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Títol del treball:

Arterial stiffness in a Mediterranean population: variation of cardio-ankle vascular index related to the presence of traditional cardiovascular risk factors and CV risk score

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Content

Summary

Cardiovascular diseases are the main cause of mortality and morbidity in high-income countries being arteriosclerosis the main pathological mechanism involved. The cardio-ankle vascular index (CAVI) is a novel stiffness measurement used to assess the extent of arteriosclerosis that enables us to detect it at early stages allowing for timely prevention treatment. In this study we want to describe the CAVI values in a Mediterranean population, as well as its associations with different clinical and behavioral risk factors. Additionally we want to analyze its relation with carotid arteriosclerosis burden assessed by other methods. A cross-sectional study was conducted with a representative sample of the general population of Girona between 40-90 years old (n=2502). The variables analyzed were: i) classical risk factors: hypertension, diabetes, obesity and hypercholesterolemia; ii) life styles: diet quality, smoking habits and physical activity; iii) carotid arteriosclerotic markers: common carotid artery intima-media thickness (CCA-IMT) and distensibility coefficient (DC); and iv) CAVI.

The group with abnormally high CAVI consisted of 43.32% of our sample and had a higher prevalence of cardiovascular risk factors, except for obesity. They also had a higher proportion of non-smokers, as well as individuals who didn't achieve the recommended cardio-protector physical activity, while no differences between groups were observed regarding the diet quality. A significant moderate correlation between CAVI and CCA-IMT and the DC was observed. We found a high prevalence of abnormally high arterial stiffness in a low coronary risk population. Since arterial stiffness is not included in the coronary risk scores, CAVI could be used to improve the classification of intermediate risk individuals sorting out those with higher risk that nowadays go undetected targeting them for timely prevention. CAVI has shown to be independently associated with aging, male sex and classical risk factors except for obesity where an inverse association was found. Physical activity in men was the only lifestyles independently associated with CAVI and did so with lower levels of CAVI. CAVI has also moderately correlated with carotid atherosclerotic burden assessed with CCA-IMT and DC.

Resum

Les malalties cardiovasculars són la principal causa de mortalitat i discapacitat en els països desenvolupats essent l'arteriosclerosi el principal mecanisme patològic. El *cardio-ankle vascular index* (CAVI) és un nou índex utilitzat per avaluar la seva progressió possibilitant la detecció en etapes primerenques permetent l'administració d'un tractament preventiu. En aquest estudi es volen descriure els valors de CAVI en una població mediterrània així com la seva associació amb diferents factors de risc tant clínics com estils de vida. També es pretén analitzar la relació de CAVI amb la càrrega arterioscleròtica de la caròtida. Es va realitzar un estudi transversal d'una mostra representativa de la població general de Girona d'entre 40 i 90 anys (n=2502). Les variables analitzades van ser: i) factors de risc clàssics: hipertensió, diabetis, obesitat i hipercolesterolèmia; ii) estils de vida: qualitat de la dieta, hàbits tabàquics i activitat física; iii) indicadors d'arteriosclerosi caròtida: *carotid artery intima-media thickness* (CCA IMT) i coeficient de distensibilitat (DC); i iv) CAVI.

El grup amb el CAVI anormalment alt consisteix del 43.32% de la mostra i tenia una major prevalença de factors de risc excepte obesitat on la prevalença era menor. També hi havia una major prevalença de no fumadors i de sedentaris que no arribaven a la activitat física mínima cardio-protectora mentre que no hi havia diferencies respecte la qualitat de la dieta. En quant a la càrrega arterioscleròtica carotídia CAVI va mostrar correlacions moderades amb el CCA-IMT màxim i amb el DC. S'ha trobat una alta prevalença de rigidesa arterial anormal en una població de baix risc coronari. Com que la rigidesa arterial no s'inclou en les equacions de risc coronari CAVI es podria afegir per millorar la classificació d'individus amb risc mitjà, reclassificant aquells amb més risc que avui en dia passen desapercebuts, permetent que rebin un tractament preventiu oportú. CAVI ha mostrat associacions positives i independents amb edat, sexe masculí i els factors de risc clàssics excepte obesitat on ha sigut inversa. La activitat física en homes ha sigut l'únic estil de vida que ha mostrat una associació independent amb CAVI. CAVI també ha obtingut correlacions moderades amb la càrrega arterioscleròtica avaluada mitjançant CCA-IMT i DC.

Resumen

Las enfermedades cardiovasculares son la principal causa de mortalidad y discapacidad en los países desarrollados siendo la arteriosclerosis el principal mecanismo patológico. El *cardio-ankle vascular index* (CAVI) es un nuevo índice para evaluar el progreso de la arteriosclerosis posibilitando la detección en etapas iniciales permitiendo la administración de un tratamiento preventivo. En este estudio se quieren describir los valores de CAVI en una población mediterránea así como sus asociaciones con diferentes factores de riesgo, tanto clínicos como estilos de vida. También se pretende analizar la relación de CAVI con la carga arteriosclerótica carotidea. Se realizó un estudio transversal de una muestra representativa de la población general de Gerona de entre 40 y 90 años (n=2502). Las variables analizadas fueron: i) factores de riesgo clásicos: hipertensión, diabetes, obesidad e hipercolesterolemia; ii) estilos de vida: calidad de la dieta, hábitos tabáquicos, y actividad física; iii) marcadores de arteriosclerosis carotidea: *carotid artery intima-media thickness* (CCA IMT) y el coeficiente de distensibilidad (DC); y iv) CAVI.

El grupo con CAVI anormalmente alto consiste del 43.32% de la muestra y tiene una mayor prevalencia de factores de riesgo excepto de obesidad donde ésta es menor. También hay una mayor prevalencia de no fumadores y de sedentarios que no llegan a la actividad física mínima cardio-protectora mientras que no hay diferencias respecto a la calidad de la dieta. En cuanto a la carga arteriosclerótica carotidea CAVI mostró correlaciones moderadas con CCA IMT y con DC. Se ha encontrado una alta prevalencia de rigidez arterial anormalmente alta en una población de bajo riesgo coronario. Como la rigidez arterial no se incluye en las ecuaciones de riesgo coronario CAVI se podría añadir para mejorar la clasificación de los individuos con riesgo intermedio, reclasificando aquellos con mayor riesgo que hoy en día pasarían desapercibidos, permitiendo la administración de un tratamiento preventivo oportuno. CAVI ha mostrado estar directa y positivamente asociado con la edad, sexo masculino y factores de riesgo clásicos excepto con obesidad donde la relación ha sido inversa. La actividad física en hombres ha sido el único estilo de vida que ha mostrado una asociación independiente con CAVI. CAVI ha su vez ha mostrado correlaciones moderadas con la carga arteriosclerótica carotidea evaluada con CCA-IMT y DC.

Introduction

Cardiovascular diseases burden

Cardiovascular diseases are the first cause of mortality (1,2) and disability in high income countries (3). In 2014, in Europe, cardiovascular diseases were responsible for 51% of mortality in women and 42% of mortality in men in 2014 (4). In Catalonia, these diseases caused 28.3% of deaths in 2013 (5). Moreover, cardiovascular diseases involve a high economic cost to society; the annual cost for the EU due to cardiovascular related diseases was €195.5 billion in 2009 (6). Therefore, due to the high impact these diseases have, it is very important to learn and understand how they develop in order to prevent them and reduce their negative effects on society to a minimum.

Arteriosclerosis

Arteriosclerosis is the main pathological mechanism of many cardiovascular diseases. It is an inflammatory process in which the arteries stiffen and thicken and is characterized by the accumulation of lipids, inflammatory cells and molecules in the subendothelial space of the artery wall causing a blood flow limitation (Figure 1). This can

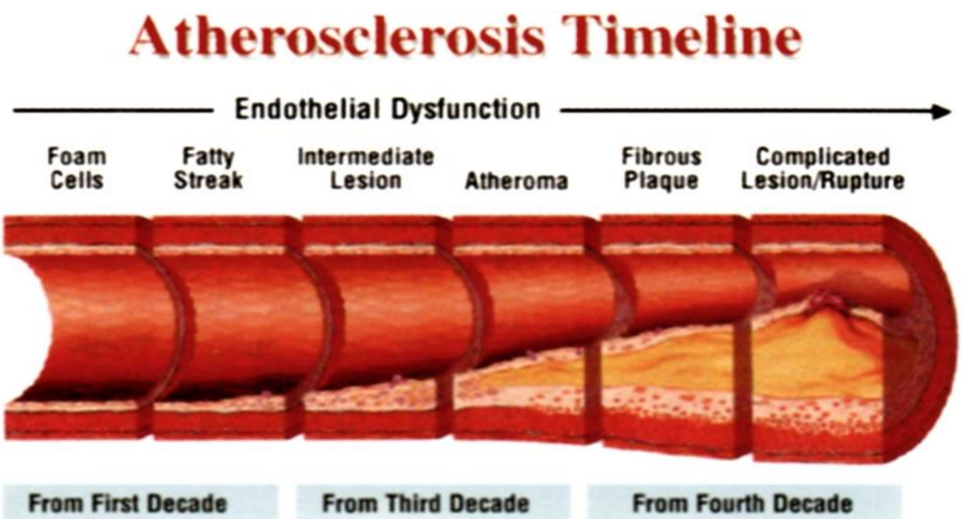

Figure 1: Atherosclerosis Timeline (79).

cause acute cardiovascular events, such as myocardial infarction, stroke or sudden cardiac death, when the occlusion of the artery lumen is acute (7) . It is a chronic process starting early on in life, with a slow progression and symptoms usually appear when it is already in advanced stages. Having a long asymptomatic phase makes prevention crucial since, if detected at early stages, its progression may be slowed down or even reversed (8).

Arteriosclerosis burden assessment

Several indexes can be used to estimate asymptomatic systemic arteriosclerosis burden being the ankle-brachial index (ABI), carotid intima-media thickness (IMT) and arterial stiffness the most commonly used in clinical practice.

ABI is the ratio between the systolic pulse in the ankle and arms, when this ratio is lower than 0.9 it is an indicator that the patient has a peripheral artery disease (PAD) in his/her lower extremities (9).

Carotid IMT is measured with ultrasounds, it is a noninvasive independent predictor of cardiovascular disease and is increasingly being used as a surrogate marker for arteriosclerosis (10). An increase of the carotid IMT of 0.1mm has been found to be associated with an increase of the future risk of myocardial infarction by 10-15% as wells as an increase of stroke risk by 13-18% (11). It has been shown that carotid IMT values tend to be higher in men than in women being age the most important factor determining a thickness increase (12). A positive correlation with carotid IMT has been found with an increase in PP, total cholesterol and, only in men, smoking. IMT has shown a negative correlation was found with HDL-C thus indicating HDL-C has a protective effect on the increase of thickness. The main limitations of this measurement are that it is highly dependent on the observer and that it requires extensive training and expensive equipment.

Arterial stiffness is the loss of elasticity of the arteries due to the degeneration of the elastic fibers as a consequence of aging as well as other pathogenic processes that affect de artery wall composition, such as arteriosclerosis (13). An increase of rigidity decreases arterial compliance causing an increase of the systolic blood pressure as well as the pulse pressure (PP) (14).

Arterial stiffness measurement

Nowadays several measurements are used to assess arterial stiffness at different sites: i) the gold standard and most commonly used method is the brachial-ankle pulse wave velocity (baPWV), this measurement has the inconvenient that its results are blood pressure dependent so they are highly variable within an individual depending on the moment of the measurement (15). Moreover, this measurement doesn't adjust well to elderly people, probably due to the healthy survivor effect (16); ii) β stiffness measures the proportional change in the luminal diameter (D) of the artery between systole and diastole (ΔD) and calculated as: (D/ΔD). It has the advantage that it is independent from blood pressure. The downside of this parameter is that it is measured using echo phase tracing system, a complex technique that requires extensive training and expensive equipment; iii) in this study we propose to use a novel measure of arterial stiffness derived from β stiffness, the cardio-ankle vascular index (CAVI) (Figure 2). The equation to determine CAVI value is as follows (17):

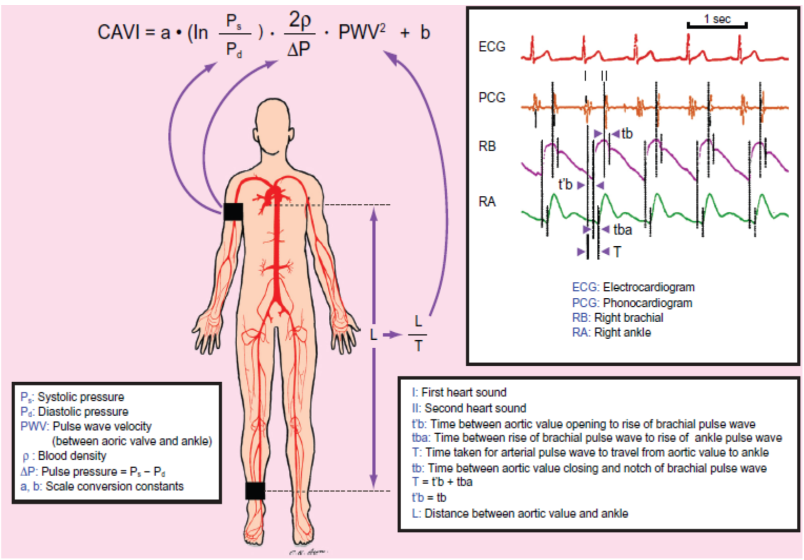

Figure 2: Schematic illustration of he acquisition of study parameters for computation of Cardio-Ankle Vascular Index (80).

$$CAVI= a[Ln(Ps/Pd) \bullet (2\rho / \Delta P) \bullet PWV^2]+b$$

Where; Ln=natural logarithm; Ps=systolic blood pressure; Pd=diastolic blood pressure; ρ=blood density (constant); ΔP=difference between Ps and Pd; PWV=pulse wave velocity; a and b=scale conversion constants. CAVI is essentially a variant of β stiffness that reflects the overall stiffness from the aorta, femoral and tibial arteries (18).

CAVI also has several limitations: i) it uses brachial-ankle instead of heart-ankle; thereby it assumes that brachial pressure is representative of carotid or thoracic arteries. This has been proved as a correct assumption since there is a moderate correlation coefficient between the CAVI values and the β values of 0.7 with the thoracic artery and 0.39 with the carotid artery with a confidence level <0.01 (19); ii) it measures the stiffness of the whole arterial tree, thus making the interpretation of the results nonspecific for any particular arterial territory. In the case of one artery being affected the pathological stiffness may be diluted by the rest of healthy arteries (16); iii) CAVI is not a good index when ABI is lower than 0.9 since the presence of peripheral arterial disease can compromise the quality of measurements giving falsely low CAVI indexes (17). These individuals, however, should already receive statin treatment to prevent future cardiovascular events (20).

On the other hand CAVI has proven to have important advantages: i) the most important one is its independence from blood pressure values. To prove this independence the CAVI value was measured before and after administering β-blocker to the same individual, being the value obtained the same in both cases (21); ii) it is highly reproducible, this has been proved measuring CAVI in a group of people six times a day for two days obtaining the same results each time without a correlation with blood pressure(16); iii) moreover, it is really simple to measure requiring very little training and provides with the stiffness of both sides of the arterial tree (16); iv) it is also an accurate indicator in a wide range of blood pressure values (50-200mmHg) (16); v) and it presents a low (<4%) intra-individual variability (17).

In summary, CAVI is a novel index, highly reproducible, easily measured and informative about the arterial stiffness of an individual and, therefore, provides valuable information about its risk of having a cardiovascular event (16).

When CAVI value is used as predictor of CV events the individuals are divided in three groups: low risk, CAVI<9; intermediate risk, CAVI≥9 and <10; high risk, CAVI≥10. Generally it is accepted that CAVI values≥9 are considered to be pathological (22). However, previous follow up studies, have found the threshold for higher risk of future cardiovascular events to be CAVI≥10 (23).

Arterial stiffness and cardiovascular risk factors

In Asiatic populations arterial stiffness has proven to be strongly related with the presence of classical risk factors: hypercholesterolemia, hypertension, diabetes and obesity. There are also non-modifiable risk factors such as age, sex and genetic background that have a great influence on arterial stiffness (24). Acquiring and maintaining healthy lifestyles is very important to slow down the progression of the arteriosclerosis before causing irreversible events, such as myocardial infarction or stroke (25). Prevention and promotion of healthy lifestyles are, therefore, the main strategies to reduce cardiovascular burden at the individual and population level.

As mentioned above there are modifiable and non-modifiable risk factors that stiffen the artery as well as healthy lifestyles that can delay and even revert the stiffening of the artery. Age is the main factor related to stiffening; aging is responsible of a 10-15% yearly stiffness increase (26). This may be due to fractures and fragmentations of the elastin fibers as a consequence of repeated stress cycles (27). This translates to a 0.05 yearly increase in the CAVI value in healthy individuals solely due to aging. Another non-modifiable factor is the sex of the individual having males a higher CAVI value by 0.2 respect women throughout all the ages, indicating that men's arteries are 4 years older than women's (28). Since we cannot change these factors we have to focus our attention on establishing healthy lifestyles in our day-to-day life and control cardio-vascular risk factors. There are several classic risk factors and in this study we are going to focus on diabetes, hypercholesterolemia, obesity and hypertension.

We are also going to evaluate whether smoking, diet and physical activity practice are related with arterial stiffness. Smoking has proven to be a hazard for cardiovascular health (29) but there is controversy whether it affects arterial stiffness or not since different studies have obtained diverse and contradictory results (30).

Nowadays there is numerous evidence pointing out the importance of a healthy diet and its protective effect on arterial stiffness (31). Positive correlations between arterial stiffness, measured with PWV, with the intake of alcohol and meat, while negative correlations with the amount of fiber, vitamins B9 and C as well as β-carotenes have been reported (32). This proves the importance of a healthy diet related to cardiovascular health and arterial stiffness in particular. Other studies have shown the protective effect the fruit and vegetable intake has on arterial stiffness, this protective effect was increased when those healthy diet habits start at childhood (33,34). Therefore it is very important to emphasize the importance of a healthy diet since early on in life.

Physical activity is any musculoskeletal movement requiring energy expenditure above resting energy expenditure (35). Regular aerobic exercise has been shown to prevent or even reverse arterial stiffness (36). Individuals that engage in physical activity on a daily basis have lower aPWV than individuals with a sedentary lifestyle (37). Physical activity decreases the risk of cardiac mortality by 31% (38), and thus enhancing the need to raise awareness and engage on regular physical activity.

Justification

The relevance of CAVI is yet to be tested on a Mediterranean population. This population is of special interest since it has a relatively low myocardial infarction incidence compared to the high prevalence of risk factors, what has been called the Mediterranean paradox (39). First, we will need to know the distribution of the CAVI values at the population level. Moreover, it is relevant to assess how CAVI relates to classical risk factors and healthy lifestyles. Finally, it is important to understand the correlation between CAVI and other atherosclerosis burden measurements, such as carotid IMT, and carotid stiffness or carotid distensibility coefficient. If we demonstrate a good correlation with other standardized methods and with healthy lifestyles we will provide evidence supporting the use of CAVI to monitor the effect of lifestyles with an easy and accessible method that can be used in routinely clinical practice.

Hypothesis

a) The prevalence of abnormal CAVI in a Mediterranean population is of 30%.
b) The presence of classical cardiovascular risk factors is associated with higher CAVI values in both sexes indicating a higher prevalence of arterial stiffness across the arterial tree.
c) Unhealthy lifestyles are associated with higher CAVI values indicating a higher prevalence of arterial stiffness across the arterial tree in both sexes.
d) There is a linear and direct association between CAVI and carotid intima-media thickness and distensibility coefficient, indicating that CAVI can be a valid surrogate marker associated with carotid stiffness and atherosis in both sexes.

Objectives

a) To assess the prevalence of abnormal CAVI in a Mediterranean population.

b) To assess the association between CAVI and classical cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia, obesity) by sex.

c) To assess the association between CAVI and lifestyles (diet, physical activity practice and smoking habits) by sex.

d) To assess the association between CAVI and carotid atherosclerosis burden (carotid IMT and carotid distensibility coefficient) by sex.

Methods

Design

This is a descriptive, population based, cross-sectional study using the population of a follow up study framed in the multicenter study Harmonization of Risk Equations in the Mediterranean in Southern Europe (HERMES) developed by the Registre Gironí del Cor (REGICOR).

Population of study

The population-based sample of this study includes 2502 individuals recruited between May 2010 and November 2013. This sample is a subpopulation belonging to a cross-sectional survey carried out between September 2007 and November 2013, as a follow up assessment of an initial cohort recruited in Girona (Catalonia, south-western Europe) from 2003 to 2006 of 6500 people, in the context of the REGICOR study, approximately 70% of the participants agreed to enroll in the study (12). Participants eligible in our study ranged between 40 and 90 years old whose CAVI measurement was performed and had an ankle-brachial index (ABI) higher than 0.9, since the presence of peripheral arterial disease can give falsely low CAVI scores (17). Participants were excluded from the study when they had a terminal disease or were institutionalized at the time of the appointment. The selected participants were summoned by phone to their primary health center where they were invited to participate in the study by signing an informed consent. With this sample we can estimate the prevalence of abnormal CAVI with a precision +/- 2% with a 95% confidence interval. It also allows us to recognize statistically significant odds ratios greater or equal than 1.25 accepting a beta risk of 0.2 when assuming a worst case scenario, proportion of exposed individuals is 50%.

Data collection

Trained nurses performed the examinations and interviews following standardized methods and homologated devices, detailed elsewhere (40).

Sociodemographic variables, smoking (current smokers, ex-smokers (>1-year), never smokers) and history of high blood pressure, dyslpidemia, and diabetes were recorded. Blood samples were extracted after 10-14 hours of fasting. Blood pressure was measured with a calibrated oscillometric sphygmomanometer (OMRON M6, HEM-7001-E), two measurements were done and the mean value was obtained. Brachial pulse pressure (PP) was calculated by subtracting the mean diastolic blood pressure (DBP) from the mean systolic blood pressure (SBP). Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides and glucose were determined by direct methodology (Roche Diagnostics, Basel, Switzerland). Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation if triglycerides were <3.4mmol/l (300 mg/dl) (41). Weight, height, body mass index (BMI) (kg/m^2), obesity (BMI ≥ 30 kg/m^2), percentage of body fat assessed with bioelectrical impedance (Tanita Corporation, BC-418MA), waist circumference (high if more than 102 cm in men and more than 88 cm in women) were also measured (42). Coronary risk was calculated using the REGICOR function (43) in those participants aged 35-74 years free of CV diseases. Hypertension was defined when (a) the participants reported a previous diagnosis or treatment for hypertension or (b) SBP and DBP were ≥140mmHg and ≥90mmHg, respectively. Diabetes was defined if the participants (a) reported a previous diagnosis or treatment for diabetes or (b) if fasting glucose concentration at recruitment was ≥ 126mg/dl. Hypercholesterolemia was defined when (a) the total cholesterol concentration was ≥ 250mg/dl or (b) participants received lipid-lowering drugs.

Physical activity was assessed using an adapted short questionnaire (Elosua R., personal communication) based on the validated Minnesota leisure-time physical activity questionnaire (44,45). Using MET based intensity code, physical activities were classified according to their energy expenditure: light physical activity <4 MET, moderate physical activity 4-5.5 MET, intense physical activity ≥6 MET. Those individuals that don't reach the minimal cardio-protective physical activity recommended by the American Heart Association, 30 minutes of moderate physical activity 5 days a week or 20 minutes of intense physical activity 3 times a week (46), were considered to be sedentary, those that reached the standards where considered active. The diet was assessed using a validated short dietary quality screener (sDQS) (47).

Intima-media thickness was measured by trained sonographers with an Aucson XP128 ultrasound machine equipped with an L75-10 MHz transducer and extended frequency software (Acuson-Siemens, Mountainview, California, United States). Measurements were made in a 1cm segment in the distal carotid artery (CCA), 1cm in the carotid bulb (CB) and 1cm in the proximal internal carotid artery (ICA) of both right and left arteries. From both sides the mean and maximal values where calculated. For the purpose of this study the maximal CCA IMT was used this is defined as the average of the maximum value observed in the right and left side. From the image files we also obtained the distensibility coefficient. When referring to this measure we considered the least distensible value of both sides to do the statistical analysis.

CAVI was used to assess arterial stiffness. Normal CAVI values are those < 8, values between 8 and 9 are considered as borderline and those higher than 9 are indicators of arteriosclerosis. These measurements where realized using VaSera VS-1500® (FukudaDenshi Co. Ltd). VaSera examinations followed the rules for the best accuracy of the measurement: participants wore light clothes and rested at least 10 minutes before the test execution. Volunteers were asked to avoid eating, smoking or consuming caffeine immediately before the examination. Participants laid face up on a bed and had arms and legs relaxed with a small cushion under their head. Cuffs at the fitted size were tightly wrapped to both brachia and ankles. Four small cushions were placed under feet and arms to avoid interaction of the inflated cuffs with the bed. Finally, electrodes were attached to right and left wrists and a heart sound microphone was fixed with a double-sided tape over the sternum in the second intercostal space. Participants were asked to keep still and silent during approximately five minutes. Only CAVI measurements obtained during at least three consecutive heartbeats were considered valid. The highest CAVI value from the left and right sides was considered for analyses.

Abnormal CAVI was defined as, CAVI≥9 or CAVI≥ mean CAVI + 1SD for the 10 year age ranges showed in Table 1. This classification affects the individuals aged 40-49 years since men between 8.48 and 9 and women between 8.13 and 9 won't be considered normal. Patients were excluded when the measurement wasn't properly done according to the VaSera guidelines.

Table 1: Threshold values to set abnormal CAVI.

	Men			Women		
Age ranges (years)	**Mean**	**SD**	**Mean+1SD**	**Mean**	**SD**	**Mean+1SD**
40-49	7.69	0.79	8.48	7.38	0.75	8.13
50-59	8.52	1.02	9.54	8.15	0.89	9.04
60-69	9.26	0.98	10.24	8.99	1.04	10.03
70-79	10.27	1.26	11.53	9.71	1.03	10.74
80-89	10.71	1.14	11.85	10.14	0.99	11.13

SD: Standard deviation

Statistical analysis

The associations between sex and CAVI groups for continuous variables were performed using T Test and ANOVA, as for categorical variables l the Chi Square test was used. We didn't differentiate between parametric and non parametric data since, according to the central limit theorem, large sample means can be treated as being normally distributed (48). A multivariate analysis with a logistic regression with the presence/absence of abnormal CAVI as the outcome was performed to determine its association with cardiovascular risk factors and lifestyles. A Pearson correlation was used to assess the association between CAVI and carotid atherosclerotic burden. A p-value lower than 0.05 was considered as statistically significant. The R-studio software was used for statistical analyses.

Ethical issues

All participants were informed and freely signed an informed consent to participate in the study, which was previously approved by the local ethics committee. The principals of experimentation in humans were respected according to the Helsinki agreement as well as the treatment of the data obtained and any results obtained. Confidentiality was kept according to 5th article of Organic law 15/1999 December 13th that regulates the automated analysis of personal data. Participant had the right to access their data and results as well as to withdraw from the study by request at any time and eliminate their data.

Results

Description of the sample

The sample consisted of 2502 participants, 46.7% were men (n=1168) and the mean age was 60.13 years (Table 2). The distribution of classical risk factors by gender is shown in Table 2.

Men had higher values of blood pressure (BP), fasting glucose, BMI, waist and REGICOR coronary risk and lower levels of total, LDL and HDL cholesterol than women. Men also had higher CAVI values as well as a higher proportion of individuals with pathological CAVI (CAVI≥9). When looking at the carotid atherosclerosis burden we also observed that men tended to have higher maximal CCA IMT and less distensibility than women.

As for the prevalence of the classical risk factors (Table 2) men had a higher prevalence of diabetes 19.35 (95% CI: 17.12-21.73) vs. 10.12 (95% CI: 8.55-11.86) and

Table 2. Main characteristics of the participants and distribution of classical risk factors distribution by sex.

	All	Men	Women	P-value
Number of subjects	2502	1168	1334	
Age, years	60.13(11.10)	60.28 (10.75)	59.99 (11.39)	0.527
Systolic BP, mmHg	130.47(19.46)	135.27 (18.00)	126.26 (19.72)	<0.001
Diastolic BP, mmHg	77.29(10.10)	80.07 (9.94)	74.86 (9.62)	<0.001
Pulse pressure, mmHg	53.17(15.72)	55.20 (15.27)	51.40 (15.89)	<0.001
Total cholesterol, mg/dl	200.78(35.22)	196.74 (35.19)	204.38 (34.87)	<0.001
Hypertension, %	48.56	56.42	41.68	<0.001
LDL-C, mg/dl	129.90(30.38)	128.53 (30.41)	131.11 (30.32)	0.036
HDL-C, mg/dl	51.26(11.46)	46.80 (10.23)	55.23 (11.03)	<0.001
Hypercholesterolemia, %	7.87	6.76	8.84	0.064
Glucose, mg/dl	96.26(22.46)	101.23 (25.99)	91.83 (17.62)	<0.001
Diabetes Mellitus, %	14.42	19.35	10.12	<0.001
Triglyceride, mg/dl	100.03(61.25)	109.97 (71.12)	91.19 (49.23)	<0.001
BMI, m/kg^2	27.29(4.40)	27.89 (3.86)	26.77 (4.76)	<0.001
Waist, cm	95.87(12.34)	100.32 (10.60)	91.98 (12.44)	<0.001
REGICOR coronary risk	3.34[1.86-5.65]	4.70 [2.72-7.29]	2.36 [1.37-3.97]	<0.001
CAVI	8.76(1.34)	8.98 (1.37)	8.58 (1.28)	<0.001
Categorical CAVI, %				<0.001
CAVI<8	30.74	25.17	35.61	
CAVI>8 and <9	28.18	28.08	28.26	
CAVI≥9 and <10	24.34	27.05	21.96	
CAVI≥10	16.75	19.69	14.17	
Abnormal CAVI	43.32	48.90	38.46	
Maximal CCA IMT, mm	0.77(0.13)	0.80 (0.15)	0.76 (0.15)	<0.001
Distensibility coefficient, cm^2 dyne^{-1}10^{-6}	26.72(10.58)	24.57 (8.75)	28.59 (11.61)	<0.001

Unless otherwise indicated, data are expressed as mean (standard deviation) or median [25th percentile-75th percentile]. BP: blood pressur HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; BMI: body mass index; CAVI: cardio-ankle vascular index; IMT: intima-media thickness; CCA: common carotid artery.

hypertension 56.42 (95% CI: 53.52-59.29) vs 41.68 (95%CI: 39.02-44.38) and as for hypercholesterolemia, even though we see that women tended to have a slightly higher prevalence, there were no significant differences. The prevalence of individuals with abnormal CAVI was 43.32% (95% CI: 41.37-45.29) while having a very low REGICOR coronary risk, 3.34%.

Lifestyles habits are shown in Table 3. When studying the diet quality no significant differences were observed. There were more men than women that had smoked at some point of their live as well as that were currently smoking (Table 3). We also saw that men smoke a higher amount of cigarettes than women according to the Brinkman index.

As for physical activity (Table 3) men spend more kcal/week than women. When breaking down the total physical activity according to the intensity in light, moderate and intense women have a higher mean expenditure in light and intense physical activity while

Table 3. Lifestyle description of the participants by sex.

	All	Men	Women	P-value
DQI	38.78(3.29)	38.66(3.28)	38.89(3.30)	0.087
Smoking, %				<0.001
Current	17.97	20.56	13.82	
Former	31.05	46.47	17.58	
Never	51.99	32.96	68.59	
Brinkman index, (cigar/day)*year				
BI<640	323.83(183.85)	338.64(179.65)	307.79(187.25)	0.029
BI>640	1154.57(586.32)	1210.62(615.24)	940.78(394.69)	<0.001
EEPA, kcal/week				
Total	1776[839-3146]	2136[987-3968]	1538[743-2556]	<0.001
Light	279[0-839]	251[0-839]	279[0-839]	0.666
Moderate	140[0-1113]	279[0-1678]	61[0-839]	<0.001
Intense	343[73-1315]	342[73-1585]	347[73-1183]	<0.001
Active, %	63.91	69.53	59.19	<0.001

Unless otherwise indicated, data are expressed as mean (standard deviation) or median [25th percentile-75th percentile]. DQI: Dietary quality index; BI: Brinkman index; EEPA: energy expenditure of *physical activity;* Brinkman index considers current and former smokers.

men have a higher mean expenditure in moderate physical activity. The proportion of individuals that achieved the recommended physical activity per week considered to be cardio-protective is higher in men than in women, 69.53 (95% CI: 66.73-72.24) vs. 59.19 (95% CI: 56.48-61.86).

Bivariate analysis by abnormal CAVI

The proportion of individuals showing an abnormal CAVI was 48.90% (95% CI: 45.98-51.80) in men and 38.46% (95% CI: 35.83-41.12) in women. We also compared within each sex those individuals that had abnormal and normal CAVI. As shown in Table 4, both men and women with normal CAVI had a mean age 10 years younger than those with abnormal CAVI.

In both sexes participants with abnormal CAVI had higher blood pressure, fasting glucose levels and REGICOR coronary risk than individuals with normal CAVI. Male participants with abnormal CAVI presented lower levels of total cholesterol as well as LDL

Table 4. Participants characteristics by sex and abnormal CAVI

	Men			Women		
	Normal	**Abnormal**	**p-value**	**Normal**	**Abnormal**	**p-value**
Number of subjects	597	571		821	513	
Age, years	54.74(8.19)	66.35(9.70)	<0.001	54.90(8.78)	68.14(10.25)	<0.001
Systolic BP, mmHg	129.17(15.16)	141.66(18.53)	<0.001	119.99(17.61)	136.28(18.69)	<0.001
Diastolic BP, mmHg	79.84(9.73)	80.32(10.15)	0.408	74.28(9.58)	75.78(9.75)	0.006
Pulse pressure, mmHg	49.33(11.68)	61.33 (16.15)	<0.001	45.70(12.16)	60.51(16.78)	<0.001
Hypertension, %	40.03	73.56	<0.001	27.41	64.52	<0.001
Total cholesterol, mg/dl	198.94(33.84)	194.44(36.44)	0.029	203.50(34.56)	205.76(35.68)	0.259
LDL-C, mg/dl	130.95(29.62)	126.00(31.05)	0.006	131.31(29.79)	130.79(31.54)	0.768
HDL-C, mg/dl	46.57(9.65)	47.05(10.82)	0.428	55.40(10.88)	54.97(11.48)	0.503
Hypercholesterolemia,%	5.68	7.71	0.255	8.77	8.97	0.981
Glucose, mg/dl	96.43(18.08)	106.27(31.50)	<0.001	89.59(14.16)	95.38(22.03)	<0.001
Diabetes Mellitus, %	11.22	27.85	<0.001	6.82	15.40	<0.001
Triglyceride, mg/dl	108.5(73.57)	111.51(68.50)	0.470	84.83(46.50)	101.32(51.76)	<0.001
BMI, m/kg^2	28.16(4.13)	27.57(3.53)	0.002	26.76 (5.09)	26.79 (4.17)	0.901
Waist, cm	100.17(11.19)	100.50(9.93)	0.597	91.14(12.98)	93.33(11.40)	0.001
REGICOR coronary risk	3.45 [2.24-5.34]	6.55 [4.51-9.98]	<0.001	1.98 [1.15-3.34]	3.51 [2.27-5.05]	<0.001
CAVI	7.94(0.68)	10.06(1.03)	<0.001	7.78(0.79)	9.85(0.79)	<0.001
Maximal CCA IMT, mm	0.75(0.12)	0.85(0.16)	<0.001	0.71(0.12)	0.83(0.15)	<0.001
Distensibility coefficient task force, cm^2 dyne^{-1}10^{-6}	27.49(8.77)	21.55(7.63)	<0.001	32.43(11.38)	22.35(8.82)	<0.001

Unless otherwise indicated, data are expressed as mean (standard deviation) or median [25th percentile-75th percentile]. BP: blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; BMI: body mass index; CAVI: cardio-ankle vascular index; IMT: intima-media thickness; CCA: common carotid artery.

cholesterol while no significant differences regarding HDL cholesterol. BMI also showed to be lower in males with abnormal CAVI but no significant differences were found regarding waist circumference or triglyceride levels. As for women, there were no significant differences regarding cholesterol or BMI while the abnormal CAVI group presented larger waists and higher levels of triglycerides (Table 4). Regarding carotid arteriosclerosis burden (Table 4) assessed using different parameters: maximal CCA IMT and distensibility coefficient, participants from both sexes with abnormal CAVI had higher values of maximal CCA IMT and lower distensibility coefficients.

In Table 5 we present the results obtained comparing lifestyles habits between the normal and the abnormal group. There were a higher proportion of smokers in the group that didn't have pathological CAVI, and in women there was a higher prevalence of non-smokers in the group that had abnormal CAVI. There weren't any differences between how much each group smoked according to the Brinkman index (Table 5). No significant differences were found between the quality of the diet in men, in women the ones with abnormal CAVI had a slightly better diet.

Regarding physical activity both groups with abnormal CAVI spent more kcal throughout the week than the healthy group, significantly in men, this expenditure was mainly related to practicing light and moderate physical activity practice. When looking at

Table 5. Participants lifestyles by sex and pathological CAVI

	Men			Women		
	Normal	Abnormal	p-value	Normal	Abnormal	p-value
DQI	38.49 (3.28)	38.84 (3.27)	0.074	38.73 (3.10)	39.14 (3.64)	0.0345
Smoking, %			<0.001			<0.001
Current	24.58	16.37		17.80	7.44	
Former	41.75	51.41		22.44	9.78	
Never	33.67	32.22		59.77	82.79	
Brinkman index, (cigar/day)*year						
BI<640	338.22(184.42)	339.31 (172.38)	0.955	310.55(191.99)	297.55(169.45)	0.584
BI>640	1136.55 (583.83)	1265.10(633.35)	0.058	950.24 (422.89)	899.38 (240.57)	0.521
EEPA, kcal/week						
Total	2027.04 [915-3516]	2269.23 [1119-4325]	0.010	1507.00 [732-2434]	1659.10 [804-3077]	0.129
Light	223.78 [0-559.44]	382.29 [0-993.00]	<0.001	223.78 [0-671.33]	419.58 [0-1006.99]	<0.001
Moderate	279.72 [0-1118.88]	524.48 [0-2097.90]	<0.001	104.9 [0-699.30]	0 [0-882.87]	0.014
Intense	657.34 [97.90-1874.13]	195.80 [24.48-1192.31]	<0.001	391.61 [89.52-1192.31]	244.76 [48.95-144.23]	0.084
Active, %	73.14	65.59	0.008	61.27	55.80	0.057

Unless otherwise indicated, data are expressed as mean (standard deviation) or median [25^{th} percentile-75^{th} percentile]. BI: Brinkman Index, considering current and former smokers; EEPA: energy expenditure of *physical activity*.

men, the normal group spent less kcal in light or moderate physical activity but the expenditure in intense physical activity was much greater than the participants with abnormal CAVI. Women with abnormal CAVI spent more kcal in light physical activity and less in moderate and intense physical activity than the healthy group. It is remarkable that men with abnormal CAVI had a significant higher proportion of individuals that didn't reach the minimal cardio-protective physical activity per week than those with normal CAVI, this tendency was also observed in women but the differences weren't significant.

Multivariate analysis:

In the multivariate logistic regression model the variables that were associated with abnormal CAVI in men and women are shown in Tables 6 and 7, respectively.

Table 6. Variables associated with abnormal CAVI scores in men. Results of the multivariate logistic regression: odds ratio (OR), 95% confidence interval (CI) and p-value of the associations.

	OR	Lower CI	Upper CI	p-value
Age	1.14	1.12	1.16	<0.001
Hypertension	2.59	1.85	3.66	<0.001
Diabetes	2.47	1.65	3.75	<0.001
Waist circumference	1.01	0.98	1.04	0.564
BMI	0.83	0.77	0.91	<0.001
Total cholesterol	1.00	0.99	1.01	0.092
Active	0.66	0.47	0.92	<0.050

BMI: body mass index.

In both sexes age, diabetes, hypertension, waist circumference, total cholesterol and a sedentary life style had an odds ratio higher than 1, being total cholesterol the only one non significant. Diabetes and hypertension are the most relevant since their presence increases the probability of having abnormal CAVI by 1.5 to 2.5 folds. BMI in turn had a significant inverse odds ratio with abnormal CAVI. Similar results were obtained in women: age, hypertension, diabetes, waist circumference and total cholesterol implied higher risks of having abnormal CAVI; in this case total cholesterol and not being sufficiently active were not significant while BMI, as in men, showed a significant

Table 7. Variables associated with abnormal CAVI scores in men. Results of the multivariate logistic regression: odds ratio (OR), 95% confidence interval (CI) and p-value of the associations.

	OR	Lower CI	Upper CI	p-value
Age	1.14	1.12	1.16	<0.001
Hypertension	2.66	1.94	3.68	<0.001
Diabetes	1.74	1.07	2.86	<0.050
Waist circumference	1.03	1.01	1.06	<0.050
BMI	0.82	0.77	0.88	<0.001
Total cholesterol	0.99	0.99	1.01	0.545
Active	0.80	0.59	1.08	0.140

BMI: body mass index

protective odds ratio on having abnormal CAVI. When a receiver operating characteristic, ROC curve, for the logistic regression models was realized an area under the curve (AUC) of 0.85 was obtained for men and of 0.86 for women. In men the best discriminant threshold when predicting abnormal CAVI was 0.46 with specificity=79.23% and sensitivity=78.53%, in women the best threshold was 0.41 with specificity=81.53% and sensitivity=75.91%.

Correlation analysis

The results of the correlation analysis are shown in figure 3, on the left for men and on the right for women. Moderate significant correlations were found in both sexes between maximal CCA IMT and distensibility coefficient with CAVI as presented in figure 3. As expected common carotid IMT showed a moderate positive correlation with CAVI, while the distensibility coefficient showed a moderate inverse correlation with CAVI. In men even though the correlations obtained were significant they were weaker than in women.

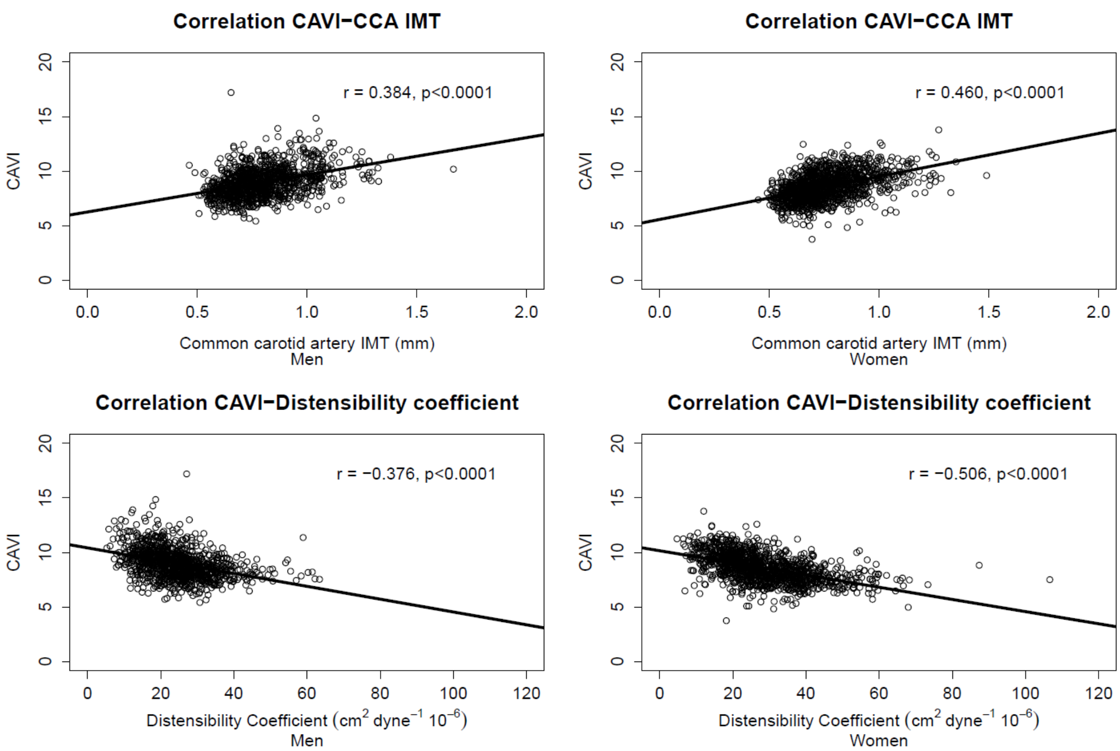

Figure 3: Relationship between CAVI and carotid arteriosclerotic burden parameters. Correlations regarding men are found on the left side and the ones regarding women are on the right. CAVI: cardio-ankle vascular index; CCA IMT: common carotid intima-media thickness.

Discussion

The results obtained in this population based study show that in our Mediterranean population men tend to have higher CAVI values than women and that as age increases CAVI increases accordingly. Of the general population studied 43.32% (95% CI: 41.37-45.29) had abnormal CAVI; this fraction of the population is older, has a higher prevalence of risk factors as well as a higher carotid atherosclerosis burden, its REGICOR risk scores are higher than the normal group but still considered of low risk, <10% (49). We have also found that the presence of diabetes and hypertension increases the probability of having abnormally high CAVI, as does physical inactivity, an increase in waist circumference or total cholesterol. However, BMI is inversely related to abnormal CAVI in both sexes. Smoking habits have shown to not have a significant relation with arterial stiffness assessed using CAVI. The models obtained realizing the multivariate analysis had good AUCs thus being good discriminators when differentiating between individuals with normal and abnormal CAVI.

The effects of being male and aging on arterial stiffness may be explained by the fact that men have higher BP, therefore, their arteries undergo higher stress levels that may cause a higher degradation of endothelial elastin (24,27,50). When assessing the association between CAVI and classical risk factors our hypothesis was validated for all the risk factors except for BMI that appeared to be inversely related with a decrease of 15% of the odds of having abnormal CAVI compared to normal weight individuals. These results regarding BMI have also been reported in other studies (51–53) although the majority find a positive correlation (28,54). A possible explanation could be that visceral fat has a higher impact on arterial stiffness than subcutaneous fat, thus being waist circumference a more accurate measure of noxious fat since BMI takes into consideration subcutaneous of fat and muscle as well as visceral fat. Waist circumference has shown to be a good indicator of visceral adiposity (55) that in turn has proven to be correlated with arterial stiffness (56). Another possibility is that our results may have a bias since assessing CAVI in overweight individuals is harder due to the fact that the cuffs are harder to place and receive a correct reading of the signals thus introducing the bias in the data tending to underestimate CAVI. Hypertension showed a positive association with the risk of having abnormal CAVI of 2.5 folds in both men and women; this fold increase has previously been reported in oriental populations (19,57,58) and is due to the hypertrophy of the intima-media caused by an increase of synthesis of extracellular matrix (59,60). A fold increase on the risk of having abnormal CAVI has also been observed with diabetes in both men and women, 2.5 and 1.8 respectively, these results have previously been seen in other studies (61,62) and can be related with the cross-linking of extracellular matrix protein and advanced glycation end-products (AGE) that in turn increase the stiffness and the normal function of the cells it affects (63,64). The accumulation of AGE and cross linking of extracellular matrix proteins are a common condition among diabetics and the elderly (65,66). Cholesterol yielded odds ratio slightly higher than one in men and women but only did so significantly in men. The effect of total cholesterol levels on CAVI across different studies have shown discrepancy in the results ranging from a significantly lower in individuals with pathological CAVI (61) to no significant differences (67) to significantly higher (68). These discrepancies are interesting since it has been established that cholesterol actively participates in the causal mechanism of atherosclerosis (7). Therefore our results presenting an OR of 1.01 in men fit the expected outcome.

When assessing the association between CAVI and lifestyles only cardio-protective physical activity in men had a significant protective odds ratio in the multivariate analysis with abnormal CAVI. When assessing the quality of the diet, the smoking status in both sexes and the cardio-protective physical activity in women there were no significant differences when adjusting the model. Smoking has long been established as an unhealthy lifestyle as it is a major cardiovascular risk factor and one of the main preventable causes of death in developed countries (69). Our results don't show a significant relation between smoking and abnormal CAVI. These results have also been observed in previous studies

(23,53,67). This may be due to the fact that these are cross-sectional studies that don't enable us to fully grasp the totality of the effects smoking has on arterial stiffness. Since smoking is a modifiable lifestyle, follow up studies would be in order to assess if there is actually a causal relation because it may be that those individuals with risk factors or of advanced age have been prescribed to modify their lifestyles and have done so recently, in which case the benefits would not be discernible. It is interesting that the effect of smoking on serum lipid profiles modifying them in a proatherogenic manner (70,71) as well as its involvement in endothelial dysfunction (72,73) have been previously validated repeatedly. It is also plausible that its influence on cardiovascular health doesn't affect stiffness but instead impacts on atheromatous plaques (74) which CAVI cannot detect since it has been observed that stiffness increases or decreases accordingly to the smoking status (75). Regarding the quality of the diet no significant differences were observed between the groups, since the DQI obtained was very similar. The results of the diet quality obtained in our study are similar to those obtained when the sDQS was validated for a general population from Spain indicating that our results are in line with those previously obtained (76). This may be due to the fact that a big part of our sample over 50 years old has abnormal CAVI, CAVI≥9, most likely as a result of aging and not caused by the diet. A cardio-protective amount of physical activity has shown to have a positive effect on cardiovascular health associated with lower central arterial stiffness (77,78). This positive effect is present in our results in men were the OR for cardio-protective PA is 0.66 (95% CI: 0.47-0.92) indicating that being active enough reduces the risk of having abnormal by 31%. In women on the other hand the effect cardio-protective physical activity also had an inverse association with arterial stiffness but in a non-significant manner since the physical activity differences between both groups weren't as important as in men.

When assessing the association between CAVI and carotid atherosclerosis burden significant correlations were found between the studied parameters and CAVI. These results are consistent with those obtained in a previous study (58) and suggest that carotid stiffness and atherosis advance alongside central stiffness.

Several limitations of our study should be considered. As it is a cross-sectional study some associations may have gone undetected and we cannot definitively state that the results obtained are due to a direct causal link with CAVI. Regarding the diet since the results obtained are very similar between groups maybe a broader questionnaire would have been better suited to assess if there were any differences between the groups. The strengths of this study in turn are: its large sample size with many clinical and behavioral factors as independent variables with enough statistical power, all the questionnaires used had been previously validated (45,47), the nurses in charge of collecting the data underwent specific training and the fact that it is the first study assessing the validity of CAVI in an occidental population.

In summary, having healthy lifestyle habits and a good control of the risk factors is associated with a better cardiovascular functionality according to CAVI. These results highlight the importance of the actual strategies to control risk factors as well as the promotion of healthy lifestyles to prevent the onset of cardiovascular events. Therefore, CAVI can be of great usefulness to detect asymptomatic atherosclerosis in routine clinical practice in the future allowing timely treatment improving prevention and reducing the incidence of cardiovascular disease. CAVI would also enable us to identify high-risk individuals that nowadays would be classified as moderate or low risk individuals with the coronary risk functions that use classical risk factors. If this identification can be validated in future studies it would allow for prevention treatment to be dispensed to individuals that otherwise wouldn't receive it.

Conclusions

The results obtained in this study show that CAVI could be a good surrogate marker of arteriosclerosis in a Mediterranean population.

1. There is a high prevalence, 43.32%, of abnormally high arterial stiffness in the Mediterranean population.
2. CAVI has shown to be positively and independently associated with aging, male sex and the risk factors hypertension, diabetes, total cholesterol and waist circumference. In contrast, CAVI was independently but inversely associated with BMI.
3. Cardio-protective physical activity is associated with lower arterial stiffness in men. Diet quality, smoking habits in both sexes and physical activity in women haven't shown an association with arterial stiffness.
4. CAVI has shown to correlate positively and moderately with maximal common carotid intima-media thickness in both sexes. Whereas with the carotid distensibility coefficient CAVI has shown to correlate negatively and moderately.

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