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Title: Adiposity measures and arterial stiffness: the MARK prospective observational study.

Authors: Leticia Gomez-Sanchez MD ^{1*}, Luis Garcia-Ortiz MD, PhD ^{1,2,3}, Maria C Patino-Alonso PhD ^{1,2,4}, Jose I Recio-Rodriguez PhD ^{1,2,5}, Rigo Fernando MD, PhD ⁶, Ruth Marti PhD ^{7,8}, Cristina Agudo-Conde ^{1,2}, Emiliano Rodriguez-Sanchez MD, PhD ^{1,2,9}, Jose A Maderuelo-Fernandez MD, PhD ^{1,2}, Rafel Ramos MD, PhD ^{7,8,10} and Manuel A Gomez-Marcos MD, PhD ^{1,2,9} for the MARK Group ¹¹

1. Primary Care Research Unit, the Alamedilla Health Center, Castilla and León Health Service (SACyL), Salamanca, Spain.
2. Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain.
3. Biomedical and diagnostic sciences department, University of Salamanca, Salamanca, Spain.
4. Statistics Department, University of Salamanca, Salamanca, Spain.
5. Department of Nursing and Physiotherapy, University of Salamanca, Salamanca, Spain.
6. San Agustín Health Center, Illes Balears Health Service (IBSALUT), Palma of Mallorca, Spain.
7. Unitat de Suport a la Recerca de Girona. Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Girona, Spain.
8. Institut d'Investigació Biomèdica of Girona Dr. Josep Trueta (IDBGI). Girona, Spain.
9. Department of Medicine, University of Salamanca, Salamanca, Spain.
10. Departament de Ciències Mèdiques, Facultat de Medicina. Universitat de Girona, Spain.
11. MARK Group. redIAPP: Research Network in Preventive Activities and Health Promotion, Girona, Spain.

***Corresponding Author:**

Leticia Gomez-Sanchez, Primary Care Research Unit, Alamedilla Health Care Center.
37003 Salamanca, Spain.

Full list of author information is available at the end of the article.

ABSTRACT

Background: Increased arterial stiffness is one possible mechanism of increased cardiovascular risk from obesity.

Objective: This study investigates the relationship of adiposity measures with arterial stiffness in Caucasian adults with intermediate cardiovascular and determines measures with greater power to explain arterial stiffness.

Setting: 6 primary care centres in in three Spanish Autonomous Communities, Spain.

Participants: This study analyzed 2354 subjects (age 35–74 years (mean 61.4±7.7 years), 61.9% male).

Methods: The study was based on cross-sectional data from the MARK study. The main outcome variables were: body mass index (BMI), waist-to-height ratio (WHtR), University clinic of Navarra body adiposity estimation (CUN-BAE) body fat percentage, and body roundness index (BRI). Vascular function was assessed by the cardio-ankle vascular index (CAVI) with the VaSera device, and brachial ankle pulse wave velocity (baPWV) was determined using a validated equation.

Results: The mean adiposity measures were a BMI of 29.2±4.4, WHtR of 0.61±0.07, CUN-BAE of 35.7±1.7, and BRI of 5.8±1.7. The mean stiffness measures were a CAVI of 8.8±1.2 and baPWV of 14.9±2.5. In multiple linear regression analysis, all adiposity measures were negatively associated with CAVI and baPWV ($p<0.01$ in all) after adjustment for cardiovascular risk factors. The proportions of CAVI variability explained in the respective by the adiposity measures were 7.5% for BMI, 7.2% for CUN-BAE, 4.3% for WHtR, and 4.1% for BRI, which were higher among diabetic, obese, younger (≤ 62 years), and non-hypertensive subjects.

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3 **Conclusions:** Adiposity measures are negatively associated with arterial stiffness measures.
4
5 The explanatory capacity of the variability of arterial stiffness is higher for BMI and CUN-
6
7 BAE and when using CAVI as a measure of stiffness rather than baPWV.
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9
10 **Trial Registration:** Clinical Trials.gov Identifier: [https://clinicaltrials.gov/ct2/show/](https://clinicaltrials.gov/ct2/show/NCT01428934)
11
12 NCT01428934. Registered 2 September 2011. Last updated September 8, 2016.
13

14 **Keywords:** Body mass index. Waist circumference. Waist-to-height ratio. Fat mass percent.
15
16 Body roundness index. Arterial stiffness.
17

18 **Strengths and limitations of this study**

- 19
- 20 • This is the first study to investigate the association between adiposity measures with
21 the cardio-ankle vascular index with and brachial ankle pulse wave velocity in
22 Caucasian adults with intermediate cardiovascular risk.
23
 - 24 • All adiposity measures were negatively associated with CAVI and baPWV.
25
 - 26 • The explanatory capacity of the variability of arterial stiffness is higher for BMI and
27 CUN-BAE and when using CAVI as a measure of stiffness rather than baPWV.
28
 - 29 • The main limitation of our study is its cross-sectional design, which does not allow us
30 to establish causal relations or the direction of influence of adiposity measures in
31 vascular function.
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INTRODUCTION

Obesity has been linked to increased all-cause and cardiovascular mortality⁽¹⁾. However, the mechanisms through which obesity can increase the frequency of cardiovascular disease beyond traditional risk factors are not clearly identified⁽²⁾. Arterial stiffness as evaluated by the brachial ankle pulse wave velocity (baPWV) is an independent predictor of coronary heart disease and mortality in both the general population⁽³⁾ and in patients with diabetes mellitus^(4; 5). Similarly, the cardio-ankle vascular index (CAVI) is associated with carotid and coronary atherosclerosis^(6; 7; 8) and is a predictor of cardiovascular events in obese patients⁽⁹⁾. Increased arterial stiffness may be one of the mechanisms by which obesity increases cardiovascular risk independently of traditional risk factors. However, the relation between adiposity and arterial stiffness remains controversial. Thus the body mass index (BMI) has been associated with arterial stiffness in the general population^(10; 11), and in diabetic patients^(12; 13). However, other works have not found this association⁽¹⁴⁾, the association disappeared after adjusting for potential confounders⁽¹³⁾, or showed a negative association^(15; 16). On the other hand, there are studies that suggest a greater correlation of measures of central or visceral adiposity than measures of general adiposity with arterial stiffness in the general population^(10; 17; 18; 19; 20), in diabetic patients⁽¹³⁾, and in diabetics and hypertensive patients⁽²¹⁾. The Whitehall II Cohort study⁽²²⁾, found that all measures of general adiposity, central adiposity, and body fat percentage were predictors of accelerated arterial stiffness in adults. Cardiovascular events are more likely to occur in patients with intermediate cardiovascular risk⁽²³⁾, and there is a lack of studies analyzing the relationship of different adiposity measures with arterial stiffness in such subjects. Thus, the primary aim of this study is to investigate the relationship between adiposity measures and arterial stiffness in Caucasian adults with intermediate cardiovascular risk. The secondary aim is to determine which adiposity measures have greater power to explain arterial stiffness.

METHODS

Study design

This trial is a cross-sectional study of subjects recruited to the *improving interMediAte RiSk management (MARK)* study (NCT01428934)⁽²⁴⁾, which is a longitudinal study designed to assess whether the ankle-brachial index, arterial stiffness (measured by CAVI), postprandial glucose, glycosylated hemoglobin, self-measured blood pressure, and the presence of comorbidities are independently associated with the occurrence of vascular events. It also investigates whether the predictive capacity of current risk equations can be improved in the intermediate risk population. The current study focuses on the baseline visit. The second step will be a 5- and 10-year follow-up trial to assess cardiovascular morbidity and mortality.

Study population

In this multicenter project, study population was performed by random sampling from subjects who met the inclusion criteria and were seeing general practitioners from July 2011 to June 2013 at six primary care centers in three Spanish Autonomous Communities. Subjects were recruited from those aged 35 to 74 years with intermediate cardiovascular risk, defined as 10-year coronary risk ranging from 5%–15% according to the adapted Framingham risk equation⁽²⁵⁾; 10-year vascular mortality risk ranging from 1%–5% according to the scoring risk in Europeans equation⁽²⁶⁾; or moderate risk according to the European Society of Hypertension guidelines for the management of arterial hypertension⁽²⁷⁾. Exclusion criteria included end-stage disease or institutionalization at the time of the visit, or history of atherosclerotic disease. This study analyzed 2354 of the 2495 subjects recruited in the MARK study, and the grounds for exclusions are shown in figure 1.

Variables and measurement instruments

A detailed description of procedures for clinical data collection and laboratory tests has been published elsewhere⁽²⁴⁾.

Anthropometric measurements

Body weight was measured twice with a certified electronic scale (Seca 770, Medical scale and measurement systems, Birmingham, United Kingdom) after adequate calibration (precision ± 0.1 kg). Readings were rounded to 100 g. Height was measured with a stadiometer (Seca 222), and the average of two measurements was recorded. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured according to the 2007 recommendations of the Spanish Society for the Study of Obesity⁽²⁹⁾. All measurements were performed with the subjects standing, wearing no shoes, and in light clothing. Waist to height ratio (WHtR) was calculated as waist circumference (cm) divided by height (cm)^(30; 31).

The body fat percentage (BF%) was calculated according University clinic of Navarra - body adiposity estimator (CUN-BAE) to the recommendations of Gomez-Ambrosi J et al⁽³²⁾.

$BF\% = -44.988 + (0.503 \times \text{Age}) + (10.689 \times \text{gender}) + (3.172 \times \text{BMI}) - (0.026 \times \text{BMI}^2) + (0.181 \times \text{BMI} \times \text{gender}) - (0.02 \text{ BMI} \times \text{Age}) - (0.005 \times \text{BMI}^2 \times \text{gender}) + (0.00021 \times \text{BMI}^2 \times \text{Age})$ where male=0 and female=1 for gender.

Body Roundness Index (BRI), was calculated using formula as $BRI = 364.2 - 365.5 \times$

$\sqrt{1 - \frac{(WC/(2\pi))^2}{(0.5 \text{ height})^2}}$ ⁽³³⁾. BRI could predict the percentage of body fat and visceral adipose tissue.

Cardio-ankle vascular index (CAVI) and brachial-ankle pulse wave velocity (baPWV)

CAVI was measured using a *VaSera VS-1500*® device (*Fukuda Denshi*)^(34; 35). CAVI values are calculated automatically by estimating the stiffness parameter β with the following equation: $\beta = 2\rho \times l / (P_s - P_d) \times \ln(P_s / P_d) \times PWV^2$, where ρ is blood density, P_s and P_d are SBP and DBP in mmHg, and PWV is measured between the aortic valve and the ankle⁽³⁶⁾.

The mean coefficient of variation of CAVI measurement is less than 5%, which is small enough to allow for clinical use of the index and confirms that CAVI has a favorable

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3 reproducibility⁽³⁵⁾. baPWV was estimated using the equation, $baPWV = (0.5934 \times \text{height (cm)} + 14.4724)/tba$ (tba is the time interval between the arm and ankle waves)⁽³⁷⁾.

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7 Measurements were performed with the patient in supine position after resting for 10 minutes
8
9 in a quiet room at a stable temperature. Subjects were instructed not to smoke or practice
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11 exercise in the hour prior to the test.

12 13 14 **Diagnosis of cardiovascular risk factors**

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16 Subjects were considered hypertensive if previously diagnosed with hypertension, if they
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18 were taking antihypertensive drugs, or if they had blood pressure levels $\geq 140/90$ mmHg.

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20 Diabetic subjects were those who had a previous diagnosed of the disease, were taking
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22 hypoglycemic drugs, or had fasting blood glucose levels ≥ 126 mg/dL or HbA1c $\geq 6.5\%$.

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24 Dyslipidemia was defined as a prior diagnosis of the condition, use of lipid-lowering drugs, or
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26 fasting total cholesterol levels ≥ 250 mg/dL.

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29 **Office or clinical blood pressure:** Office blood pressure measurement involved three
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31 measurements of SBP and DBP with a validated OMRON model M10-IT
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33 sphygmomanometer (Omron Health Care, Kyoto, Japan). The measurements followed the
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35 recommendations of the European Society of Hypertension⁽³⁸⁾, and the averages of the last
36
37 two measurements were used.

38 39 40 **Lifestyles**

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43 **Tobacco:** Smoking history was assessed by asking questions about the participant's smoking
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45 status (smoker/non-smoker). We considered smokers to include those who currently smoke or
46
47 who have stopped smoking within the past year.

48 49 50 **Laboratory determinations**

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52 Venous blood sampling was performed between 08:00 and 09:00 hours after the individuals
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54 fasted and abstained from smoking and the consumption of alcohol and caffeinated beverages
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56 for the previous 12 hours. Fasting plasma glucose, serum, high-density lipoprotein (HDL)

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3 cholesterol concentrations and triglyceride concentrations were measured using standard
4 enzymatic automated methods. Low-density lipoprotein cholesterol was estimated by the
5 Friedewald equation when the direct parameter will be not available. Atherogenic index was
6 estimated (total cholesterol / HDL cholesterol). Blood samples were collected in the Health
7 Center and analyzed at the hospital of reference.
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14 The researchers who performed the different tests were blinded to the clinical data of the
15 subjects. All assessments were made within a period of 10 days.
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18 **Data analysis**

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20 Continuous variables were expressed as the mean \pm standard deviation. Frequency
21 distributions were used for categorical data. Comparisons between quantitative variables were
22 performed using the student t-test, while the chi-squared test and Fisher's exact test were used
23 for qualitative variables. Pearson's correlation coefficient was used to estimate the
24 relationship of the adiposity measures with CAVI and baPWV.
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32 Four different multiple linear regression models were used to analyze the associations of each
33 adiposity measure with CAVI, and another four models were used with baPWV. CAVI and
34 baPWV were the dependent variables, and the adiposity measures as independent variables in
35 each model. All models were adjusted for age (years), gender (0 = male and 1 = female),
36 systolic blood pressure (SBP), smoking status (0 = No and 1 = Yes), atherogenic index, and
37 HbA1c. The explanatory capacity of the model was measured by R^2 , and the proportion
38 attributed to each variable was estimated by the change in R^2 . The analysis was also
39 performed by age groups, diagnosis of diabetes, hypertension, and obesity.
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50 ANCOVA models were used to test the differences in the mean values of CAVI and baPWV
51 with the quartiles of the four measures of adiposity after adjusting for the confounding
52 variables used in the regression analysis. Pairwise post hoc comparisons were examined using
53 the Bonferroni test. Data were analyzed using SPSS Statistics for Windows version 23.0
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3 (IBM Corp, Armonk, NY). Values of $p < 0.05$ were considered statistically significant.
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5 Continuous variables were expressed as the mean \pm standard deviation. Frequency
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7 distributions were used for categorical data. Comparisons between quantitative variables were
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24 systolic blood pressure (SBP), smoking status (0 = No and 1 = Yes), atherogenic index, and
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26 HbA1c. The explanatory capacity of the model was measured by R^2 , and the proportion
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40 the Bonferroni test. Data were analyzed using SPSS Statistics for Windows version 23.0
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42 (IBM Corp, Armonk, NY). Values of $p < 0.05$ were considered statistically significant.
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45 **Ethics statement**

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47 All participants were informed of the objectives and procedures of the study and signed the
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49 informed consent form to participate. The study was approved by the Clinical Research Ethics
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51 Committee of the Primary Care Research Institute Jordi Gol, the Health Care Area of
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53 Salamanca and Palma of Mallorca. The study was conducted following the recommendations
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55 of the Declaration of Helsinki⁽²⁸⁾. The confidentiality of information provided by participants
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3 was ensured, complying with the rules established by Spanish Organic Law 15/1999, of 13
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5 December on the Protection of Personal Data.
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7 8 **RESULTS**

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10 The anthropometric measures, clinical characteristics, and vascular function measures of the
11 subjects are presented in Table 1. The mean age of the patients was 61.4 ± 7.7 years, and
12
13 61.9% were male. Male subjects had a higher percentage of smokers (31.5 vs. 22.7) and
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15 hypertension (80.1 vs. 75.4). In contrast, females had a higher prevalence of obesity (40.4 vs.
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17 33.4), dyslipidemia (73.1 vs. 63.6), and diabetes (36.5 vs. 31.8). The mean value of CAVI was
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19 8.8 ± 1.2 (8.9 in males and 8.6 in females) and the mean baPWV was 14.9 ± 2.5 (14.8 in males
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21 and 15.0 in females). All of the analyzed adiposity measures except for waist circumference
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23 presented higher values in women.
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27 Pearson's correlation coefficients between the adiposity measures and the vascular function
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29 parameters are shown in table 2. All adiposity measures were negatively correlated with
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31 CAVI, and this correlation increases after adjusting for age, sex, and SBP.
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34 Fig. 2 shows the estimated marginal means of CAVI (A) and baPWV (B) by quartiles of the
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36 different adiposity measures. After adjustment for the variables used in the multiple linear
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38 regression analysis, the mean CAVI values decrease as the quartiles of the four adiposity
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40 measurements increased.
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43 In the multiple linear regression analysis, CAVI and baPWV show negative associations with
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45 all adiposity measures ($p < 0.01$ in all) after adjustment for age, gender, systolic blood
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47 pressure, smoking, atherogenic index, and HbA1c (Table 3). The proportions of CAVI
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49 variability explained in the respective multiple linear regression analyses by the adiposity
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51 measures were 7.5% for BMI, 7.2% for CUN-BAE, 4.3% for WHtR, and 4.1% for BRI. In the
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53 case of baPWV, the variance explained by the measures of adiposity was 0.8% BMI, 0.7% for
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55 CUN-BAE, and 0.1% for WHtR and BRI. (Table 3).
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3 In subgroup analysis, the proportion of CAVI variability explained by adiposity measures
4 was higher among diabetics, the obese, non-hypertensive subjects, and subjects 62 years or
5 younger (Table 1S) (supplementary material).
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9 **DISCUSSION**

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11 The results of this study show that adiposity measures have a negative association with
12 arterial stiffness, especially CAVI. BMI and CUN-BAE have greater explanatory power of
13 the variability of CAVI and baPWV than the WHtR and the BRI, and the explanatory
14 capacity was higher in the case of CAVI. We found a negative association of different
15 adiposity measures with CAVI and baPWV after adjustment for other cardiovascular risk
16 factors.
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25 This negative association with CAVI has already been described in previous studies. BMI has
26 shown a negative association in work performed on children ⁽³⁹⁾, as well as in hypertensive
27 and type 2 diabetes patients in Ghana ⁽⁴⁰⁾. Similarly, waist circumference has shown a
28 negative relation in subjects with metabolic syndrome ^(41; 42).
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34 However, other authors have described a positive association of different adiposity measures
35 with the β -stiffness parameter, but after adjustment for age and other possible confounding
36 factors, the association remained for only men with type 2 diabetes mellitus ⁽⁴³⁾. On the other
37 hand, other studies have also found no association between BMI and CAVI ⁽⁴⁴⁾.
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43 Studies analyzing the association of adiposity measures with baPWV have been performed in
44 mainly Eastern populations and have focused on assessing the association of BMI and waist
45 circumference as measures of adiposity. The results have also not been concordant, with some
46 finding a negative association with BMI. Studies analyzing the association of adiposity
47 measures with baPWV have been performed in mainly Eastern populations and have focused
48 on assessing the association of BMI and waist circumference as measures of adiposity. The
49 results have also not been concordant, with some finding a negative association with BMI ⁽⁴⁵⁾,
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3 and with waist circumference in men only or in women only ^{(46), (47)}. However, other studies
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5 have found a positive correlation with BMI and waist circumference ⁽⁴⁸⁾. One study that
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7 analyzed the association of different adiposity parameters with baPWV in middle-aged adults
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9 found a positive association with waist circumference and visceral fat but not with body fat
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11 percentage ⁽⁴⁹⁾.
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14 The results are also not consistent in works that have used carotid-femoral pulse wave
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16 velocity (cfPWV) as a measure of stiffness. Some studies have described a greater association
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18 with measures of central or visceral adiposity in both the general population and in diabetics
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20 (10; 13; 17; 18; 19; 21). However, Strasser et al. ⁽⁴⁹⁾ found no association with body fat percentage,
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22 and other studies also found no association between BMI and cfPWV ^(13; 14). Rodrigues et al
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24 ⁽¹⁵⁾, reported that BMI was negatively associated with cfPWV ($\beta = -0.103$) in a large
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26 population sample.
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30 Arterial stiffness depends on the elasticity of the arterial wall and the diameter of the arterial
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32 wall, and a positive correlation has been found between BMI and aortic diameter measured by
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34 nuclear magnetic resonance ⁽⁵⁰⁾. This could be a factor that explains the negative association
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36 between measures of adiposity and arterial stiffness. These discrepancies between studies may
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38 be partially explained by different methods of arterial stiffness measurement and the
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40 adjustment variables used. The reason may be that CAVI reflects central and peripheral
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42 arterial stiffness and is less influenced by blood pressure values at the time of measurement
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44 ^(36; 51; 52). In contrast, arterial stiffness assessed with baPWV is a measure of peripheral arterial
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46 stiffness ⁽³⁷⁾. Other possible reasons that may influence the observed differences are age, sex,
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48 race, prevalent cardiovascular risk, and drugs used for treatment of the different risk factor ^{(12;}
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50 ^{45; 47; 48)}.
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54 The proportion of baPWV variability explained by adiposity measurements in our study was
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56 small (between 0.8% for BMI and 0.1% for WHtR and BRI). The results are also lower than
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3 those published for the general population by Wohlfahrt et al. (5% for WHtR and 3% for
4 BMI) ⁽¹⁰⁾ but similar to the results reported by Rodrigues et al. (BMI 0.7%) ⁽¹⁵⁾. The
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6 proportion of CAVI variability explained by adiposity measures was higher than 7% with
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8 CUN-BAE and BMI and higher than 4% with WHtR and BRI. In the subgroup analysis, the
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10 proportion of CAVI variability explained by adiposity measures was higher in diabetic, obese,
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12 younger, and non-hypertensive subjects. Our results indicate the influence of adiposity
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14 measurements on CAVI is greater than on baPWV. We have not found previous work that has
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16 analyzed this aspect with CAVI. The novel results of this study may have important clinical
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18 relevance since they show the associations of both general and abdominal obesity measures
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20 with CAVI and baPWV in subjects with intermediate cardiovascular risk. In addition, the
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22 results provide information that could be used in new prospective studies and could
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24 potentially help to improve cardiovascular risk equations.
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30 The main limitation of our study is its cross-sectional design, which does not allow us to
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32 establish causal relations or the direction of influence of adiposity measures in vascular
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34 function. Another limitation that must be mentioned is that the population was ethnically
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36 homogeneous (all subjects were Caucasians with intermediate cardiovascular risk). Therefore,
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38 the extrapolation of our findings may be limited.
39

40 **Conclusion**

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42 In conclusion, the adiposity measures analyzed show a negative association with measures of
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44 arterial stiffness. The explanatory capacity of the variability of arterial stiffness is greater with
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46 BMI and CUN-BAE and when using CAVI as a measure of stiffness rather than baPWV.
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48 These results suggest that measures of general adiposity and body fat percentage better
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50 explain the variability of CAVI than measures of abdominal and visceral adiposity.
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5 Lead author for this group: Rafel Ramos, Research Unit, Primary Health Care, Girona, Jordi
6
7 Gol Institute for Primary Care Research (IDIAP Jordi Gol), Catalonia, Spain, E-mail:
8
9 rramos.girona.ics@gencat.net. Coordinating Center: Rafel Ramos, Ruth Martí, Dídac
10
11 Parramon, Anna Ponjoan, Miquel Quesada, Maria Garcia-Gil, Martina Sidera and Lourdes
12
13 Camós, Research Unit, Primary Health Care, Jordi Gol Institute for Primary Care Research
14
15 (IDIAP Jordi Gol), C/Maluquer Salvador, 11, 17002-Girona, Catalonia, Spain. Fernando
16
17 Montesinos, Ignacio Montoya, Carlos López, Anna Agell, Núria Pagès of the Primary Care
18
19 Services, Girona, Catalan Institute of Health (ICS), Catalonia, Spain. Irina Gil, Anna Maria-
20
21 Castro of the Primary Care Services, Girona, Institut d'Assistència Sanitaria (IAS), Catalonia,
22
23 Spain. Fernando Rigo, Guillermo Frontera, Antònia Rotger, Natalia Feuerbach, Susana Pons,
24
25 Natividad Garcia, John Guillaumet, Micaela Llull and Mercedes Gutierrez of the Health
26
27 Center Primary Care San Agustín, Ibsalut Balears, Spain. Cristina Agudo-Conde, Leticia
28
29 Gómez-Sanchez, Carmen Castaño-Sanchez, Carmela Rodriguez-Martín, Benigna Sanchez-
30
31 Salgado, Angela de Cabo-Laso, Emiliano Rodriguez-Sanchez, Jose Angel Maderuelo-
32
33 Fernandez, Marta Gómez-Sánchez, Emilio Ramos-Delgado, Carmen Patino-Alonso, Jose I
34
35 Recio-Rodriguez, Manuel A Gomez-Marcos and Luis Garcia-Ortiz, Primary Care Research
36
37 Unit of The Alamedilla, Salamanca, Spain, Castilla and León Health Service–SACYL.

38
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40
41 interpreted the results, prepared the manuscript draft, performed all analytical testing,
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44
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48
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50
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3 **Figure legends**
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5 **Figure 1:** Flow chart of this MARK substudy.
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7 Abbreviations: N, number; CAVI, cardio-ankle vascular index; baPWV, brachial-ankle pulse
8 wave velocity; ABI, ankle-brachial index; WC, waist circumference.
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3 **Figure 2:** Data are given as medias estimadas de CAVI y baPWV por cuartiles de BMI,
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5 WHtR, CUN-BAE and BRI.

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7 Adjusted for age (years), gender (0=male and 1=female), systolic blood pressure smoking
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9 (0=No and 1=Yes), atherogenic index, and HbA1c.

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11 baPWV and CAVI levels were compared using an ANOVA test, followed by post hoc
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13 analysis using a Bonferroni test.

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16 CAVI: $p < 0.05$ entre cuartiles, excepto entre el cuartil 2 y el cuartil 3 con el BRI y la WHtR
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18 (p= 0.999).

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21 baPWV: $p < 0.05$ entre cuartiles con BMI entre los cuartiles 1 - 4, 2 - 4 y 3-4 and con CUN-
22
23 BAE entre los cuartiles 1 - 3, 1 - 4 and 2 -4. $P > 0.05$ entre cuartiles de BRI y de WHtR.

24
25 Abbreviations: CAVI, cardio-ankle vascular index. ba-PWV, brachial-ankle pulse wave
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27 velocity. BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, University clinic
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29 of Navarra - body adiposity estimator. BRI, body roundness index.
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Table 1 General characteristics of all the sample and by gender.

Variables	Global (n=2354)	Males (n=1456)	Females (n=898)	p Value
Age (years)	61.4±7.7	61.1±8.1	61.8±7.0	0.030
Smoking n (%)	658 (28.0)	456 (31.5)	202 (22.7)	<0.001
Alcohol (gr/week)	72.2±117.5	102.2±133.3	23.6±59.9	<0.001
Height (cm)	165±9	170±7	156±6	<0.001
Weight (kg)	79.4±14.6	83.9±13.4	72.2±13.3	<0.001
BMI (kg/m ²)	29.2±4.4	29.1±3.9	29.5±5.1	0.035
BMI ≥ 30 n (%)	847 (36.0)	485 (33.4)	362 (40.4)	0.001
Waist circumference (cm)	100.9±11.6	102.9±10.5	97.6±12.5	<0.001
WHtR	0.61±0.07	0.61±0.06	0.62±0.08	<0.001
CUN-BAE	35.7±1.7	31.1±4.5	43.1±5.1	<0.001
BRI	5.8±1.7	5.7±1.5	6.1±2.1	<0.001
SBP (mmHg)	137.1±17.4	138.9±17.1	134.2±17.5	<0.001
DBP (mmHg)	84.4±10.2	85.5±10.4	82.7±9.7	<0.001
Heart rate (beats minutes)	74.2±10.2	73.3±12.7	75.8±11.6	<0.001
Hypertension n (%)	1712 (72.7)	1122 (80.1)	590 (75.4)	<0.001
Antihypertensive drugs n (%)	1199 (50.9)	729 (50.2)	470 (52.6)	0.289
Total Cholesterol (mg/dl)	225.8±40.9	220.8±39.1	233.9±42.5	<0.001
LDL Cholesterol (mg/dl)	140.4±34.9	138.9±34.2	142.8±35.8	0.011
HDL Cholesterol (mg/dl)	49.8±12.9	47.9±11.9	52.9±13.8	<0.001
Triglycerides (mg/dl)	145.5±96.6	150.3±106.3	137.7±77.9	0.001
Atherogenic index	4.8±1.3	4.8±1.3	4.7±1.3	0.002
Dyslipidemia n (%)	1585 (67.3)	927 (63.6)	658 (73.1)	<0.001
Lipid lowering drugs n (%)	671 (28.5)	392 (26.8)	279 (31.0)	0.034
FPG (mg/dl)	107.2±34.8	106.9±33.9	107.6±36.1	0.659
HbA1c	4.8±1.3	5.9±1.4	6.1±1.4	0.001
Diabetes n (%)	791 (33.6)	463 (31.8)	328 (36.5)	0.020
Antidiabetic drugs n (%)	474 (20.1)	269 (18.5)	205 (22.9)	0.011
CAVI	8.8±1.2	8.9±1.2	8.6±1.1	<0.001
baPWV (m/s)	14.9±2.5	14.8±2.5	15.0±2.6	0.107

Values are means and (standard deviations) for continuous data and number and (proportions) for categorical data.

Abbreviations: BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, University clinic of Navarra - body adiposity estimator. BRI, body roundness index. SBP, systolic blood pressure. DBP, diastolic blood pressure. LDL, low density lipoprotein. HDL, high density lipoprotein. FPG, fasting plasma glucose. HbA1c, glycosylated hemoglobin. CAVI, cardio ankle vascular index. baPWV, braquial-ankle pulse wave velocity.

p value differences between male and females

Table 2: Bivariate correlations of adiposity measures with CAVI and baPWV.

	CAVI		baPWV	
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a
BMI	-0.264**	-0.303**	-0.035	-0.068**
WHtR	-0.119**	-0.222**	0.090**	0.001
CUN-BAE	-0.187**	-0.297**	0.054*	-0.063**
BRI	-0.125**	-0.218**	0.078**	0.005

Abbreviations: CAVI, cardio-ankle vascular index. baPWV, brachial-ankle pulse wave velocity. BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, University clinic of Navarra - body adiposity estimator. BRI, body roundness index.

^a Adjusted for age, sex and systolic blood pressure.

p-values by Pearson correlation. * p<0.05, ** p< 0.01.

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Table 3: Multiple regression analysis: association between adiposity measures with CAVI and baPWV.

	R ²	β(95%CI)	Partial R ²	p value
CAVI				
BMI	0.410	-0.075 (-0.084 to -0.066)	0.075	<0.001
WHtR	0.378	-3.580 (-4.137 to -3.022)	0.043	<0.001
CUN-BAE	0.407	-0.067 (-0.075 to -0.060)	0.072	<0.001
BRI	0.376	-0.142 (-0.165 to -0.119)	0.041	<0.001
baPWV				
BMI	0.398	-0.055 (-0.073 to -0.036)	0.008	<0.001
WHtR	0.391	-1.402 (-2.594 to -0.211)	0.001	0.021
CUN-BAE	0.397	-0.056 (-0.073 to -0.039)	0.007	<0.001
BRI	0.391	-0.060 (-0.109 to -0.012)	0.001	0.014

Four different multiple linear regression models were used to analyze the associations of adiposity measures with CAVI and baPWV.

Adjusted for age (years), gender (0=male and 1=female), systolic blood pressure smoking (0=No and 1=Yes), atherogenic index, and HbA1c.

Abbreviations: CAVI, cardio-ankle vascular index. baPWV, brachial-ankle pulse wave velocity. BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, University clinic of Navarra - body adiposity estimator. BRI, body roundness index.

Table 4: Association between adiposity measures with CAVI in different groups.

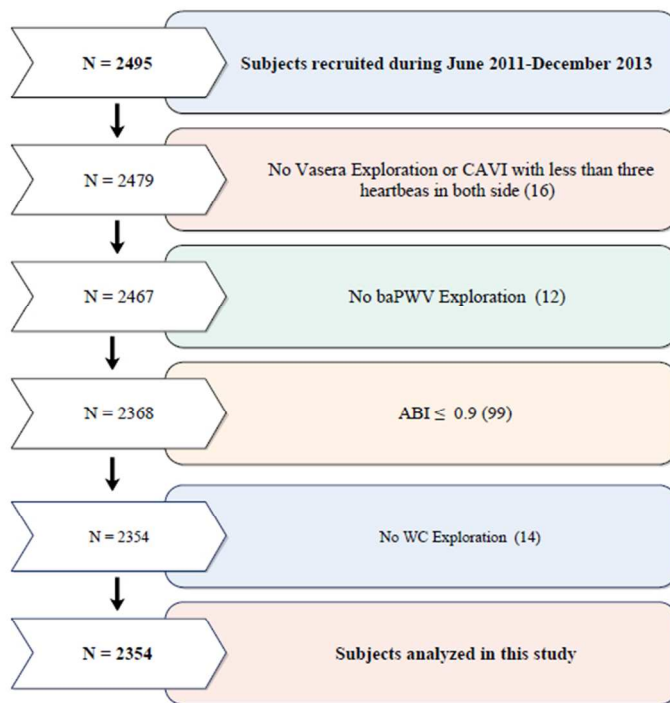
	R ²	β(95%CI)	Partial R ²	p value
CAVI				
Hypertensive				
BMI	0.408	-0.075 (-0.086 to -0.064)	0.068	<0.001
WHtR	0.383	-3.816 (-4.512 to -3.120)	0.043	<0.001
CUN-BAE	0.406	-0.068 (-0.078 to -0.058)	0.066	<0.001
BRI	0.383	-0.157 (-0.186 to -0.129)	0.043	<0.001
Non hypertensive				
BMI	0.376	-0.073 (-0.088 to -0.058)	0.095	<0.001
WHtR	0.326	-2.999 (-3.915 to -2.083)	0.056	<0.001
CUN-BAE	0.407	-0.068 (-0.082 to -0.054)	0.091	<0.001
BRI	0.321	-0.110 (-0.147 to -0.074)	0.050	<0.001
Diabetics				
BMI	0.434	-0.088 (-0.102 to -0.076)	0.102	<0.001
WHtR	0.395	-4.478 (-5.411 to -3.545)	0.039	<0.001
CUN-BAE	0.436	-0.084 (-0.097 to -0.070)	0.104	<0.001
BRI	0.387	-0.154 (-0.200 to -0.127)	0.047	<0.001
Non diabetics				
BMI	0.392	-0.067 (-0.078 to -0.056)	0.054	<0.001
WHtR	0.365	-3.057 (-3.764 to -2.350)	0.027	<0.001
CUN-BAE	0.393	-0.060 (-0.070 to -0.050)	0.055	<0.001
BRI	0.365	-0.128 (-0.158 to -0.099)	0.027	<0.001
Obese				
BMI	0.414	-0.090 (-0.109 to -0.071)	0.057	<0.001
WHtR	0.376	-2.985 (-4.512 to -3.120)	0.036	<0.001
CUN-BAE	0.413	-0.098 (-0.120 to -0.077)	0.058	<0.001
BRI	0.376	-0.112 (-4.125 to -1.846)	0.036	<0.001
Non obese				
BMI	0.371	-0.063 (-0.083 to -0.043)	0.016	<0.001
WHtR	0.358	-1.397 (-2.371 to -0.424)	0.002	0.005
CUN-BAE	0.370	-0.050 (-0.067 to -0.034)	0.015	<0.001
BRI	0.357	0.048 (-0.089 to -0.007)	0.001	0.021
≤ 62 years				
BMI	0.358	-0.077 (-0.087 to -0.066)	0.107	<0.001
WHtR	0.321	-4.017 (-4.731 to -3.302)	0.070	<0.001
CUN-BAE	0.352	-0.067 (-0.077 to -0.058)	0.101	<0.001
BRI	0.318	-0.158 (-0.059 to -0.092)	0.067	<0.001
> 62 years				
BMI	0.231	-0.074 (-0.088 to -0.060)	0.074	<0.001
WHtR	0.191	-3.108 (-3.9980 to -2.235)	0.034	<0.001
CUN-BAE	0.230	-0.071 (-0.085 to -0.058)	0.073	<0.001
BRI	0.190	0.125 (-0.161 to -0.089)	0.033	<0.001

Multiple linear regression models were used to analyze the associations of adiposity measures with CAVI by groups.

Adjusted for age (years), gender (0=male and 1=female), Systolic blood pressure, smoking (0=No and 1=Yes), atherogenic index, and HbA1c.

Abbreviations: CAVI, cardio-ankle vascular index. BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, University clinic of Navarra - body adiposity estimator. BRI, body roundness index.

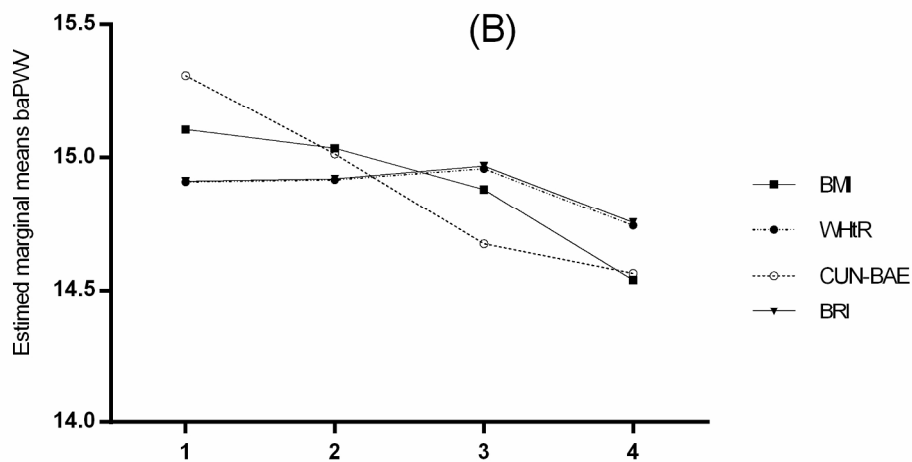
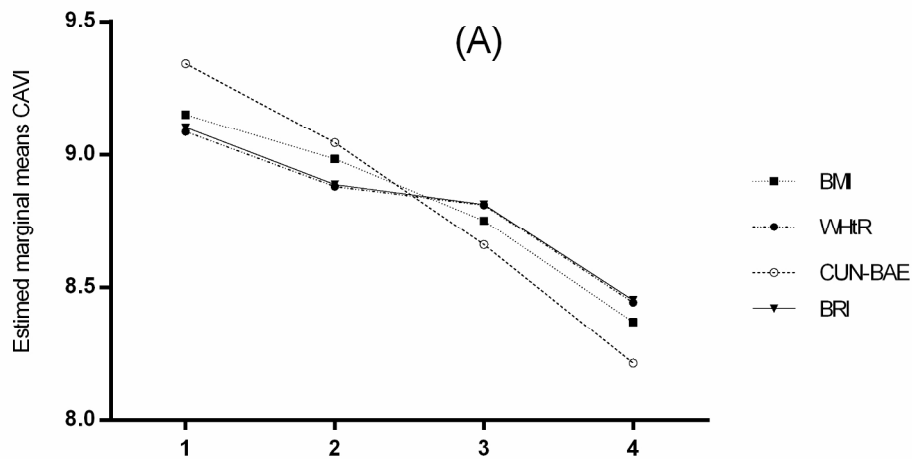
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195x236mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract Yes, Page 1 (b) Provide in the abstract an informative and balanced summary of what was done and what was found Yes, Page 2
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Yes, Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses Yes, Page 4
Methods		
Study design	4	Present key elements of study design early in the paper Yes, Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Yes, Page 5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants Yes, Page 5 (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case Yes, Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Yes, Page 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Yes, Page 6-9
Bias	9	Describe any efforts to address potential sources of bias Yes, Page 8-9
Study size	10	Explain how the study size was arrived at Yes, Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Yes, Page 8-9

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- Statistical methods 12 (a) Describe all statistical methods, including those used to control for confounding **Yes, Page 8-9**
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- (b) Describe any methods used to examine subgroups and interactions **Yes, Page 8-9**
-
- (c) Explain how missing data were addressed **Yes, Page 8-9**
-
- (d) *Cohort study*—If applicable, explain how loss to follow-up was addressed
Case-control study—If applicable, explain how matching of cases and controls was addressed
Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
-
- (e) Describe any sensitivity analyses **Yes, Page 8-9**

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Yes, Page 10-11 (b) Give reasons for non-participation at each stage Yes, Page 10-11 (c) Consider use of a flow diagram Yes, Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Yes, Page 10 (b) Indicate number of participants with missing data for each variable of interest Yes, Page 10 (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Yes, Tables (b) Report category boundaries when continuous variables were categorized Yes, tables (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Yes, Page 10

Discussion

Key results	18	Summarise key results with reference to study objectives Yes, Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Yes, Page 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Yes, Page 11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results Yes, Page 11

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Yes, Page 15
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Title: Adiposity measures and arterial stiffness in primary care: the MARK prospective observational study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016422.R1
Article Type:	Research
Date Submitted by the Author:	27-Apr-2017
Complete List of Authors:	Gomez-Sanchez, Leticia; La Alamedilla health centre., Primary Care Research Unit; 2. Biomedical Research Institute of Salamanca (IBSAL) Garcia-Ortiz, Luis; La Alamedilla health centre. University of Salamanca, Primary care research unit La Alamedilla Patino-Alonso, Maria; University of Salamanca, Salamanca, Spain , Statistics Department, Recio-Rodriguez, Jose; La Alamedilla Health Center, Primary Care Research Unit, The Alamedilla Health Center, Castilla and León Health Service (SACYL), Biomedical Research Institute of Salamanca (IBSAL) Frigo, Fernando; Illes Balears Health Service (IBSALUT) Marti, Rhut; Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Unitat de Suport a la Recerca de Girona. Agudo-Conde, Cristina; La Alamedilla Health Center,, Research Unit Rodriguez-Sanchez, Emiliano; Primary Care Research Unit, The Alamedilla Health Center, Salamanca, Spain, ; Biomedical Research Institute of Salamanca (IBSAL), Maderuelo-Fernandez, Jose; Salamanca Institute for Biomedical Research. (IBSAL)., Primary care research unit La Alamedilla, Castilla and León Health Service (SACYL). Network of Preventive Activities and Health Promotion in Primary Care (REDIAPP) Ramos, Rafel; Universidad de Girona. Departament Medicina Gomez-Marcos, Manuel; the Alamedilla Health Center, Castilla and León Health Service (SACYL), Primary Care Research Unit; University of Salamanca,, Department of Medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Diabetes and endocrinology, Epidemiology, Nutrition and metabolism
Keywords:	Body mass index, Waist circumference, Waist-to-height ratio, Fat mass percent., Body roundness index., Arterial stiffness

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Manuscripts

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3 1 **Title: Adiposity measures and arterial stiffness in primary care: the MARK prospective**
4 **observational study**

5
6 3 **Authors:** Leticia Gomez-Sanchez MD ^{1,2*}, Luis Garcia-Ortiz MD, PhD ^{1,2,3}, Maria C Patino-
7
8 4 Alonso PhD ^{1,2,4}, Jose I Recio-Rodriguez PhD ^{1,2,5}, Rigo Fernando MD, PhD ⁶, Ruth Marti
9
10 5 PhD ^{7,8}, Cristina Agudo-Conde ^{1,2}, Emiliano Rodriguez-Sanchez MD, PhD ^{1,2,9}, Jose A
11
12 6 Maderuelo-Fernandez MD, PhD ^{1,2}, Rafel Ramos MD, PhD ^{7,8,10} and Manuel A Gomez-
13
14 7 Marcos MD, PhD ^{1,2,9} for the MARK Group ¹¹

15
16 8 1. Primary Care Research Unit, the Alamedilla Health Center, Castilla and León Health
17
18 9 Service (SACyL), Salamanca, Spain

19
20 10 2. Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain

21
22 11 3. Biomedical and diagnostic sciences department, University of Salamanca, Salamanca,
23
24 12 Spain

25
26 13 4. Statistics Department, University of Salamanca, Salamanca, Spain

27
28 14 5. Department of Nursing and Physiotherapy, University of Salamanca, Salamanca, Spain

29
30 15 6. San Agustín Health Center, Illes Balears Health Service (IBSALUT), Palma of Mallorca,
31
32 16 Spain

33
34 17 7. Unitat de Suport a la Recerca de Girona. Institut Universitari d'Investigació en Atenció
35
36 18 Primària Jordi Gol (IDIAP Jordi Gol), Girona, Spain

37
38 19 8. Institut d'Investigació Biomèdica of Girona Dr. Josep Trueta (IDBGI). Girona, Spain

39
40 20 9. Department of Medicine, University of Salamanca, Salamanca, Spain

41
42 21 10. Departament de Ciències Mèdiques, Facultat de Medicina. Universitat de Girona, Spain.

43
44 22 11. MARK Group. redIAPP: Research Network in Preventive Activities and Health
45
46 23 Promotion, Girona, Spain

47
48 24 ***Corresponding Author:**

49
50 25 Leticia Gomez-Sanchez, Primary Care Research Unit, Alamedilla Health Care Center

51
52 26 37003 Salamanca, Spain

53
54 27 E-mail addresses: leticiagmzsncz@gmail.com

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56 28 Full list of author information is available at the end of the article.

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1
2
3 **ABSTRACT**
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5 **Background:** The cardiovascular risk in obesity is potentially increased by arterial stiffness.
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7 **Objective:** This study investigates the relationship of adiposity measures with arterial
8 stiffness in Caucasian adults with intermediate cardiovascular risk and determines the best
9
10 measures to explain arterial stiffness.
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14 **Setting:** Six primary care centers in three Spanish Autonomous Communities, Spain
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16 **Participants:** This study analyzed 2354 subjects (age range, 35–74 years; mean age, 61.4±7.7
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18 years, 61.9% male).
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20 **Methods:** The study was based on cross-sectional data from the MARK study. The main
21
22 outcome variables were: body mass index (BMI), waist-to-height ratio (WHtR), University
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24 clinic of Navarra body adiposity estimation (CUN-BAE) body fat percentage, and body
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26 roundness index (BRI). Vascular function was assessed by the cardio-ankle vascular index
27
28 (CAVI) with the VaSera device, and brachial ankle pulse wave velocity (baPWV) was
29
30 determined using a validated equation.
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34 **Results:** The mean adiposity measures were a BMI of 29.2±4.4, WHtR of 0.61±0.07, CUN-
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36 BAE of 35.7±1.7, and BRI of 5.8±1.7. The mean stiffness measures were a CAVI of 8.8±1.2
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38 and baPWV of 14.9±2.5. In multiple linear regression analysis, all adiposity measures were
39
40 negatively associated with CAVI and baPWV (p<0.01 for all) after adjustment for
41
42 cardiovascular risk factors. The proportion of CAVI variability explained by the adiposity
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44 measures were 5.5% for BMI, 5.8% for CUN-BAE, 3.8% for WHtR, and 3.7% for BRI,
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46 which were higher among diabetic, obese, younger (≤ 62 years), and non-hypertensive
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48 subjects who had similar activity and sedentary profiles.
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51 **Conclusions:** Adiposity measures are negatively associated with arterial stiffness measures.
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53 The arterial stiffness variability is better explained for BMI and CUN-BAE and when CAVI
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55 is used as a measure of stiffness rather than baPWV.
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3 1 **Trial Registration:** Clinical Trials.gov Identifier: [https://clinicaltrials.gov/ct2/show/](https://clinicaltrials.gov/ct2/show/NCT01428934)
4
5 2 NCT01428934. Registered 2 September 2011. Last updated September 8, 2016.
6

7 3 **Keywords:** body mass index, waist circumference, waist-to-height ratio, fat mass percent,
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9 4 body roundness index, arterial stiffness
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14 6 **Strengths and limitations of this study**

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16 7 • This is the first study to investigate the association between adiposity measures using a
17
18 8 cardio-ankle vascular index with and brachial ankle pulse wave velocity in Caucasian adults
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20 9 with intermediate cardiovascular risk.
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22
23 10 • All adiposity measures were negatively associated with CAVI and baPWV.
24

25 11 • The arterial stiffness variability was better explained for BMI and CUN-BAE and
26
27 12 when CAVI was used as a measure of stiffness rather than baPWV.
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29 13 • The main limitation of our study is its cross-sectional design, which does not allow
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31 14 establishment of causal relationships or the direction in which adiposity measures influence
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33 15 vascular function.
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1 INTRODUCTION

2 Obesity has been linked to increased all-cause and cardiovascular mortality ⁽¹⁾. However, the
3 mechanisms through which obesity can increase the frequency of cardiovascular disease
4 beyond traditional risk factors are not clearly identified ⁽²⁾. Arterial stiffness as evaluated by
5 the brachial ankle pulse wave velocity (baPWV) is an independent predictor of coronary heart
6 disease and mortality in both the general population ⁽³⁾ and in patients with diabetes mellitus
7 ^(4; 5). Similarly, the cardio-ankle vascular index (CAVI) is associated with carotid and
8 coronary atherosclerosis ^(6; 7; 8) and is a predictor of cardiovascular events in obese patients ⁽⁹⁾.

9 Increased arterial stiffness may be a mechanism by which obesity increases cardiovascular
10 risk independently of traditional risk factors. However, the relationship between adiposity and
11 arterial stiffness remains controversial. The body mass index (BMI) has been associated with
12 arterial stiffness in the general population ^(10; 11) and in diabetic patients ^(12; 13). However, other
13 research has not found this association ⁽¹⁴⁾, the association disappeared after adjusting for
14 potential confounders ⁽¹³⁾, or it showed a negative association ^(15; 16). Additionally, there are
15 studies that suggest a stronger correlation of measures of central or visceral adiposity than
16 measures of general adiposity with arterial stiffness in the general population ^(10; 17; 18; 19; 20), in
17 diabetic patients ⁽¹³⁾, and in diabetics and hypertensive patients ⁽²¹⁾. The Whitehall II Cohort
18 study ⁽²²⁾ showed that all measures of general adiposity, central adiposity, and body fat
19 percentage were predictors of accelerated arterial stiffness in adults.

20 Cardiovascular events are more likely to occur in patients with intermediate cardiovascular
21 risk ⁽²³⁾, and there is a lack of studies analyzing the relationship of different adiposity
22 measures with arterial stiffness in these subjects. Thus, the primary aim of this study was to
23 investigate the relationship between adiposity measures and arterial stiffness in Caucasian
24 adults with intermediate cardiovascular risk. The secondary aim was to determine which
25 adiposity measures best explain arterial stiffness.

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5 2 **METHODS**6
7 3 **Study design**

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9 4 This trial is a cross-sectional study of subjects recruited to the *improving interMediAte RisK*
10 5 *management (MARK)* study ([NCT01428934](#))⁽²⁴⁾, which is a longitudinal study designed to
11 6 assess whether the ankle-brachial index, arterial stiffness (measured by CAVI), postprandial
12 7 glucose, glycosylated hemoglobin, self-measured blood pressure, and the presence of
13 8 comorbidities are independently associated with the occurrence of vascular events. It also
14 9 investigates whether the predictive capacity of current risk equations can be improved in the
15 10 intermediate risk population. The current study focuses on the baseline visit. The second step
16 11 will be a 5- and 10-year follow-up trial to assess cardiovascular morbidity and mortality.
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29 13 **Study population**

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31 14 In this multicenter project, the study population was obtained by random sampling from
32 15 subjects who met the inclusion criteria and who were seeing general practitioners from July
33 16 2011 to June 2013 at six primary care centers in three Spanish Autonomous Communities.
34 17 Subjects were recruited from those aged 35 to 74 years with an intermediate cardiovascular
35 18 risk, defined as a 10-year coronary risk ranging from 5%–15% according to the adapted
36 19 Framingham risk equation⁽²⁵⁾; a 10-year vascular mortality risk ranging from 1%–5%
37 20 according to the scoring risk in Europeans equation⁽²⁶⁾; or a moderate risk according to the
38 21 European Society of Hypertension guidelines for the management of arterial hypertension⁽²⁷⁾.
39 22 Exclusion criteria included end-stage disease or institutionalization at the time of the visit, or
40 23 history of atherosclerotic disease. This study analyzed 2354 of the 2495 subjects recruited in
41 24 the MARK study, and the exclusion criteria are shown in Figure 1.
42 25

1 Variables and measurement instruments

2 A detailed description of procedures for clinical data collection and laboratory tests has been
3 published elsewhere⁽²⁴⁾.

5 Anthropometric measurements

6 Body weight was measured twice using a certified electronic scale (Seca 770, Medical scale
7 and measurement systems, Birmingham, United Kingdom) after adequate calibration
8 (precision ± 0.1 kg). Readings were rounded-off to the nearest 100 g. Height was measured
9 using a stadiometer (Seca 222), and the average of two measurements was recorded. Body
10 mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist
11 circumference was measured according to the 2007 recommendations of the Spanish Society
12 for the Study of Obesity⁽²⁸⁾. All measurements were performed with the subjects standing,
13 wearing no shoes, and in light clothing. Waist-to-height ratio (WHtR) was calculated as the
14 waist circumference (cm) divided by height (cm)^(29;30).

15 The body fat percentage (BF%) was calculated according to the University clinic of
16 Navarra - body adiposity estimator (CUN-BAE) using the recommendations of Gomez-
17 Ambrosi et al.⁽³¹⁾: $BF\% = -44.988 + (0.503 \times \text{Age}) + (10.689 \times \text{sex}) + (3.172 \times \text{BMI}) - (0.026$
18 $\times \text{BMI}^2) + (0.181 \times \text{BMI} \times \text{sex}) - (0.02 \text{ BMI} \times \text{Age}) - (0.005 \times \text{BMI}^2 \times \text{sex}) +$
19 $(0.00021 \times \text{BMI}^2 \times \text{Age})$, where male=0 and female=1 for sex.

20 Body roundness index (BRI) was calculated using the following formula: $BRI = 364.2 -$

21 $365.5 \times \sqrt{1 - \left(\frac{WC/(2\pi)}{0.5 \text{height}}\right)^2}$ ⁽³²⁾. BRI can predict the percentage of body fat and visceral adipose
22 tissue.

24 Cardio-ankle vascular index (CAVI) and brachial-ankle pulse wave velocity (baPWV)

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3 1 CAVI was measured using a *VaSera VS-1500®* device (*Fukuda Denshi*)^(33; 34). CAVI values
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5 2 are calculated automatically by estimating the stiffness parameter β using the following
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7 3 equation: $\beta = 2\rho \times 1 / (Ps - Pd) \times \ln (Ps / Pd) \times PWV^2$, where ρ is blood density, Ps and Pd
8
9 4 are SBP and DBP in mmHg, and PWV is measured between the aortic valve and the ankle
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11 5 ⁽³⁵⁾. The mean coefficient of variation of CAVI measurement is less than 5%, which is small
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13 6 enough to allow for clinical use of the index and confirms that CAVI has a favorable
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15 7 reproducibility⁽³⁴⁾.

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19 8 CAVI was calculated using the *VaSera VS-1500®* device (*Fukuda Denshi*), and with the
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21 9 values obtained, the baPWV was estimated using the equation $baPWV = (0.5934 \times \text{height}$
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23 10 $(\text{cm}) + 14.4724) / tba$ (where tba is the time interval between the arm and ankle waves)⁽³⁶⁾.

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26 11 Measurements were performed with the patient in the supine position after resting for 10
27
28 12 minutes in a quiet room at a stable temperature. Subjects were instructed not to smoke or
29
30 13 exercise in the hour before the test.

34 15 **Diagnosis of cardiovascular risk factors**

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37 16 Subjects were considered to be hypertensive if they were previously diagnosed with
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39 17 hypertension, if they were taking antihypertensive drugs, or if they had blood pressure levels
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41 18 $\geq 140/90$ mmHg. Diabetic subjects were those who had been previously diagnosed with the
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43 19 disease, were taking hypoglycemic drugs, or had a fasting blood glucose levels ≥ 126 mg/dL
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45 20 or HbA1c $\geq 6.5\%$. Dyslipidemia was defined if they were treated with lipid-lowering drugs or
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47 21 had altered LDL ≥ 130 mg/dl, HDL ≤ 45 mg/dl in men and ≤ 55 in women, and TG ≥ 150
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49 22 mg/dl, as established by the European Society of Cardiology (ESC) and the European
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51 23 Atherosclerosis Society (EAS) 2011⁽³⁷⁾.

56 25 **Office or clinical blood pressure**

1 Office blood pressure measurement involved three measurements of SBP and DBP using a
2 validated OMRON model M10-IT sphygmomanometer (Omron Health Care, Kyoto, Japan).
3 The measurements followed the recommendations of the European Society of Hypertension
4 ⁽³⁸⁾, and the average of the last two measurements was used.

6 **Lifestyles**

7 **Tobacco**

8 Smoking history was assessed by asking questions about the participant's smoking status
9 (smoker/non-smoker). We considered smokers to include those who currently smoke or who
10 had stopped smoking within the past year.

12 **Leisure time physical activity**

13 Leisure time physical activity (LTPA) was collected using the Minnesota LTPA Questionnaire
14 ⁽³⁹⁾ that was validated for Spanish men and women^(40; 41). The questionnaire was administered
15 by trained interviewers who spent about 10 to 20 min per participant collecting detailed
16 information about physical activity (PA) during the preceding year, the number of times this
17 activity was performed, and the average duration of each activity on each occasion. Each PA
18 has an intensity code, based on the ratio between the metabolic rate during PA practice and the
19 basal metabolic rate (MET) ⁽⁴²⁾. We assumed that 1 MET approximately corresponds to 1
20 kcal/min of energy expenditure. Therefore, we can calculate the total energy expenditure in
21 leisure time of PA (EEPA_{total}) in kilocalories per week. Moreover, based on the PA intensity
22 code, we could quantify the energy expenditure in physical activity (EEPA) according to the
23 activity's classification as intense, moderate, or light intensity as follows: light PA intensity is
24 below 4 METs, such as walking (EEPA_{light}). Moderate PA intensity is 4–5.5 METs, such as
25 brisk walking (EEPA_{moderate}). Intense PA intensity is greater than or equal to 6 METs, such as
26 jogging (EEPA_{intense}). Thus, for each particular subject: EEPA_{total} = EEPA_{light} + EEPA_{moderate}
27 + EEPA_{intense}.

28 Based on recommendations from the American Heart Association ⁽⁴³⁾, we considered those
29 participants who do not meet the recommendations of moderate-intensity aerobic PA practice

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3 1 for a minimum of 30 min on 5 days each week (EEPA_{moderate} < 675 kcal/week) or high-
4 2 intensity aerobic PA practice for a minimum of 20 min on 3 days each week (EEPA_{intense} <
5 3 420 kcal/week) to be sedentary.
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10 5 **Laboratory determinations**

11
12 6 Venous blood sampling was performed between 08:00 and 09:00 hours after the individuals
13 7 fasted and abstained from smoking and the consumption of alcohol and caffeinated beverages
14 8 for the previous 12 hours. Fasting plasma glucose, serum, high-density lipoprotein (HDL)
15 9 cholesterol concentrations and triglyceride concentrations were measured using standard
16 10 enzymatic automated methods. Low-density lipoprotein cholesterol was estimated using the
17 11 Friedewald equation when the direct parameter was not available. The atherogenic index was
18 12 estimated (total cholesterol / HDL cholesterol). Blood samples were collected at the Health
19 13 Center and analyzed at the hospital of reference.
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30 14 The researchers who performed the different tests were blinded to the clinical data of the
31 15 subjects. All assessments were made within a period of 10 days.
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37 17 **Data analysis**

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39 18 Continuous variables were expressed as the mean \pm standard deviation. Frequency
40 19 distributions were used for categorical data. Comparisons between quantitative variables were
41 20 performed using the Student's *t*-test, while the chi-squared test and Fisher's exact test were
42 21 used for qualitative variables. Pearson's correlation coefficient was used to estimate the
43 22 relationship of the adiposity measures to CAVI and baPWV.
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50 23 Four different multiple linear regression models were used to analyze the associations of
51 24 each adiposity measure with CAVI, and four other models were used with baPWV. CAVI and
52 25 baPWV were the dependent variables, and the adiposity measures were the independent
53 26 variables in each model. All models were adjusted for age (years), sex (0 = male and 1 =
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1 female), systolic blood pressure (SBP), smoking status (0 = No and 1 = Yes), atherogenic
2 index, HbA1c, and METs/min/week. The explanatory capacity of the model was measured by
3 R^2 , and the proportion attributed to each variable was estimated by the change in R^2 . The
4 analysis was also performed by age groups, diagnosis of diabetes, hypertension, and obesity.

5 ANCOVA models were used to test the differences in the mean values of CAVI and
6 baPWV with the quartiles of the four adiposity measures after adjusting for the confounding
7 variables that were used in the regression analysis. Pairwise post hoc comparisons were
8 examined using the Bonferroni test. Data were analyzed using SPSS Statistics for Windows
9 version 23.0 (IBM Corp, Armonk, NY). Values of $p < 0.05$ were considered statistically
10 significant.

11

12 **Ethics statement**

13 All participants were informed of the study objectives and procedures and they all signed an
14 informed consent form to participate. The study was approved by the Clinical Research Ethics
15 Committee of the Primary Care Research Institute Jordi Gol, the Health Care Area of
16 Salamanca, and Palma of Mallorca. The study was conducted following the recommendations
17 of the Declaration of Helsinki⁽⁴⁴⁾. The confidentiality of information provided by participants
18 was ensured, complying with the rules established by Spanish Organic Law 15/1999, of 13
19 December on the Protection of Personal Data.

20

21 **RESULTS**

22 Anthropometric measures, clinical characteristics, and vascular function measures of the
23 subjects are presented in Table 1. The mean age of the patients was 61.4 ± 7.7 years, and
24 61.9% were male. Male subjects had a higher percentage of smokers (31.5 vs. 22.7) and
25 hypertension (80.1 vs. 75.4) compared with females. However, females had a higher

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3 1 prevalence of obesity (40.4 vs. 33.4), sedentariness (53.7 vs. 37.0), dyslipidemia (73.1 vs.
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5 2 63.6), and diabetes (36.5 vs. 31.8) compared with males. The mean value of CAVI was
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7 3 8.8±1.2 (8.9 in males and 8.6 in females, $p<0.001$) and the mean baPWV was 14.9±2.5 (14.8
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9 4 in males and 15.0 in females). All of the analyzed adiposity measures except for waist
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11 5 circumference presented higher values in women compared with men.

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14 6 Pearson's correlation coefficient results between the adiposity measures and the vascular
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16 7 function parameters are shown in Table 2. All adiposity measures were negatively correlated
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18 8 with CAVI, and this correlation increases after adjusting for age, sex, and SBP. The
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20 9 correlation between CAVI and baPWV was $r=0.745$ ($p<0.001$).

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23 10 Figure 1S (Supplementary material) shows the estimated marginal means of CAVI (a) and
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25 11 baPWV (b) by quartiles of the different adiposity measures. After adjustment for the variables
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27 12 used in the multiple linear regression analysis, the mean CAVI values decreased as the
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29 13 quartiles of the four adiposity measurements increased ($p<0.05$). However, the same is not
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31 14 true of baPWV with WHtR and BRI ($p>0.05$).

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34 15 In the multiple linear regression analysis, CAVI and baPWV showed negative associations
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36 16 with all adiposity measures ($p<0.01$ for all) after adjustment for age, sex, SBP, smoking,
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38 17 atherogenic index, HbA1c, and METs/min/week (Table 3). The proportion of CAVI
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40 18 variability explained in the respective multiple linear regression analyses by the adiposity
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42 19 measures was 5.5% for BMI, 5.8% for CUN-BAE, 3.8% for WHtR, and 3.7% for BRI. For
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44 20 baPWV, the variance explained by the measures of adiposity were 0.7% for BMI, and CUN-
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46 21 BAE, and 0.1% for WHtR, and 0.2 for BRI.

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49 22 In the multiple linear regression analysis by subgroup, the proportion of CAVI variability
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51 23 explained by adiposity measures was higher among diabetics, obese, non-hypertensive, and
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53 24 subjects 62 years of age or younger, and was similar in active and sedentary people (Table 4).
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1 DISCUSSION

2 The results of this study show that adiposity measures have a negative association with
3 arterial stiffness, especially CAVI. BMI and CUN-BAE better explain the variability of CAVI
4 and baPWV than the WHtR and the BRI, and the explanatory capacity was higher in the case
5 of CAVI. We found a negative association of different adiposity measures with CAVI and
6 baPWV after adjustment for other cardiovascular risk factors.

7 In this study the mean value of CAVI was higher in males, which is in agreement with
8 published data indicating that CAVI increases linearly with age, and is higher in males than in
9 females (approximately 0.2, which is equivalent to 4–5 years old)^(22; 45). Similarly, there was
10 no difference in the mean baPWV between the sexes, which is consistent with data published
11 by Tomiyama et al.⁽⁴⁶⁾ who showed that the effect of age on baPWV is different according to
12 sex. Females have a higher arterial stiffness than prepubertal males, and this increases after
13 menopause. Men, however, experience a linear increase in arterial stiffness from puberty.
14 This suggests that women have large arteries that are intrinsically more rigid compared with
15 men, but these effects are mitigated by sex steroids during the reproductive years^(47; 48).

16 This negative association with CAVI has already been described in previous studies. BMI
17 has shown a negative association in children⁽⁴⁹⁾, and in hypertensive and type 2 diabetes
18 patients in Ghana⁽⁵⁰⁾. Similarly, waist circumference has shown a negative relationship in
19 subjects with metabolic syndrome^(51; 52).

20 However, other authors have described a positive association of different adiposity
21 measures with the β -stiffness parameter, but after adjustment for age and other possible
22 confounding factors, the association remained for only men with type 2 diabetes mellitus⁽⁵³⁾.
23 Other studies have also found no association between BMI and CAVI⁽⁵⁴⁾.

24 Studies analyzing the association of adiposity measures with baPWV have also been
25 performed mainly in Eastern populations and have focused on assessing the association

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3 1 between BMI and waist circumference as measures of adiposity. The results are controversial,
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5 2 with some finding a negative association with BMI ⁽⁴⁶⁾, and with waist circumference in men
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7 3 only or in women only ^{(55), (56)}. However, other studies have found a positive correlation with
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9 4 BMI and waist circumference ⁽⁵⁷⁾. One study that analyzed the association of different
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11 5 adiposity parameters with baPWV in middle-aged adults found a positive association with
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13 6 waist circumference and visceral fat but not with body fat percentage ⁽⁵⁸⁾.

16 7 The results are also not consistent in studies that used the carotid-femoral pulse wave
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18 8 velocity (cfPWV) as a measure of stiffness. Some studies have described a greater association
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20 9 with measures of central or visceral adiposity in both the general population and in diabetics
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22 10 ^(10; 13; 17; 18; 19; 21). However, Strasser et al. ⁽⁵⁸⁾ found no association with body fat percentage,
23
24 11 and other studies also found no association between BMI and cfPWV ^(13; 14). Rodrigues et al.
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26 12 ⁽¹⁵⁾, reported that BMI was negatively associated with cfPWV ($\beta = -0.103$) in a large
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28 13 population sample. The Whitehall II Cohort study ⁽²²⁾, which was completed based on staff
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30 14 lists from offices located in central London, showed that all measures of adiposity were robust
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32 15 predictors of accelerated CFPWV, after adjusting them for potential confounding factors. The
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34 16 use of different measures to measure arterial stiffness such as CAVI and baPWV, as well as
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36 17 being a population with intermediate cardiovascular risk could explain some of the
37
38 18 discrepancies with our study.

42 19 Arterial stiffness depends on arterial wall elasticity and diameter, and a positive correlation
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44 20 was found between BMI and aortic diameter measured by nuclear magnetic resonance ⁽⁵⁹⁾.
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46 21 This could partially explain the negative association between measures of adiposity and
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48 22 arterial stiffness. These discrepancies between studies may be partially explained by different
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50 23 methods of arterial stiffness measurement and the adjustment variables used. This may be
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52 24 because CAVI reflects central and peripheral arterial stiffness and is less influenced by blood
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54 25 pressure values at the time of measurement ^(35; 60; 61). Conversely, arterial stiffness assessed

1 using baPWV is a measure of peripheral arterial stiffness ⁽³⁶⁾. Other potential influences on
2 the observed differences are age, sex, race, prevalent cardiovascular risk, and drugs used for
3 treatment of the different risk factor ^(12; 46; 56; 57).

4 The proportion of baPWV variability explained by adiposity measurements in our study
5 was less than 1% (between 0.7% for BMI and 0.1% for CUN-BAE). The results are also
6 lower than those published for the general population by Wohlfahrt et al. (5% for WHtR and
7 3% for BMI) ⁽¹⁰⁾, but similar to the results reported by Rodrigues et al. (BMI 0.7%) ⁽¹⁵⁾. The
8 proportion of CAVI variability that is explained by adiposity measures was higher than 5%
9 with CUN-BAE and BMI and higher than 3.5% with WHtR and BRI. In the subgroup
10 analysis, the proportion of CAVI variability explained by adiposity measures was higher in
11 diabetic, obese, younger, and non-hypertensive subjects. Our results show that the influence
12 of adiposity measurements on CAVI is greater than on baPWV. To our knowledge, no other
13 study has analyzed this aspect using CAVI. The novel results of this study may have
14 important clinical relevance because they show the associations of both general and
15 abdominal obesity measures with CAVI and baPWV in subjects with intermediate
16 cardiovascular risk. Additionally, the results provide information that could be used in new
17 prospective studies and could potentially help to improve cardiovascular risk equations.

18 In summary, our results showed the correlation between measures of adiposity and
19 measures of arterial stiffness is greater with CAVI than with baPWV. The different measures
20 of adiposity better explain the variability of arterial stiffness evaluated using CAVI than using
21 baPWV. All this suggests that the relationship with adiposity measures is greater if the
22 arterial stiffness is measured using CAVI than using baPWV. This is likely because CAVI
23 measures rigidity at the central and peripheral levels and it is not influenced by blood pressure
24 at the time of measurement ^(35; 60; 62; 63).

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3 1 The main limitation of our study is its cross-sectional design, which does not allow us to
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5 2 establish causal relationships or the direction of influence of adiposity measures in vascular
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7 3 function. Another limitation is that the population was ethnically homogeneous (all subjects
8
9 4 were Caucasians with intermediate cardiovascular risk). Therefore, the extrapolation of our
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11 5 findings may be limited.
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14 6 15 16 7 **CONCLUSION**

17
18 8 In conclusion, the adiposity measures analyzed show a negative association with arterial
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20 9 stiffness measures. Arterial stiffness is better explained with BMI and CUN-BAE and when
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22 10 using CAVI as a measure of stiffness rather than baPWV. These results suggest that measures
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24 11 of general adiposity and body fat percentage better explain the variability of CAVI compared
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26 12 with measures of abdominal and visceral adiposity.
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34 15 Lead author for this group: Rafel Ramos, Research Unit, Primary Health Care, Girona, Jordi
35
36 16 Gol Institute for Primary Care Research (IDIAP Jordi Gol), Catalonia, Spain, E-mail:
37
38 17 rramos.girona.ics@gencat.net. Coordinating Center: Rafel Ramos, Ruth Martí, Dídac
39
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41
42 19 Camós, Research Unit, Primary Health Care, Jordi Gol Institute for Primary Care Research
43
44 20 (IDIAP Jordi Gol), C/Maluquer Salvador, 11, 17002-Girona, Catalonia, Spain. Fernando
45
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47
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49
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51
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3 1 Center Primary Care San Agustín, Ibsalut Balears, Spain. Cristina Agudo-Conde, Leticia
4
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6
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12
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21 9 interpreted the results, prepared the manuscript draft, performed all analytical testing,
22
23 10 interpreted the results and reviewed the manuscript and corrected the final version of the
24
25 11 manuscript. JIR-R, RM, FR, and CA-C participated in the study design, data collection and
26
27 12 manuscript review. ER-S and JAM-F participated in the study design, interpretation of results
28
29 13 and manuscript review. MCP-A participated in the analysis of results and final review of the
30
31 14 manuscript. LG-O, RR, and MAG-M participated in the protocol design, fundraising, analysis
32
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56 25 **Competing interests:** None declared.
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5 **Ethics approval:** The Clinical Research Ethics Committee of the Primary Care Research
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7 Institute Jordi Gol, the Health Care Area of Salamanca and Palma of Mallorca.
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16 **Data sharing statement:** No additional data are available.
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21 **Informed consent:** Obtained.
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3 **1 Figure legends**
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5 **2 Figure 1.** Flow chart of this MARK substudy
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7 Abbreviations: N, number; CAVI, cardio-ankle vascular index; baPWV, brachial-ankle pulse
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9 wave velocity; ABI, ankle-brachial index; WC, waist circumference
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For peer review only

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3 **Figure 1S (Supplementary material).** Data are given as medias estimadas de CAVI y
4
5 baPWV por cuartiles de BMI, WHtR, CUN-BAE and BRI.

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7 Adjusted for age (years), sex (0=male and 1=female), systolic blood pressure smoking
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9 (0=No and 1=Yes), atherogenic index, HbA1c, and METs/min/week
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baPWV and CAVI levels were compared using an ANOVA.

Abbreviations: CAVI, cardio-ankle vascular index; ba-PWV, brachial-ankle pulse wave
velocity; BMI, body mass index; WHtR, waist-to-height ratio; CUN-BAE, University clinic
of Navarra - body adiposity estimator; BRI, body roundness index

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Table 1 General characteristics of all the sample and by gender.

Variables	Global (n=2354)	Males (n=1456)	Females (n=898)	p Value
Age (years)	61.4±7.7	61.1±8.1	61.8±7.0	0.030
Smoking n (%)	658 (28.0)	456 (31.5)	202 (22.7)	<0.001
Alcohol (gr/week)	72.2±117.5	102.2±133.3	23.6±59.9	<0.001
Physical activity (METs/min/week)	2481±2512	2886±1831	1825±1691	<0.001
Sedentary n (%)	1020 (43.3)	538 (37.0)	482 (53.7)	<0.001
Height (cm)	165±9	170±7	156±6	<0.001
Weight (kg)	79.4±14.6	83.9±13.4	72.2±13.3	<0.001
BMI (kg/m ²)	29.2±4.4	29.1±3.9	29.5±5.1	0.035
BMI ≥ 30 n (%)	847 (36.0)	485 (33.4)	362 (40.4)	0.001
Waist circumference (cm)	100.9±11.6	102.9±10.5	97.6±12.5	<0.001
WHtR	0.61±0.07	0.61±0.06	0.62±0.08	<0.001
CUN-BAE	35.7±1.7	31.1±4.5	43.1±5.1	<0.001
BRI	5.8±1.7	5.7±1.5	6.1±2.1	<0.001
SBP (mmHg)	137.1±17.4	138.9±17.1	134.2±17.5	<0.001
DBP (mmHg)	84.4±10.2	85.5±10.4	82.7±9.7	<0.001
Heart rate (beats minutes)	74.2±10.2	73.3±12.7	75.8±11.6	<0.001
Hypertension n (%)	1712 (72.7)	1122 (80.1)	590 (75.4)	<0.001
Antihypertensive drugs n (%)	1199 (50.9)	729 (50.2)	470 (52.6)	0.289
Total Cholesterol (mg/dl)	225.8±40.9	220.8±39.1	233.9±42.5	<0.001
LDL Cholesterol (mg/dl)	140.4±34.9	138.9±34.2	142.8±35.8	0.011
HDL Cholesterol (mg/dl)	49.8±12.9	47.9±11.9	52.9±13.8	<0.001
Triglycerides (mg/dl)	145.5±96.6	150.3±106.3	137.7±77.9	0.001
Atherogenic index	4.8±1.3	4.8±1.3	4.7±1.3	0.002
Dyslipidemia n (%)	2151 (91.4)	1311 (90.0)	840 (93.5)	<0.001
Lipid lowering drugs n (%)	671 (28.5)	392 (26.8)	279 (31.0)	0.034
FPG (mg/dl)	107.2±34.8	106.9±33.9	107.6±36.1	0.659
HbA1c	4.8±1.3	5.9±1.4	6.1±1.4	0.001
Diabetes n (%)	791 (33.6)	463 (31.8)	328 (36.5)	0.020
Antidiabetic drugs n (%)	474 (20.1)	269 (18.5)	205 (22.9)	0.011
CAVI	8.8±1.2	8.9±1.2	8.6±1.1	<0.001
baPWV (m/s)	14.9±2.5	14.8±2.5	15.0±2.6	0.107

3 Values are means and (standard deviations) for continuous data and number and (proportions) for
4 categorical data.

5 Abbreviations: METs-min/week, metabolic equivalent minutes per week. BMI, body mass index.
6 WHtR, waist-to-height ratio. CUN-BAE, University clinic of Navarra - body adiposity estimator. BRI,
7 body roundness index. SBP, systolic blood pressure. DBP, diastolic blood pressure. LDL, low density
8 lipoprotein. HDL, high density lipoprotein. FPG, fasting plasma glucose. HbA1c, glycosylated
9 hemoglobin. CAVI, cardio ankle vascular index. baPWV, braquial-ankle pulse wave velocity.

10 p value differences between male and females

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1 **Table 2: Bivariate correlations of adiposity measures with CAVI and baPWV.**

	CAVI		baPWV	
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a
BMI	-0.264**	-0.303**	-0.035	-0.068**
WHtR	-0.119**	-0.222**	0.090**	0.001
CUN-BAE	-0.187**	-0.297**	0.054*	-0.063**
BRI	-0.125**	-0.218**	0.078**	0.005

2 Abbreviations: CAVI, cardio-ankle vascular index. baPWV, brachial-ankle pulse wave velocity. BMI,
3 body mass index. WHtR, waist-to-height ratio. CUN-BAE, University clinic of Navarra - body
4 adiposity estimator. BRI, body roundness index.

5 ^a Adjusted for age, sex and systolic blood pressure.
6 p-values by Pearson correlation.* p<0.05, ** p< 0.01.
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3 **Table 3: Multiple regression analysis: association between adiposity measures with CAVI and**
4 **baPWV.**

	R²	β(95%CI)	Partial R²	p value
CAVI				
BMI	0.412	-0.075 (-0.083 to -0.066)	0.055	<0.001
WHtR	0.381	-3.650 (-4.207 to -3.093)	0.038	<0.001
CUN-BAE	0.410	-0.069 (-0.077 to -0.061)	0.058	<0.001
BRI	0.378	-0.142 (-0.165 to -0.120)	0.037	<0.001
baPWV				
BMI	0.402	-0.057 (-0.076 to -0.038)	0.007	<0.001
WHtR	0.394	-1.557 (-2.748 to -0.366)	0.001	0.021
CUN-BAE	0.401	-0.050 (-0.068 to -0.033)	0.007	<0.001
BRI	0.394	-0.066 (-0.115 to -0.018)	0.002	0.014

3 Four different multiple linear regression models were used to analyze the associations of adiposity
4 measures with CAVI and baPWV.

5 Adjusted for age (years), gender (0=male and 1=female), systolic blood pressure, smoking (0=No and
6 1=Yes), METs/min/week, atherogenic index, HbA1c and METs/min/week.

7 Abbreviations: METs-min/week, metabolic equivalent minutes per week. CAVI, cardio-ankle vascular
8 index. baPWV, brachial-ankle pulse wave velocity. BMI, body mass index. WHtR, waist-to-height
9 ratio. CUN-BAE, University clinic of Navarra - body adiposity estimator. BRI, body roundness index.
10 METs-min/week, metabolic equivalent minutes per week.

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1 **Table 4: Association between adiposity measures with CAVI in different groups.**

	R²	β(95%CI)	Partial R²	p value
	CAVI			
Hypertensive				
BMI	0.409	-0.076 (-0.087 to -0.065)	0.023	<0.001
WHtR	0.383	-3.838 (-4.527 to -3.148)	0.035	<0.001
CUN-BAE	0.368	-0.068 (-0.078 to -0.058)	0.051	<0.001
BRI	0.382	-0.165 (-0.193 to -0.137)	0.035	<0.001
Non hypertensive				
BMI	0.372	-0.071 (-0.086 to -0.057)	0.052	<0.001
WHtR	0.324	-2.939 (-3.850 to -2.028)	0.046	<0.001
CUN-BAE	0.405	-0.065 (-0.080 to -0.052)	0.080	<0.001
BRI	0.318	-0.108 (-0.144 to -0.072)	0.042	<0.001
Diabetics				
BMI	0.439	-0.087 (-0.101 to -0.073)	0.95	<0.001
WHtR	0.399	-4.613 (-5.523 to -3.702)	0.069	<0.001
CUN-BAE	0.437	-0.083 (-0.096 to -0.070)	0.70	<0.001
BRI	0.391	-0.169 (-0.205 to -0.134)	0.062	<0.001
Non diabetics				
BMI	0.396	-0.067 (-0.078 to -0.056)	0.049	<0.001
WHtR	0.369	-3.088 (-3.781 to -2.396)	0.034	<0.001
CUN-BAE	0.395	-0.059 (-0.069 to -0.050)	0.064	<0.001
BRI	0.369	-0.130 (-0.159 to -0.099)	0.034	0.016
Obese				
BMI	0.414	-0.093 (-0.112 to -0.074)	0.054	<0.001
WHtR	0.377	-2.945 (-4.085 to -1.804)	0.003	<0.001
CUN-BAE	0.418	-0.100 (-0.122 to -0.079)	0.026	<0.001
BRI	0.377	-0.110 (-0.153 to -0.069)	0.003	<0.001
Non obese				
BMI	0.373	-0.058 (-0.078 to -0.038)	0.013	<0.001
WHtR	0.361	-1.394 (-2.366 to -0.421)	0.003	0.005
CUN-BAE	0.373	-0.050 (-0.066 to -0.033)	0.020	<0.001
BRI	0.358	-0.044 (-0.085 to -0.003)	0.002	0.034
≤ 62 years				
BMI	0.368	-0.078 (-0.089 to -0.077)	0.77	0.003
WHtR	0.332	-4.142 (-4.854 to -3.431)	0.065	<0.001
CUN-BAE	0.361	-0.068 (-0.078 to -0.058)	0.070	<0.001
BRI	0.324	-0.163 (-0.192 to -0.134)	0.062	<0.001
> 62 years				
BMI	0.234	-0.074 (-0.088 to -0.060)	0.056	<0.001
WHtR	0.195	-3.086 (-3.948 to -2.224)	0.029	<0.001
CUN-BAE	0.234	-0.071 (-0.084 to -0.059)	0.065	<0.001
BRI	0.193	-0.124 (-0.159 to -0.089)	0.028	<0.001
Assets				
BMI	0.414	-0.072 (-0.084 to -0.060)	0.053	<0.001
WHtR	0.392	-3.570 (-4.335 to -2.805)	0.034	<0.001
CUN-BAE	0.413	-0.066 (-0.075 to -0.053)	0.060	<0.001
BRI	0.391	-0.148 (-0.180 to -0.116)	0.034	<0.001
Sedentary				
BMI	0.408	-0.080 (-0.092 to -0.068)	0.055	<0.001
WHtR	0.364	-3.776 (-4.598 to -2.954)	0.045	<0.001
CUN-BAE	0.404	-0.074 (-0.086 to -0.062)	0.059	<0.001
BRI	0.360	-0.144 (-0.176 to -0.111)	0.043	<0.001

2 Multiple linear regression models were used to analyze the associations of adiposity measures with CAVI by
3 groups.

4 Adjusted for age (years), gender (0=male and 1=female), Systolic blood pressure, smoking (0=No and 1=Yes),
5 METs/min/week, atherogenic index, HbA1c and METs/min/week.

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1 Abbreviations: METs-min/week, metabolic equivalent minutes per week. CAVI, cardio-ankle vascular
2 index. BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, University clinic of Navarra - body
3 adiposity estimator. BRI, body roundness index.
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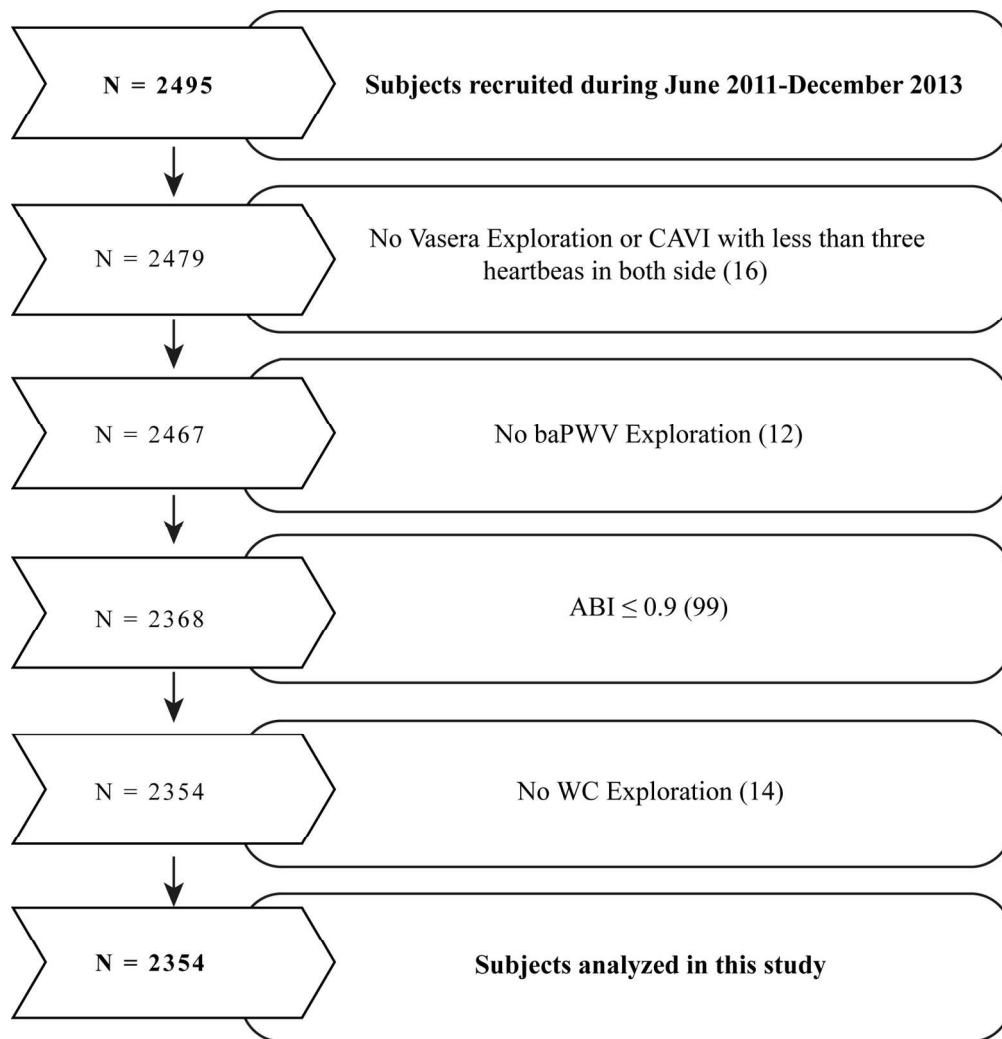
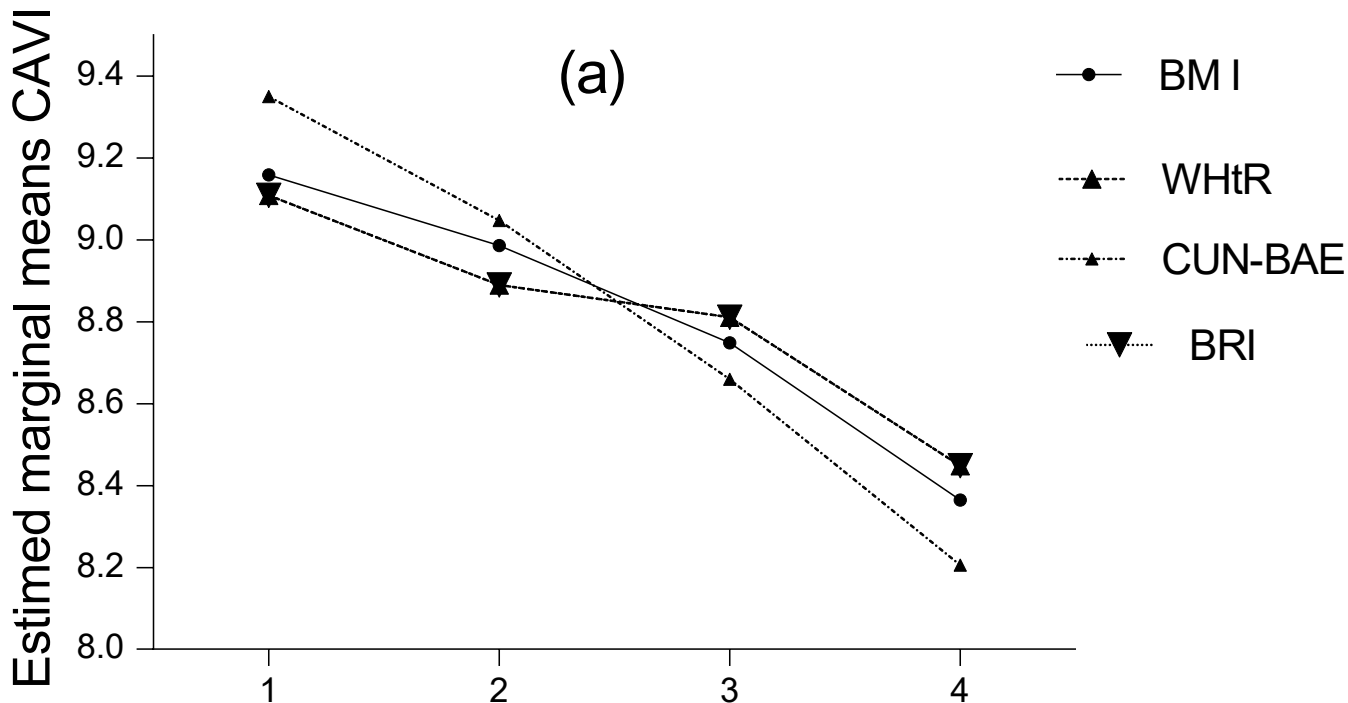


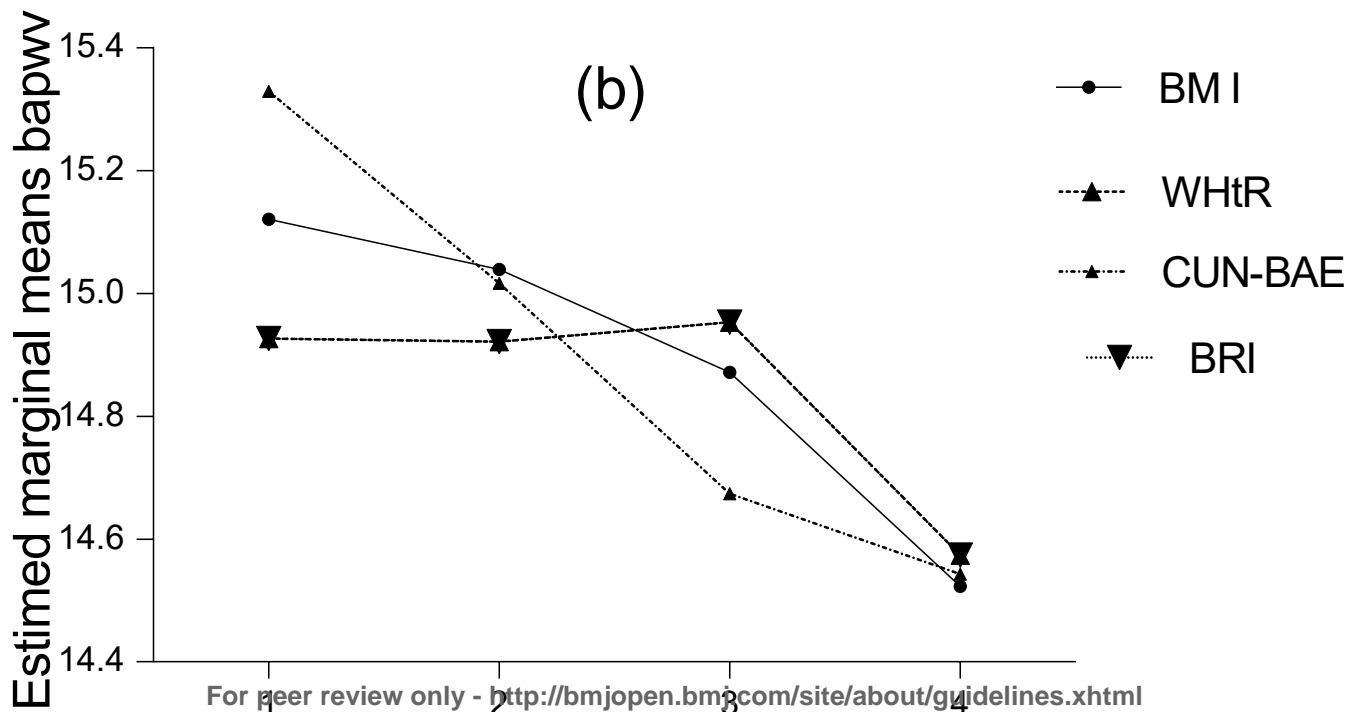
Figure 1. Flow chart of this MARK substudy
 Abbreviations: N, number; CAVI, cardio-ankle vascular index; baPWV, brachial-ankle pulse wave velocity; ABI, ankle-brachial index; WC, waist circumference

145x149mm (300 x 300 DPI)

(a)



(b)



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract Yes, Page 1 (b) Provide in the abstract an informative and balanced summary of what was done and what was found Yes, Page 2
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Yes, Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses Yes, Page 4
Methods		
Study design	4	Present key elements of study design early in the paper Yes, Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Yes, Page 5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants Yes, Page 5 (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case Yes, Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Yes, Page 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Yes, Page 6-9
Bias	9	Describe any efforts to address potential sources of bias Yes, Page 8-9
Study size	10	Explain how the study size was arrived at Yes, Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Yes, Page 8-9

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2 Statistical methods

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(a) Describe all statistical methods, including those used to control for confounding

Yes, Page 8-9

(b) Describe any methods used to examine subgroups and interactions **Yes, Page 8-9**

(c) Explain how missing data were addressed **Yes, Page 8-9**

(d) *Cohort study*—If applicable, explain how loss to follow-up was addressed*Case-control study*—If applicable, explain how matching of cases and controls was addressed*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy

(e) Describe any sensitivity analyses **Yes, Page 8-9**

Continued on next page

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Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Yes, Page 10-11 (b) Give reasons for non-participation at each stage Yes, Page 10-11 (c) Consider use of a flow diagram Yes, Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Yes, Page 10 (b) Indicate number of participants with missing data for each variable of interest Yes, Page 10 (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Yes, Tables (b) Report category boundaries when continuous variables were categorized Yes, tables (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Yes, Page 10

Discussion

Key results	18	Summarise key results with reference to study objectives Yes, Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Yes, Page 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Yes, Page 11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results Yes, Page 11

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Yes, Page 15
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Adiposity measures and arterial stiffness in primary care: the MARK prospective observational study

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Complete List of Authors:	Gomez-Sanchez, Leticia; La Alamedilla health centre., Primary Care Research Unit; 2. Biomedical Research Institute of Salamanca (IBSAL) Garcia-Ortiz, Luis; La Alamedilla health centre. University of Salamanca, Primary care research unit La Alamedilla Patino-Alonso, Maria; University of Salamanca, Salamanca, Spain , Statistics Department, Recio-Rodriguez, Jose; La Alamedilla Health Center, Primary Care Research Unit, The Alamedilla Health Center, Castilla and León Health Service (SACYL), Biomedical Research Institute of Salamanca (IBSAL) Frigo, Fernando Marti, Rhut; Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP Jordi Gol), Unitat de Suport a la Recerca de Girona. Agudo-Conde, Cristina; La Alamedilla Health Center,, Research Unit Rodriguez-Sanchez, Emiliano; Primary Care Research Unit, The Alamedilla Health Center, Salamanca, Spain, ; Biomedical Research Institute of Salamanca (IBSAL), Maderuelo-Fernandez, Jose; Salamanca Institute for Biomedical Research. (IBSAL)., Primary care research unit La Alamedilla, Castilla and León Health Service (SACYL). Network of Preventive Activities and Health Promotion in Primary Care (REDIAPP) Ramos, Rafel; Universidad de Girona. Departament Medicina Gomez-Marcos, Manuel; the Alamedilla Health Center, Castilla and León Health Service (SACYL), Primary Care Research Unit; University of Salamanca,, Department of Medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Diabetes and endocrinology, Epidemiology, Nutrition and metabolism
Keywords:	Body mass index, Waist circumference, Waist-to-height ratio, Fat mass percent., Body roundness index., Arterial stiffness

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Manuscripts

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3 1 **Title: Adiposity measures and arterial stiffness in primary care: the MARK prospective**
4 **observational study**

5
6 3 **Authors:** Leticia Gomez-Sanchez MD ^{1,2*}, Luis Garcia-Ortiz MD, PhD ^{1,2,3}, Maria C Patino-
7
8 4 Alonso PhD ^{1,2,4}, Jose I Recio-Rodriguez PhD ^{1,2,5}, Fernando Frigo MD, PhD ⁶, Rhut Martí
9
10 5 PhD ^{7,8}, Cristina Agudo-Conde ^{1,2}, Emiliano Rodriguez-Sanchez MD, PhD ^{1,2,9}, Jose A
11
12 6 Maderuelo-Fernandez MD, PhD ^{1,2}, Rafel Ramos MD, PhD ^{7,8,10} and Manuel A Gomez-
13
14 7 Marcos MD, PhD ^{1,2,9} for the MARK Group ¹¹

15
16 8 1. Primary Care Research Unit, the Alamedilla Health Center, Castilla and León Health
17
18 9 Service (SACyL), Salamanca, Spain

19
20 10 2. Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain

21
22 11 3. Biomedical and diagnostic sciences department, University of Salamanca, Salamanca,
23
24 12 Spain

25
26 13 4. Statistics Department, University of Salamanca, Salamanca, Spain

27
28 14 5. Department of Nursing and Physiotherapy, University of Salamanca, Salamanca, Spain

29
30 15 6. San Agustín Health Center, Illes Balears Health Service (IBSALUT), Palma of Mallorca,
31
32 16 Spain

33
34 17 7. Unitat de Suport a la Recerca de Girona. Institut Universitari d'Investigació en Atenció
35
36 18 Primària Jordi Gol (IDIAP Jordi Gol), Girona, Spain

37
38 19 8. Institut d'Investigació Biomèdica of Girona Dr. Josep Trueta (IDBGI). Girona, Spain

39
40 20 9. Department of Medicine, University of Salamanca, Salamanca, Spain

41
42 21 10. Departament de Ciències Mèdiques, Facultat de Medicina. Universitat de Girona, Spain.

43
44 22 11. MARK Group. redIAPP: Research Network in Preventive Activities and Health
45
46 23 Promotion, Girona, Spain

47
48 24 ***Corresponding Author:**

49
50 25 Leticia Gomez-Sanchez, Primary Care Research Unit, Alamedilla Health Care Center

51
52 26 37003 Salamanca, Spain

53
54 27 E-mail addresses: leticiagmzsnchz@gmail.com

55
56 28 Full list of author information is available at the end of the article.

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58 29

1
2
3 **1 ABSTRACT**

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5 **2 Background:** The cardiovascular risk in obesity is potentially increased by arterial stiffness.

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7 **3 Objective:** This study investigates the relationship of adiposity measures with arterial
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10 stiffness in Caucasian adults with intermediate cardiovascular risk.

11 **5 Setting:** Six primary care centers in three Spanish Autonomous Communities, Spain

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13 **6 Participants:** This study analyzed 2354 subjects (age range, 35–74 years; mean age, 61.4±7.7
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16 years, 61.9% male).

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18 **8 Methods:** The study was based on cross-sectional data from the MARK study. The main
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outcome variables were: body mass index (BMI), waist-to-height ratio (WHtR), Clínica
Universidad de Navarra-body adiposity estimation (CUN-BAE) body fat percentage, and
body roundness index (BRI). Vascular function was assessed by the cardio-ankle vascular
index (CAVI) with the VaSera device, and brachial ankle pulse wave velocity (baPWV) was
determined using a validated equation.

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14 Results: The mean adiposity measures were a BMI of 29.2±4.4, WHtR of 0.61±0.07, CUN-
BAE of 35.7±1.7, and BRI of 5.8±1.7. The mean stiffness measures were a CAVI of 8.8±1.2
and baPWV of 14.9±2.5. In multiple linear regression analysis, all adiposity measures were
negatively associated with CAVI and baPWV ($p<0.01$ for all) after adjustment for
cardiovascular risk factors. The proportion of CAVI variability by the adiposity measures
were 5.5% for BMI, 5.8% for CUN-BAE, 3.8% for WHtR, and 3.7% for BRI, which were
higher among diabetic, obese, younger (≤ 62 years), and non-hypertensive subjects who had
similar activity and sedentary profiles.

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22 Conclusions: Adiposity measures are negatively associated with arterial stiffness measures.
The percentage of variation in CAVI explained by its relation to the different measures of
adiposity ranges from 5.8% (CUN-BAE) to 3.7% (BRI). In the case of baPWV it oscillates
between 0.7% (CUN-BAE and BMI) and 0.1% (WHtR).

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5 2 **Trial Registration:** Clinical Trials.gov Identifier: [https://clinicaltrials.gov/ct2/show/](https://clinicaltrials.gov/ct2/show/NCT01428934)
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7 3 NCT01428934. Registered 2 September 2011. Last updated September 8, 2016.

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9 4 **Keywords:** body mass index, waist circumference, waist-to-height ratio, fat mass percent,
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11 5 body roundness index, arterial stiffness
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14 7 **Strengths and limitations of this study**

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16 8 • This is the first study to investigate the association between adiposity measures using a
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18 9 cardio-ankle vascular index with and brachial ankle pulse wave velocity in Caucasian adults
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20 10 with intermediate cardiovascular risk.
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24 11 • All adiposity measures were negatively associated with CAVI and baPWV.
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27 12 • The arterial stiffness variability was better explained for BMI and CUN-BAE and
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29 13 when CAVI was used as a measure of stiffness rather than baPWV.
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31 14 • The main limitation of our study is its cross-sectional design, which does not allow
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33 15 establishment of causal relationships or the direction in which adiposity measures influence
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35 16 vascular function.
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1 INTRODUCTION

2 Obesity has been linked to increased all-cause and cardiovascular mortality⁽¹⁾. However, the
3 mechanisms through which obesity can increase the frequency of cardiovascular disease
4 beyond traditional risk factors are not clearly identified⁽²⁾. It has been suggested that,
5 increased arterial stiffness may be a mechanism by which obesity increases cardiovascular
6 risk independently of traditional risk factors⁽³⁾. It is known that arterial stiffness as evaluated
7 by the brachial ankle pulse wave velocity (baPWV) is an independent predictor of coronary
8 heart disease and mortality in both the general population⁽⁴⁾ and in patients with diabetes
9 mellitus^(5; 6). Similarly, the cardio-ankle vascular index (CAVI) is associated with carotid and
10 coronary atherosclerosis^(7; 8; 9) and is a predictor of cardiovascular events in obese patients
11⁽¹⁰⁾. I. However, the relationship between adiposity and arterial stiffness remains
12 controversial. In this respect, there are studies that show that the body mass index (BMI) has
13 been associated with arterial stiffness in the general population^(3; 11) and in diabetic patients
14^(12; 13). However, other research has not found this association⁽¹⁴⁾, or the association
15 disappeared after adjusting for potential confounders⁽¹³⁾, or it showed a negative association
16^(15; 16). Additionally, there are studies that suggest a stronger correlation of measures of central
17 or visceral adiposity than measures of general adiposity with arterial stiffness in the general
18 population^(3; 17; 18; 19; 20), in diabetic patients⁽¹³⁾, and in diabetics and hypertensive patients⁽²¹⁾.
19 Finally the Whitehall II Cohort study⁽²²⁾ showed that all measures of general adiposity,
20 central adiposity, and body fat percentage were predictors of accelerated arterial stiffness in
21 adults. In this context the analysis of the relationship between arterial stiffness and different
22 measures of adiposity can help to understand the role of obesity in cardiovascular disease.

23 Our study was designed bearing in mind that cardiovascular events are more likely to occur in
24 patients with intermediate cardiovascular risk⁽²³⁾, and that there is a lack of studies analyzing
25 the relationship of different adiposity measures with arterial stiffness in these subjects. We
26 have established the following objectives: the primary aim of this study was to investigate the

1 relationship between adiposity measures and arterial stiffness in Caucasian adults with
2 intermediate cardiovascular risk. The secondary aim was to analyze the differences between
3 the associations of adiposity measures with distinct arterial stiffness markers. (Page 4 and 5).

4 5 **METHODS**

6 **Study design**

7 This trial was a cross-sectional study of subjects recruited to the *improving interMediAte RisK*
8 *management (MARK)* study (NCT01428934)⁽²⁴⁾, which was a longitudinal study designed to
9 assess whether the ankle-brachial index, arterial stiffness (measured by CAVI), postprandial
10 glucose, glycosylated hemoglobin, self-measured blood pressure, and the presence of
11 comorbidities were independently associated with the occurrence of vascular events. It also
12 investigated whether the predictive capacity of current risk equations could be improved in
13 the intermediate risk population. The current study focused on the baseline visit. The second
14 step will be a 5- and 10-year follow-up trial to assess cardiovascular morbidity and mortality.

15 16 **Study population**

17 In this multicenter project, the study population was obtained by random sampling from
18 subjects who met the inclusion criteria and who were seeing general practitioners from July
19 2011 to June 2013 at six primary care centers in three Spanish Autonomous Communities.
20 Subjects were recruited from those aged 35 to 74 years with an intermediate cardiovascular
21 risk, defined as a 10-year coronary risk ranging from 5%–15% according to the adapted
22 Framingham risk equation⁽²⁵⁾; 10-year vascular mortality risk greater than 1% and less than
23 5% according to the SCORE equation⁽²⁶⁾; or a moderate risk according to the European
24 Society of Hypertension guidelines for the management of arterial hypertension⁽²⁷⁾. Exclusion
25 criteria included end-stage disease or institutionalization at the time of the visit, or history of
26 atherosclerotic disease. This study analyzed 2354 of the 2495 subjects recruited in the MARK

1 study. For the present analysis, we excluded 141 individuals, with $ABI \leq 0.9$ (n=99), or which
2 CAVI (n=16), baPWV (n=12) and WC (n = 14) measurements were incomplete (Figure 1).

4 **Variables and measurement instruments**

5 A detailed description of procedures for clinical data collection and laboratory tests has been
6 published elsewhere ⁽²⁴⁾.

8 **Anthropometric measurements**

9 Body weight was measured twice using a certified electronic scale (Seca 770, Medical scale
10 and measurement systems, Birmingham, United Kingdom) after adequate calibration
11 (precision ± 0.1 kg). Readings were rounded-off to the nearest 100 g. Height was measured
12 using a stadiometer (Seca 222), and the average of two measurements was recorded. Body
13 mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist
14 circumference was measured according to the 2007 recommendations of the Spanish Society
15 for the Study of Obesity ⁽²⁸⁾. All measurements were performed with the subjects standing,
16 wearing no shoes, and in light clothing. Waist-to-height ratio (WHtR) was calculated as the
17 waist circumference (cm) divided by height (cm) ^(29; 30).

18 The body fat percentage (BF%) was calculated according to the Clínica Universidad de
19 Navarra-body adiposity estimator (CUN-BAE) using the recommendations of Gomez-
20 Ambrosi et al. ⁽³¹⁾: $BF\% = -44.988 + (0.503 \times \text{Age}) + (10.689 \times \text{sex}) + (3.172 \times \text{BMI}) - (0.026$
21 $\times \text{BMI}^2) + (0.181 \times \text{BMI} \times \text{sex}) - (0.02 \text{ BMI} \times \text{Age}) - (0.005 \times \text{BMI}^2 \times \text{sex}) +$
22 $(0.00021 \times \text{BMI}^2 \times \text{Age})$, where male=0 and female=1 for sex.

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3 1 Body roundness index (BRI) was calculated using the following formula: $BRI = 364.2 -$
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5 $365.5 \times \sqrt{1 - \frac{(WC/(2\pi))^2}{(0.5height)^2}}$ (32). BRI can predict the percentage of body fat and visceral adipose
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5 **Cardio-ankle vascular index (CAVI) and brachial-ankle pulse wave velocity (baPWV)**

6 CAVI was measured using a *VaSera VS-1500®* device (*Fukuda Denshi*) (33; 34). CAVI values
7 were calculated automatically by estimating the stiffness parameter β using the following
8 equation: $\beta = 2\rho \times 1 / (Ps - Pd) \times \ln (Ps / Pd) \times PWV^2$, where ρ was blood density, Ps and Pd
9 were SBP and DBP in mmHg, and PWV was measured between the aortic valve and the
10 ankle (35). The mean coefficient of variation of CAVI measurement was less than 5%, which is
11 small enough to allow for clinical use of the index and confirmed that CAVI was reproducible
12 index (34).

13 CAVI was calculated using the *VaSera VS-1500®* device (*Fukuda Denshi*), and with the
14 values obtained, the baPWV was estimated using the equation $baPWV = (0.5934 \times \text{height}$
15 $(\text{cm}) + 14.4724) / tba$ (where tba was the time interval between the arm and ankle waves) (36).

16 Measurements were performed with the patient in the supine position after resting for 10
17 minutes in a quiet room at a stable temperature. Subjects were instructed not to smoke or
18 exercise in the hour before the test.

20 **Diagnosis of cardiovascular risk factors**

21 Subjects were considered to be hypertensive if they were previously diagnosed hypertension,
22 if they were taking antihypertensive drugs, or if they had blood pressure levels $\geq 140/90$
23 mmHg. Diabetic subjects were those who had been previously diagnosed with the disease,
24 were taking hypoglycemic drugs, or had a fasting blood glucose levels ≥ 126 mg/dL or
25 $HbA1c \geq 6.5\%$. Dyslipidemia was defined if they were treated with lipid-lowering drugs or

1 had altered LDL \geq 130 mg/dl, HDL \leq 45 mg/dl in men and \leq 55 in women, and TG \geq 150
2 mg/dl, as established by the European Society of Cardiology (ESC) and the European
3 Atherosclerosis Society (EAS) 2011 ⁽³⁷⁾.

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5 **Office or clinical blood pressure**

6 Office blood pressure measurement involved three measurements of SBP and DBP using a
7 validated OMRON model M10-IT sphygmomanometer (Omron Health Care, Kyoto, Japan).
8 The measurements followed the recommendations of the European Society of Hypertension
9 ⁽³⁸⁾, and the average of the last two measurements was used.

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11 **Lifestyles**

12 **Tobacco**

13 Smoking history was assessed by asking questions about the participant's smoking status
14 (smoker/non-smoker). We considered smokers to include those who currently smoke or who
15 had stopped smoking within the past year.

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17 **Leisure time physical activity**

18 Leisure time physical activity (LTPA) was collected using the Minnesota LTPA Questionnaire
19 ⁽³⁹⁾ that was validated for Spanish men and women ^(40; 41). The questionnaire was administered
20 by trained interviewers who spent about 10 to 20 min per participant collecting detailed
21 information about physical activity (PA) during the preceding year, the number of times this
22 activity was performed, and the average duration of each activity on each occasion. Each PA
23 has an intensity code, based on the ratio between the metabolic rate during PA practice and the
24 basal metabolic rate (MET) ⁽⁴²⁾. It is assumed that 1 MET corresponds to approximately 1
25 kcal/min of energy expenditure. Therefore, we can calculate the total energy expenditure in
26 leisure time of PA (EEPA_{total}) in kilocalories per week. Moreover, based on the PA intensity
27 code, we could quantify the energy expenditure in physical activity (EEPA) according to the
28 activity's classification as intense, moderate, or light intensity as follows: light PA intensity

1 was below 4 METs, such as walking (EEPA_{light}). Moderate PA intensity was 4–5.5 METs, such as brisk walking (EEPA_{moderate}). Intense PA intensity was greater than or equal to 6 METs, such as jogging (EEPA_{intense}). Thus, for each particular subject: EEPA_{total} = EEPA_{light} + EEPA_{moderate} + EEPA_{intense}.

Based on recommendations from the American Heart Association⁽⁴³⁾, we considered those participants who do not meet the recommendations of moderate-intensity aerobic PA practice for a minimum of 30 min on 5 days each week (EEPA_{moderate} < 675 kcal/week) or high-intensity aerobic PA practice for a minimum of 20 min on 3 days each week (EEPA_{intense} < 420 kcal/week) to be sedentary.

Laboratory determinations

Venous blood sampling was performed between 08:00 and 09:00 hours after the individuals fasted and abstained from smoking and the consumption of alcohol and caffeinated beverages for the previous 12 hours. Fasting plasma glucose, serum, high-density lipoprotein (HDL) cholesterol concentrations and triglyceride concentrations were measured using standard enzymatic automated methods. Low-density lipoprotein cholesterol was estimated using the Friedewald equation when the direct parameter was not available. The atherogenic index was estimated (total cholesterol / HDL cholesterol). Blood samples were collected at the Health Center and analyzed at the hospital of reference.

The researchers who performed the different tests were blinded to the clinical data of the subjects. All assessments were made within a period of 10 days.

Data analysis

Continuous variables were expressed as the mean ± standard deviation. Frequency distributions were used for categorical data. Comparisons between quantitative variables were performed using the Student's *t*-test, while the chi-squared test and Fisher's exact test were used for qualitative variables. Pearson's correlation coefficient was used to estimate the

1 relationship of the adiposity measures to CAVI and baPWV. We used Steiger's Z statistics for
2 testing the significance of the difference between correlations coefficients ⁽⁴⁴⁾.

3 Four different multiple linear regression models were used to analyze the associations of
4 each adiposity measure with CAVI, and four other models were used with baPWV. CAVI and
5 baPWV were the dependent variables, and the adiposity measures were the independent
6 variables in each model. All models were adjusted for age (years), sex (0 = male and 1 =
7 female), systolic blood pressure (SBP), smoking status (0 = No and 1 = Yes), atherogenic
8 index, HbA1c, and METs/min/week. The explanatory capacity of the model was measured by
9 R^2 , and the proportion attributed to each variable was estimated by the change in R^2 . The
10 analysis was also performed by age groups, diagnosis of diabetes, hypertension, and obesity.

11 ANCOVA models were used to test the differences in the mean values of CAVI and
12 baPWV with the quartiles of the four adiposity measures after adjusting for the confounding
13 variables that were used in the regression analysis. Pairwise post hoc comparisons were
14 examined using the Bonferroni test. Data were analyzed using SPSS Statistics for Windows
15 version 23.0 (IBM Corp, Armonk, NY). Values of $p < 0.05$ were considered statistically
16 significant.

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18 **Ethics statement**

19 All participants were informed of the study objectives and procedures and they all signed an
20 informed consent form to participate. The study was approved by the Clinical Research Ethics
21 Committee of the Primary Care Research Institute Jordi Gol, the Health Care Area of
22 Salamanca, and Palma of Mallorca. The study was conducted following the recommendations
23 of the Declaration of Helsinki ⁽⁴⁵⁾. The confidentiality of information provided by participants
24 was ensured, complying with the rules established by Spanish Organic Law 15/1999, of 13
25 December on the Protection of Personal Data.

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5 2 **RESULTS**

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7 Anthropometric measures, clinical characteristics, and vascular function measures of the
8 subjects are presented in Table 1. The mean age of the patients was 61.4 ± 7.7 years, and
9 61.9% were male. Male subjects had a higher percentage of smokers (31.5 vs. 22.7) and
10 hypertension (80.1 vs. 75.4) compared with females. However, females had a higher
11 prevalence of obesity (40.4 vs. 33.4), sedentariness (53.7 vs. 37.0), dyslipidemia (73.1 vs.
12 63.6), and diabetes (36.5 vs. 31.8) compared with males. The mean value of CAVI was
13 8.8 ± 1.2 (8.9 in males and 8.6 in females, $p < 0.001$) and the mean baPWV was 14.9 ± 2.5 (14.8
14 in males and 15.0 in females). All of the analyzed adiposity measures except for waist
15 circumference presented higher values in women compared with men.

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17 Pearson's correlation coefficient results between the adiposity measures and the vascular
18 function parameters are shown in Table 2. All adiposity measures were negatively correlated
19 with CAVI, and this correlation increases after adjusting for age, sex, and SBP. The
20 correlation between CAVI and baPWV was $r = 0.745$ ($p < 0.001$). We found differences in
21 correlation coefficients between CAVI, baPWV and measures of adiposity ($p < 0.001$ in all
22 cases).

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24 Figure 1S (Supplementary material) shows the estimated marginal means of CAVI (a) and
25 baPWV (b) by quartiles of the different adiposity measures. After adjustment for the variables
used in the multiple linear regression analysis, the mean CAVI values decreased as the
quartiles of the four adiposity measurements increased ($p < 0.05$). However, the same is not
true of baPWV with WHtR and BRI ($p > 0.05$).

In the multiple linear regression analysis, CAVI and baPWV showed negative associations
with all adiposity measures ($p < 0.01$ for all) after adjustment for age, sex, SBP, smoking,
atherogenic index, HbA1c, and METs/min/week (Table 3). The proportion of CAVI

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3 1 variability which can be attributed to the variation in the adiposity measures was 5.5% for
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5 2 BMI, 5.8% for CUN-BAE, 3.8% for WHtR, and 3.7% for BRI. 5.5% for BMI, 5.8% for
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7 3 CUN-BAE, 3.8% for WHtR, and 3.7% for BRI. For baPWV, the variability by the measures
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9 4 of adiposity were 0.7% for BMI, and CUN-BAE, and 0.1% for WHtR, and 0.2 for BRI. The
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11 5 association between adiposity measurements and CAVI revealed standardized β between -
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13 6 0.450 (CUN-BAE) and -0.221 (WHtR). In the case of baPWV the values oscillate between -
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15 7 0.152 (CUN-BAE) and -0.044 (WHtR).

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18 8 In the multiple linear regression analysis by subgroup, the proportion of CAVI variability
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20 9 by adiposity measures was higher among diabetics, obese, non-hypertensive, and subjects 62
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22 10 years of age or younger, and was similar in active and sedentary people (Table 4).
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25 26 27 12 **DISCUSSION**

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29 13 The results of this study show that adiposity measures have a negative association with
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31 14 arterial stiffness, especially CAVI. BMI and CUN-BAE are the ones with the highest
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33 15 coefficient of determination. We found a negative association of different adiposity measures
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35 16 with CAVI and baPWV after adjustment for other cardiovascular risk factors.
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39 17 In this study the mean value of CAVI was higher in males, which is in agreement with
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41 18 published data indicating that CAVI increases linearly with age, and is higher in males than in
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43 19 females (approximately 0.2, which is equivalent to 4–5 years old)^(22; 46). Similarly, there was
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45 20 no difference in the mean baPWV between the sexes, which is consistent with data published
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47 21 by Tomiyama et al.⁽⁴⁷⁾ who showed that the effect of age on baPWV is different according to
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49 22 sex. Females have a higher arterial stiffness than prepubertal males, and this increases after
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51 23 menopause. Men, however, experience a linear increase in arterial stiffness from puberty.
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53 24 This suggests that women have large arteries that are intrinsically more rigid compared with
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55 25 men, but these effects are mitigated by sex steroids during the reproductive years^(48; 49).
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3 1 This negative association with CAVI has already been described in previous studies. BMI
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5 2 has shown a negative association in children ⁽⁵⁰⁾, and in hypertensive and type 2 diabetes
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7 3 patients in Ghana ⁽⁵¹⁾. Similarly, waist circumference has shown a negative relationship in
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9 4 subjects with metabolic syndrome ^(52; 53).

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11 5 However, other authors have described a positive association of different adiposity
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13 6 measures with the β -stiffness parameter, but after adjustment for age and other possible
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15 7 confounding factors, the association remained for only men with type 2 diabetes mellitus ⁽⁵⁴⁾.
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17 8 Other studies have also found no association between BMI and CAVI ⁽⁵⁵⁾.

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20 9 Studies analyzing the association of adiposity measures with baPWV have also been
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22 10 performed mainly in Eastern populations and have focused on assessing the association
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24 11 between BMI and waist circumference as measures of adiposity. The results are controversial,
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26 12 with some finding a negative association with BMI ⁽⁴⁷⁾, and with waist circumference in men
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28 13 only or in women only ^(56; 57). However, other studies have found a positive correlation with
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30 14 BMI and waist circumference ⁽⁵⁸⁾. One study that analyzed the association of different
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32 15 adiposity parameters with baPWV in middle-aged adults found a positive association with
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34 16 waist circumference and visceral fat but not with body fat percentage ⁽⁵⁹⁾.

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38 17 The results are also not consistent in studies that used the carotid-femoral pulse wave
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40 18 velocity (cfPWV) as a measure of stiffness. Some studies have described a greater association
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42 19 with measures of central or visceral adiposity in both the general population and in diabetics
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44 20 ^(3; 13; 17; 18; 19; 21). However, Strasser et al. ⁽⁵⁹⁾ found no association with body fat percentage,
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46 21 and other studies also found no association between BMI and cfPWV ^(13; 14). Rodrigues et al.
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48 22 ⁽¹⁵⁾, reported that BMI was negatively associated with cfPWV ($\beta = -0.103$) in a large
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50 23 population sample. The Whitehall II Cohort study ⁽²²⁾, which was completed based on staff
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52 24 lists from offices located in central London, showed that all measures of adiposity were robust
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54 25 predictors of accelerated cfPWV, after adjusting them for potential confounding factors. The
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1 use of different measures to measure arterial stiffness such as CAVI and baPWV, as well as
2 being a population with intermediate cardiovascular risk could explain some of the
3 discrepancies with our study.

4 Arterial stiffness depends on arterial wall elasticity and diameter, and a positive correlation
5 was found between BMI and aortic diameter measured by nuclear magnetic resonance ⁽⁶⁰⁾.
6 This could partially explain the negative association between measures of adiposity and
7 arterial stiffness. These discrepancies between studies may be partially explained by different
8 methods of arterial stiffness measurement and the adjustment variables used. This may be
9 because CAVI reflects central and peripheral arterial stiffness and is less influenced by blood
10 pressure values at the time of measurement ^(35; 61; 62). Conversely, arterial stiffness assessed
11 using baPWV is a measure of peripheral arterial stiffness ⁽³⁶⁾. Other potential influences on
12 the observed differences are age, sex, race, prevalent cardiovascular risk, and drugs used for
13 treatment of the different risk factor ^(12; 47; 56; 58). These differences between CAVI and
14 baPWV, as measures of rigidity, could explain the results shown in this study, suggesting a
15 greater association of adiposity measurements with CAVI than with baPWV.

16 The proportion of baPWV variability explained by adiposity measurements in our study
17 was less than 1% (between 0.7% for BMI and 0.1% for CUN-BAE). The results are also
18 lower than those published for the general population by Wohlfahrt et al. (5% for WHtR and
19 3% for BMI) ⁽³⁾, but similar to the results reported by Rodrigues et al. (BMI 0.7%) ⁽¹⁵⁾. The
20 proportion of CAVI variability that is explained by adiposity measures was higher than 5%
21 with CUN-BAE and BMI and higher than 3.5% with WHtR and BRI. In the subgroup
22 analysis, the proportion of CAVI variability explained by adiposity measures was higher in
23 diabetic, obese, younger, and non-hypertensive subjects. Our results show that the influence
24 of adiposity measurements on CAVI is greater than on baPWV. To our knowledge, no other
25 study has analyzed this aspect using CAVI. The novel results of this study may have

1 important clinical relevance because they show the associations of both general and
2 abdominal obesity measures with CAVI and baPWV in subjects with intermediate
3 cardiovascular risk. Additionally, the results provide information that could be used in new
4 prospective studies and could potentially help to improve cardiovascular risk equations.

5 Numerous studies have been carried out analyzing the effect of weight loss on arterial
6 stiffness, most of which have been collected in two meta-analyses. The first analyzed the
7 results of 20 studies (1259 participants), and showed that modest weight loss (8% of initial
8 body weight) achieved with diet and lifestyle interventions seems to improve PWV. The
9 standardized mean difference (SMD) for the overall effect of weight loss on baPWV
10 measured at all sites was -0.32 (95% CI, -0.41, -0.24; P=0.0001). cfPWV (SMD, -0.35; 95%
11 CI, -0.44, -0.26; P=0.0001; 16 studies) and baPWV (SMD, -0.48; 95% CI, -0.78, -0.18;
12 P=0.002; 5 studies) improved with weight loss.⁽⁶³⁾ In the second meta-analysis, 43 studies
13 (4231 participants) were included and it was found that the average weight loss was
14 approximately 11% of the initial body weight and that weight loss improved CAVI (SMD= -
15 0.48; p = 0.04)⁽⁶⁴⁾.

16 In summary, our results showed the correlation between measures of adiposity and
17 measures of arterial stiffness is greater with CAVI than with baPWV. The different measures
18 of adiposity better explained the variability of arterial stiffness evaluated using CAVI than
19 using baPWV. All this suggests that the relationship with adiposity measures is greater if the
20 arterial stiffness is measured using CAVI than using baPWV. This is likely because CAVI
21 measures rigidity at the central and peripheral levels and it is not influenced by blood pressure
22 at the time of measurement^(35; 61; 65; 66).

23 The main limitation of our study is its cross-sectional design, which does not allow us to
24 establish causal relationships or the direction of influence of adiposity measures in vascular
25 function. Another limitation is that the population was ethnically homogeneous (all subjects

1 were Caucasians with intermediate cardiovascular risk). Therefore, the extrapolation of our
2 findings may be limited.

4 **CONCLUSION**

5 In conclusion, the adiposity measures analyzed show a negative association with arterial
6 stiffness measures. The percentage of variation in CAVI explained by its relation to the
7 different measures of adiposity ranges from 5.8% (CUN-BAE) to 3.7% (BRI). In the case of
8 baPWV it oscillates between 0.7% (CUN-BAE and BMI) and 0.1% (WHtR). These results
9 suggest that measures of general adiposity and body fat percentage better explain the
10 variability of CAVI compared with measures of abdominal and visceral adiposity.

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13 Lead author for this group: Rafel Ramos, Research Unit, Primary Health Care, Girona, Jordi
14 Gol Institute for Primary Care Research (IDIAP Jordi Gol), Catalonia, Spain, E-mail:
15 rramos.girona.ics@gencat.net. Coordinating Center: Rafel Ramos, Ruth Martí, Dídac
16 Parramon, Anna Ponjoan, Miquel Quesada, Maria Garcia-Gil, Martina Sidera and Lourdes
17 Camós, Research Unit, Primary Health Care, Jordi Gol Institute for Primary Care Research
18 (IDIAP Jordi Gol), C/Maluquer Salvador, 11, 17002-Girona, Catalonia, Spain. Fernando
19 Montesinos, Ignacio Montoya, Carlos López, Anna Agell, Núria Pagès of the Primary Care
20 Services, Girona, Catalan Institute of Health (ICS), Catalonia, Spain. Irina Gil, Anna Maria-
21 Castro of the Primary Care Services, Girona, Institut d'Assistència Sanitària (IAS), Catalonia,
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25 Gómez-Sanchez, Carmen Castaño-Sanchez, Carmela Rodriguez-Martín, Benigna Sanchez-

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2
3 1 Salgado, Angela de Cabo-Laso, Emiliano Rodriguez-Sanchez, Jose Angel Maderuelo-
4
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6
7 3 Recio-Rodriguez, Manuel A Gomez-Marcos and Luis Garcia-Ortiz, Primary Care Research
8
9 4 Unit of The Alamedilla, Salamanca, Spain, Castilla and León Health Service–SACYL.
10
11
12 5

13
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15
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17
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19
20 9 manuscript. JIR-R, RM, FR, and CA-C participated in the study design, data collection and
21
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23
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25
26 12 manuscript. LG-O, RR, and MAG-M participated in the protocol design, fundraising, analysis
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1 **Figure legends**

2 **Figure 1.** Flow chart of this MARK substudy

3 Abbreviations: N, number; CAVI, cardio-ankle vascular index; baPWV, brachial-ankle pulse

4 wave velocity; ABI, ankle-brachial index; WC, waist circumference

5

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1 **Figure 1S (Supplementary material).** Data are given as medias estimadas de CAVI y
2 baPWV por cuartiles de BMI, WHtR, CUN-BAE and BRI.

3 Adjusted for age (years), sex (0=male and 1=female), systolic blood pressure smoking
4 (0=No and 1=Yes), atherogenic index, HbA1c, and METs/min/week

5 baPWV and CAVI levels were compared using an ANOVA.

6 Abbreviations: CAVI, cardio-ankle vascular index; ba-PWV, brachial-ankle pulse wave
7 velocity; BMI, body mass index; WHtR, waist-to-height ratio; CUN-BAE, Clínica
8 Universidad de Navarra - body adiposity estimator; BRI, body roundness index

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Table 1 General characteristics of all the sample and by gender.

Variables	Global (n=2354)	Males (n=1456)	Females (n=898)	p Value
Age (years)	61.4±7.7	61.1±8.1	61.8±7.0	0.030
Smoking n (%)	658 (28.0)	456 (31.5)	202 (22.7)	<0.001
Alcohol (gr/week)	72.2±117.5	102.2±133.3	23.6±59.9	<0.001
Physical activity (METs/min/week)	2481±2512	2886±1831	1825±1691	<0.001
Sedentary n (%)	1020 (43.3)	538 (37.0)	482 (53.7)	<0.001
Height (cm)	165±9	170±7	156±6	<0.001
Weight (kg)	79.4±14.6	83.9±13.4	72.2±13.3	<0.001
BMI (kg/m ²)	29.2±4.4	29.1±3.9	29.5±5.1	0.035
BMI ≥ 30 n (%)	847 (36.0)	485 (33.4)	362 (40.4)	0.001
Waist circumference (cm)	100.9±11.6	102.9±10.5	97.6±12.5	<0.001
WHtR	0.61±0.07	0.61±0.06	0.62±0.08	<0.001
CUN-BAE	35.7±1.7	31.1±4.5	43.1±5.1	<0.001
BRI	5.8±1.7	5.7±1.5	6.1±2.1	<0.001
SBP (mmHg)	137.1±17.4	138.9±17.1	134.2±17.5	<0.001
DBP (mmHg)	84.4±10.2	85.5±10.4	82.7±9.7	<0.001
Heart rate (beats minutes)	74.2±10.2	73.3±12.7	75.8±11.6	<0.001
Hypertension n (%)	1712 (72.7)	1122 (80.1)	590 (75.4)	<0.001
Antihypertensive drugs n (%)	1199 (50.9)	729 (50.2)	470 (52.6)	0.289
Total Cholesterol (mg/dl)	225.8±40.9	220.8±39.1	233.9±42.5	<0.001
LDL Cholesterol (mg/dl)	140.4±34.9	138.9±34.2	142.8±35.8	0.011
HDL Cholesterol (mg/dl)	49.8±12.9	47.9±11.9	52.9±13.8	<0.001
Triglycerides (mg/dl)	145.5±96.6	150.3±106.3	137.7±77.9	0.001
Atherogenic index	4.8±1.3	4.8±1.3	4.7±1.3	0.002
Dyslipidemia n (%)	2151 (91.4)	1311 (90.0)	840 (93.5)	<0.001
Lipid lowering drugs n (%)	671 (28.5)	392 (26.8)	279 (31.0)	0.034
FPG (mg/dl)	107.2±34.8	106.9±33.9	107.6±36.1	0.659
HbA1c	4.8±1.3	5.9±1.4	6.1±1.4	0.001
Diabetes n (%)	791 (33.6)	463 (31.8)	328 (36.5)	0.020
Antidiabetic drugs n (%)	474 (20.1)	269 (18.5)	205 (22.9)	0.011
CAVI	8.8±1.2	8.9±1.2	8.6±1.1	<0.001
baPWV (m/s)	14.9±2.5	14.8±2.5	15.0±2.6	0.107

Values are means and (standard deviations) for continuous data and number and (proportions) for categorical data.

Abbreviations: METs-min/week, metabolic equivalent minutes per week. BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, Clínica Universidad de Navarra-body adiposity estimator. BRI, body roundness index. SBP, systolic blood pressure. DBP, diastolic blood pressure. LDL, low density lipoprotein. HDL, high density lipoprotein. FPG, fasting plasma glucose. HbA1c, glycosylated hemoglobin. CAVI, cardio ankle vascular index. baPWV, braquial-ankle pulse wave velocity.

p value differences between male and females

1 **Table 2: Bivariate correlations of adiposity measures with CAVI and baPWV.**

	CAVI		baPWV	
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a
BMI	-0.264**	-0.303**	-0.035	-0.068**
WHtR	-0.119**	-0.222**	0.090**	0.001
CUN-BAE	-0.187**	-0.297**	0.054*	-0.063**
BRI	-0.125**	-0.218**	0.078**	0.005

2 The correlation coefficients between CAVI, baPWV and adiposity measurements showed
3 significant differences (p <0.001 in all cases).

4 Abbreviations: CAVI, cardio-ankle vascular index. baPWV, brachial-ankle pulse wave velocity. BMI,
5 body mass index. WHtR, waist-to-height ratio. CUN-BAE, Clínica Universidad de Navarra-body
6 adiposity estimator. BRI, body roundness index.

7 ^a Adjusted for age, sex and systolic blood pressure.

8 p-values by Pearson correlation.* p<0.05, ** p< 0.01.

9

1 **Table 3: Multiple regression analysis: association between adiposity measures with CAVI and**
 2 **baPWV.**

	R²	Standardized β	No Standardized β(95% CI)	Partial R²	p value
CAVI					
BMI	0.412	-0.289	-0.075 (-0.083 to -0.066)	0.055	<0.001
WHtR	0.381	-0.221	-3.650 (-4.207 to -3.093)	0.038	<0.001
CUN-BAE	0.410	-0.450	-0.069 (-0.077 to -0.061)	0.058	<0.001
BRI	0.378	-0.215	-0.142 (-0.165 to -0.120)	0.037	<0.001
baPWV					
BMI	0.402	-0.100	-0.057 (-0.076 to -0.038)	0.007	<0.001
WHtR	0.394	-0.044	-1.557 (-2.748 to -0.366)	0.001	0.021
CUN-BAE	0.401	-0.152	-0.050 (-0.068 to -0.033)	0.007	<0.001
BRI	0.394	-0.046	-0.066 (-0.115 to -0.018)	0.002	0.014

3 Four different multiple linear regression models were used to analyze the associations of adiposity
 4 measures with CAVI and baPWV.

5 Adjusted for age (years), gender (0=male and 1=female), systolic blood pressure, smoking (0=No and
 6 1=Yes), atherogenic index, HbA1c and METs/min/week.

7 Abbreviations: CAVI, cardio-ankle vascular index. baPWV, brachial-ankle pulse wave velocity. BMI,
 8 body mass index. WHtR, waist-to-height ratio. CUN-BAE, Clínica Universidad de Navarra-body
 9 adiposity estimator. BRI, body roundness index. METs-min/week, metabolic equivalent minutes per
 10 week.

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1 **Table 4: Association between adiposity measures with CAVI in different groups.**

	R²	β(95% CI)	Partial R²	p value
	CAVI			
Hypertensive				
BMI	0.409	-0.076 (-0.087 to -0.065)	0.023	<0.001
WHtR	0.383	-3.838 (-4.527 to -3.148)	0.035	<0.001
CUN-BAE	0.368	-0.068 (-0.078 to -0.058)	0.051	<0.001
BRI	0.382	-0.165 (-0.193 to -0.137)	0.035	<0.001
Non hypertensive				
BMI	0.372	-0.071 (-0.086 to -0.057)	0.052	<0.001
WHtR	0.324	-2.939 (-3.850 to -2.028)	0.046	<0.001
CUN-BAE	0.405	-0.065 (-0.080 to -0.052)	0.080	<0.001
BRI	0.318	-0.108 (-0.144 to -0.072)	0.042	<0.001
Diabetics				
BMI	0.439	-0.087 (-0.101 to -0.073)	0.95	<0.001
WHtR	0.399	-4.613 (-5.523 to -3.702)	0.069	<0.001
CUN-BAE	0.437	-0.083 (-0.096 to -0.070)	0.70	<0.001
BRI	0.391	-0.169 (-0.205 to -0.134)	0.062	<0.001
Non diabetics				
BMI	0.396	-0.067 (-0.078 to -0.056)	0.049	<0.001
WHtR	0.369	-3.088 (-3.781 to -2.396)	0.034	<0.001
CUN-BAE	0.395	-0.059 (-0.069 to -0.050)	0.064	<0.001
BRI	0.369	-0.130 (-0.159 to -0.099)	0.034	0.016
Obese				
BMI	0.414	-0.093 (-0.112 to -0.074)	0.054	<0.001
WHtR	0.377	-2.945 (-4.085 to -1.804)	0.003	<0.001
CUN-BAE	0.418	-0.100 (-0.122 to -0.079)	0.026	<0.001
BRI	0.377	-0.110 (-0.153 to -0.069)	0.003	<0.001
Non obese				
BMI	0.373	-0.058 (-0.078 to -0.038)	0.013	<0.001
WHtR	0.361	-1.394 (-2.366 to -0.421)	0.003	0.005
CUN-BAE	0.373	-0.050 (-0.066 to -0.033)	0.020	<0.001
BRI	0.358	-0.044 (-0.085 to -0.003)	0.002	0.034
≤ 62 years				
BMI	0.368	-0.078 (-0.089 to -0.077)	0.77	0.003
WHtR	0.332	-4.142 (-4.854 to -3.431)	0.065	<0.001
CUN-BAE	0.361	-0.068 (-0.078 to -0.058)	0.070	<0.001
BRI	0.324	-0.163 (-0.192 to -0.134)	0.062	<0.001
> 62 years				
BMI	0.234	-0.074 (-0.088 to -0.060)	0.056	<0.001
WHtR	0.195	-3.086 (-3.948 to -2.224)	0.029	<0.001
CUN-BAE	0.234	-0.071 (-0.084 to -0.059)	0.065	<0.001
BRI	0.193	-0.124 (-0.159 to -0.089)	0.028	<0.001
Assets				
BMI	0.414	-0.072 (-0.084 to -0.060)	0.053	<0.001
WHtR	0.392	-3.570 (-4.335 to -2.805)	0.034	<0.001
CUN-BAE	0.413	-0.066 (-0.075 to -0.053)	0.060	<0.001
BRI	0.391	-0.148 (-0.180 to -0.116)	0.034	<0.001
Sedentary				
BMI	0.408	-0.080 (-0.092 to -0.068)	0.055	<0.001
WHtR	0.364	-3.776 (-4.598 to -2.954)	0.045	<0.001
CUN-BAE	0.404	-0.074 (-0.086 to -0.062)	0.059	<0.001
BRI	0.360	-0.144 (-0.176 to -0.111)	0.043	<0.001

2 Multiple linear regression models were used to analyze the associations of adiposity measures with CAVI by
3 groups.

4 Adjusted for age (years), gender (0=male and 1=female), Systolic blood pressure, smoking (0=No and 1=Yes),
5 atherogenic index, HbA1c and METs/min/week.

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3 1 Abbreviations: METs-min/week, metabolic equivalent minutes per week. CAVI, cardio-ankle vascular
4 2 index. BMI, body mass index. WHtR, waist-to-height ratio. CUN-BAE, Clínica Universidad de Navarra-body
5 3 adiposity estimator. BRI, body roundness index.
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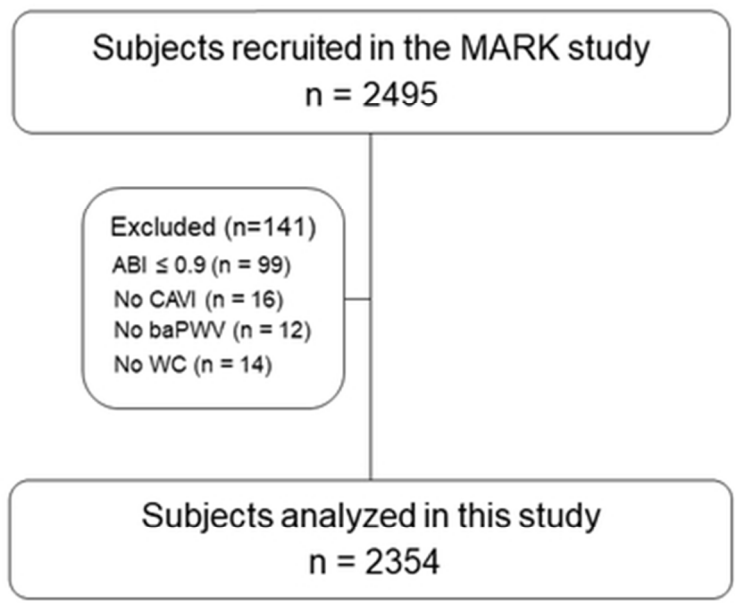
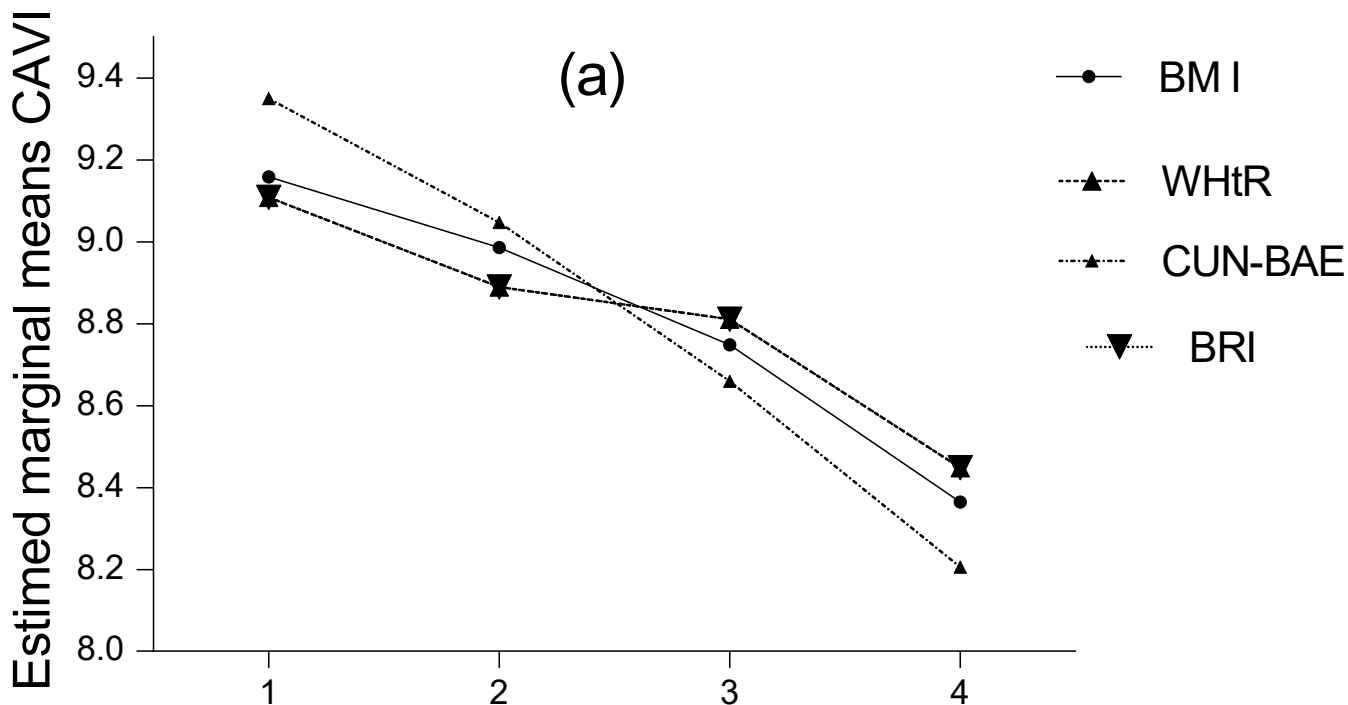


Figure 1. Flow chart of this MARK substudy
Abbreviations: N, number; CAVI, cardio-ankle vascular index; baPWV, brachial-ankle pulse wave velocity; ABI, ankle-brachial index; WC, waist circumference

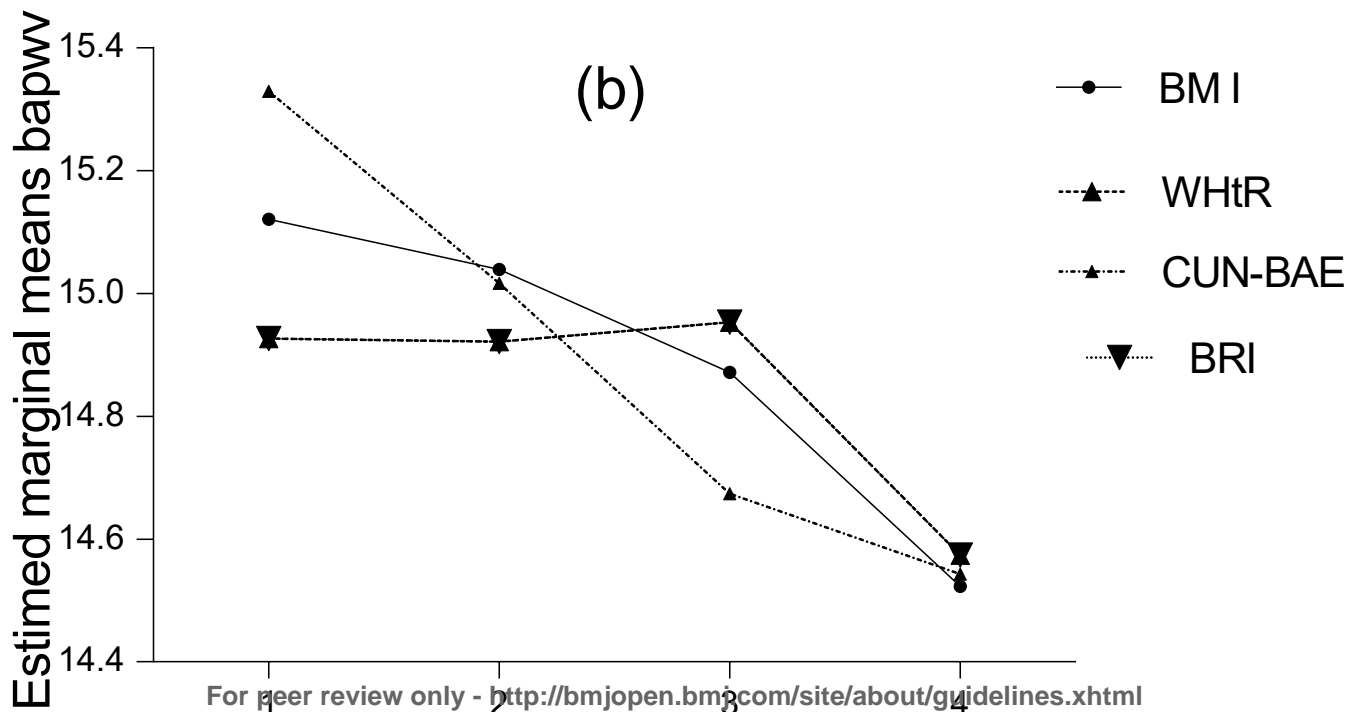
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(a)



(b)



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract Yes, Page 1 (b) Provide in the abstract an informative and balanced summary of what was done and what was found Yes, Page 2
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Yes, Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses Yes, Page 4
Methods		
Study design	4	Present key elements of study design early in the paper Yes, Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Yes, Page 5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants Yes, Page 5 (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case Yes, Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Yes, Page 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Yes, Page 6-9
Bias	9	Describe any efforts to address potential sources of bias Yes, Page 8-9
Study size	10	Explain how the study size was arrived at Yes, Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Yes, Page 8-9

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- Statistical methods 12 (a) Describe all statistical methods, including those used to control for confounding **Yes, Page 8-9**
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- (b) Describe any methods used to examine subgroups and interactions **Yes, Page 8-9**
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- (c) Explain how missing data were addressed **Yes, Page 8-9**
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- (d) *Cohort study*—If applicable, explain how loss to follow-up was addressed
Case-control study—If applicable, explain how matching of cases and controls was addressed
Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
-
- (e) Describe any sensitivity analyses **Yes, Page 8-9**

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Yes, Page 10-11 (b) Give reasons for non-participation at each stage Yes, Page 10-11 (c) Consider use of a flow diagram Yes, Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Yes, Page 10 (b) Indicate number of participants with missing data for each variable of interest Yes, Page 10 (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Yes, Tables (b) Report category boundaries when continuous variables were categorized Yes, tables (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Yes, Page 10

Discussion

Key results	18	Summarise key results with reference to study objectives Yes, Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Yes, Page 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Yes, Page 11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results Yes, Page 11

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Yes, Page 15
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.