]***	9.78 [4.09,15.8]***
p for trend	1080	<0.001	<0.001	<0.001
<b>Glycated hemoglobin (%)</b>				
Categories				
<5.7	531	0 (reference)	0 (reference)	0 (reference)
5.7 to 6.4	423	9.27 [3.32,15.6]**	7.26 [1.67,13.2]*	6.42 [0.94,12.2]*
≥6.5	126	70.1 [56.2,85.2]***	66.9 [53.9,81.0]***	61.8 [49.2,75.4]***
p for trend	1080	<0.001	<0.001	<0.001
<b>Urine albumin (mg/l)</b>				
Categories				
<10	789	0 (reference)	0 (reference)	0 (reference)
10 to 20	173	9.71 [1.76,18.3]*	6.58 [-0.87,14.6]	5.65 [-1.63,13.5]
≥20	118	37.4 [25.7,50.1]***	29.6 [19.0,41.2]***	26.6 [16.4,37.7]***
p for trend	1080	<0.001	<0.001	<0.001
<b>High-sensitivity C-reactive protein (mg/l)</b>				
Categories				
<1	482	0 (reference)	0 (reference)	0 (reference)
1 to 3	307	-1.98 [-8.27,4.74]	-0.40 [-6.50,6.10]	-0.80 [-6.76,5.54]
>3	291	15.3 [7.80,23.4]***	17.4 [10.0,25.2]***	14.4 [7.38,22.0]***
p for trend	1080	<0.001	<0.001	<0.001

\*p<0.05. \*\*p<0.01. \*\*\*p<0.001.

<sup>a</sup> Model 1: Linear regression model adjusted for sex, age, and educational level (primary or less, secondary, or university).

<sup>b</sup> Model 2: As Model 1 and further adjusted for smoking status (never, former, or current), alcohol consumption (never, former, moderate [≤10 g/day in women and ≤20 g/day in men], or heavy), diet quality (Mediterranean Diet Adherence Screener), energy intake (kcal/day), light physical activity (min/day), moderate-to-vigorous physical activity (min/day), and sedentary behavior (min/day).

<sup>c</sup> HOMA-IR ≥ 2.25 in men and ≥ 2.38 in women.

**Supplemental Figure 1.** Participants' flow chart.

