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Visceral adiposity index and 10-year Cardiovascular Disease incidence: the ATTICA Study

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Abstract

Background/Aims: Visceral adiposity index (VAI) has been proposed as a marker of visceral adipose tissue accumulation/dysfunction. Our aim was to evaluate potential associations between the VAI and the 10-year cardiovascular disease (CVD) incidence. **Methods and Results:** During 2001-2002, 3042 Greek adults (1514 men; age: ≥ 18 years) without previous CVD were recruited into the ATTICA study, whilst the 10-year study follow-up was performed in 2011-2012, recording the fatal/non-fatal CVD incidence in 2020 (1010 men) participants. The baseline VAI scores for these participants were calculated based on anthropometric and lipid variables, while VAI tertiles were extracted for further analyses. During the study follow-up a total of 317 CVD events (15.7%) were observed. At baseline, the participants' age and the prevalence of hypertension, diabetes, hypercholesterolemia and metabolic syndrome increased significantly across the VAI tertiles. After adjusting for multiple confounders, VAI exhibited a significantly independent positive association with the 10-year CVD incidence (OR= 1.05, 95%CI: 1.01, 1.10), whereas the association of the body mass index (HR= 1.03, 95%CI: 0.99, 1.08), or the waist circumference (HR= 1.01, 95%CI: 0.99, 1.02) was less prominent. Sex-specific analysis further showed that VAI remained significantly predictive of CVD in men alone (HR= 1.06, 95%CI: 1.00, 1.11) but not in women (HR= 1.06, 95%CI: 0.96, 1.10). **Conclusions:** Our findings show for the first time in a large-sample, long-term, prospective study in Europe that the VAI is independently associated with elevated 10-year CVD risk, particularly in men. This suggests that the VAI may be utilized as an additional indicator of long-term CVD risk for Caucasian/Mediterranean men without previous CVD.

Key words: Cardiovascular Disease; CVD; visceral adiposity; obesity; visceral adiposity index; VAI

Abbreviations

CVD	Cardiovascular disease
VAI	Visceral adiposity index
BMI	Body Mass Index
WC	Waist circumference
MetS	Metabolic Syndrome
CHD	Coronary heart disease
TG	Triglycerides
HDL	High-density lipoprotein
AlkaMeSY	Alkam Metabolic Syndrome Study
WHR	Waist to hip ratio
WHtR	Waist to height ratio
IPAQ	International Physical Activity Questionnaire
CRP	C-reactive protein
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor α
SD	Standard deviation
HR	Hazard Ratio
CI	Confidence Interval
SPSS	Statistical Package for Social Sciences
PCOS	Polycystic ovary syndrome

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, representing 31% of the total global mortality in 2012 [1]. Abdominal (visceral) fat adiposity together with insulin resistance and metabolic syndrome (MetS) components (*e.g.* type 2 diabetes, hyperlipidemia, hypertension) are key risk factors in the development of CVD [2-4]. Notably, the distribution of excess adipose tissue is regarded as a more important CVD risk factor than obesity *per se* [5]. Indeed, although epidemiological data show that there is higher prevalence of cardiometabolic complications associated with increasing body mass index (BMI), there is often remarkable heterogeneity amongst individuals with similar BMI values [6, 7]. As such, data from the INTERHEART study, evaluating the impact of obesity on CVD, have shown that visceral adiposity contributes more than BMI to CVD risk [7]. Moreover, Yusuf *et al.* further specified that the association between visceral obesity and coronary heart disease (CHD) risk was significant in all 52 countries of the INTERHEART study [8].

Recently, the Visceral Adiposity Index (VAI) has been identified as a simple, reliable marker of visceral adiposity dysfunction that reflects cardiometabolic risk [9]. VAI was introduced as a sex-specific index, based on simple anthropometric measures [waist circumference (WC) and BMI] and common lipidemic parameters [triglycerides (TG), and high-density lipoprotein (HDL) cholesterol], expressing visceral fat function. Indeed, in 2010 Amato *et al.* first showed in a population of 1498 Caucasian adults from the retrospective Alkam Metabolic Syndrome (AlkaMeSy) Study that increased VAI was independently associated with cardiovascular (2.5-fold increase) and cerebrovascular events (1.5-fold increase),

whilst this novel index also proved to be a better indicator for incident diabetes than its individual components (*i.e.,* WC, BMI, TG, HDL) [10].

Importantly, VAI has been proposed as a prognostic tool of early cardiometabolic risk even when overt MetS manifestations are absent [11]. However, to date there is a paucity of long-term, prospective studies evaluating the CVD risk predictive power of VAI. Thus, the aim of the present work was to evaluate potential associations between the VAI values at the baseline/entry examination of the ATTICA Study and the documented 10-year CVD incidence in this cohort of Caucasian adults without previous CVD.

2. Materials and Methods

2.1 ATTICA Study Cohort

The ATTICA Study is a prospective, population-based study conducted in the greater metropolitan area of Athens (Greece). The study baseline examination was performed during 2001-2002, as previously described [12]. Briefly, the study was designed to enrol only one participant per household through a random, multistage process based on the age/sex distribution of the Attica region (2001 Census). A total of 3042 Caucasian adults [1514 men (18-87 years); 1528 women (18-89 years)] agreed to participate and were assessed by trained personnel, as per study protocol [12]. Exclusion of CVD at baseline was performed through a detailed clinical evaluation by the study physicians.

2.2 Baseline measurements

The baseline study data included demographic characteristics (age; sex; years of education), personal/family history of hypertension, hypercholesterolemia and diabetes, CVD family history, as well as dietary and other lifestyle habits, such as smoking status (pack years) and physical activity. The dietary evaluation was based on a validated semi-quantitative food-frequency questionnaire, *i.e.* the EPIC-Greek questionnaire [13], which was kindly provided by the Unit of Nutrition of Athens Medical School. The MedDietScore was also applied to assess adherence to the Mediterranean diet (range 0-55; higher score values indicate better adherence) [14]. To assess the physical activity status of study participants the International Physical Activity Questionnaire was used (IPAQ), as an index of weekly energy expenditure, and participants not reporting any physical activities were defined as physically inactive [15]. Moreover, simple anthropometric indices were measured/calculated for all study participants, including BMI (kg/m^2), WC (cm), waist to hip ratio (WHR), and waist to height ratio (WHtR). Resting arterial blood pressure (BP; mean of 3 recordings) was also measured at the end of the baseline physical examination and participants with average BP ≥140/90 mmHg [or on antihypertensive medication(s)] were classified as hypertensive. Finally, blood samples were collected between 8 to 10 a.m., after 12 hours of fasting and alcohol abstinence. Total serum cholesterol, HDL-cholesterol, and TG were measured using a chromatographic enzymatic method in a Technicon automatic analyser RA-1000 (Dade Behring, Marburg, Germany). Hypercholesterolemia was defined as total cholesterol levels >200 mg/dl (or use of lipid lowering agents). Blood glucose levels (mg/dl) were measured with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA, USA) and diabetes mellitus was defined according to the American Diabetes Association criteria (*i.e.*, fasting

blood glucose >125 mg/dl). Furthermore, to assess circulating pro-inflammatory biomarkers, measurements were performed for: C-reactive protein (CRP) by particle-enhanced immunonephelometry (N Latex; Dade-Behring Marburg GmbH, Marburg, Germany); interleukin-6 (IL-6) by a high-sensitivity enzyme-linked immunosorbent assay (ELISA; R&D Systems Europe Ltd, Abingdon, United Kingdom); and tumor necrosis factor-alpha (TNF-α) by ELISA (Quantikine HS/human TNF-α, R&D Systems, Inc. Minneapolis, MN), following the manufacturer's protocols.

For the purposes of this study, according to the criteria for the clinical diagnosis of the MetS proposed in 2009 by the joint statement of the International Diabetes Federation (IDF); National Heart, Lung, and Blood Institute (NHLBI); American Heart Association (AHA); World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity [16], the study participants were also classified according to MetS presence at baseline, based on whether three or more of the following components were present: (i) WC ≥102 cm for males or ≥88 cm for females (WC thresholds for abdominal obesity in the 2009 MetS definition recommended by the European Cardiovascular Societies for European populations [16]); (ii) TG ≥150 mg/dl (or on drug treatment for elevated TG); (iii) HDL-cholesterol <40 mg/dL for males or <50 mg/dL for females (or on drug treatment for reduced HDL-cholesterol); (iv) systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg (or on antihypertensive drug treatment); (v) fasting blood glucose ≥100 mg/dL (or on antidiabetic treatment).

2.3 Visceral adiposity index (VAI) assessment

The VAI was calculated for each study participant at the baseline study

examination, as previously described by Amato and colleagues [9]. Briefly, using BMI (kg/m^2), WC (cm), TG (mmol/l) and HDL (mmol/l), the proposed equations for calculating VAI in males and females are as follows:

$$\text{Males: VAI} = \left(\frac{\text{WC}}{39.68 + (1.88 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{1.03}\right) \times \left(\frac{1.31}{\text{HDL}}\right)$$

$$\text{Females: VAI} = \left(\frac{\text{WC}}{36.58 + (1.89 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL}}\right)$$

For the analyses of this study, VAI tertiles (<2.4; 2.4-4.5; >4.5) were also extracted for the study sample.

2.4 Follow-up ATTICA Study examination (2011-2012)

During 2011-12, the ATTICA Study 10-year follow-up (mean follow-up: 8.41 years) was performed, as previously described [17]. Of the 3042 initially enrolled participants, 2583 were re-evaluated for this follow-up [85% participation rate; mean age at baseline (years±SD): 45±14 and 46±14 years for women and men, respectively, with no difference to the overall study sample]. Detailed evaluation of the medical records of all participants (*n*=2583) for their 10-year CVD status was performed and data (with WHO-ICD coding) were collected for: (a) vital status (death from any cause or due to CVD); (b) development of CVD, including myocardial infarction, angina pectoris, other identified forms of ischemia, heart failure of different types, chronic arrhythmias and stroke [17]. Regarding CVD status at the follow-up, complete and clinically accurate data were obtained from 2020 participants. The scope of the present work focuses on the role of VAI as a predictor of CVD risk, thus herein we present our data in relation to the 10-year incidence of fatal/non-fatal CVD.

2.5 Bioethics

The study was approved by the Institutional Bioethics Committee and was carried out in accordance to the Declaration of Helsinki (1989) of the World Medical Association. Prior to any study procedures, all participants were informed about the study protocol, and provided written signed consent.

2.6 Statistical analysis

Crude, non-fatal and fatal incidence rates of combined CVD (*i.e.*, CHD or stroke) were calculated as the ratio of new cases to the number of participants in the follow-up. Continuous variables are presented as mean values ± standard deviation (SD) and categorical variables as frequencies. Associations between categorical variables were tested using the chi-square test, while between continuous variables using Pearson *r* or Spearman's *rho* coefficients for the normally and skewed variables, respectively. Comparisons of mean values of normally distributed variables between those study participants who developed a CVD event and the rest of the participants were performed using Student's t-test, after controlling for equality of variances using the Levene's test. Comparisons of continuous variables without normal distribution were performed using the non-parametric U-test proposed by Mann and Whitney. Continuous variables were tested for normality through P-P plots. The hazard ratios (HR) and the corresponding 95% confidence intervals (CI) of developing a CVD event during the 10-year follow-up period, according to the participants' baseline characteristics were estimated using Cox proportional hazards models. Moreover, Hosmer-Lemeshow statistic and *-2logLikelihood* were calculated to evaluate model's

goodness-of-fit, and Negelkerke R-square as a proxy of models discriminant power. The time to CVD event was recorded on an annual basis. All reported *p*-values are based on two-sided tests and the corresponding 95% CI. The Statistical Package for Social Sciences (SPSS) version 21 (SPSS Inc, Chicago, IL, U.S.A.) was used for all statistical analyses.

3. Results

3.1 10-year CVD incidence and baseline VAI correlations

The 10-year fatal or non-fatal CVD event rate was 157 cases/1000 participants [*i.e.,* n=317 subjects; men: n=198 (195 cases/1000 participants); women: n=119 (118 cases/1000 participants); p for gender difference <0.001]. VAI scores at baseline were positively correlated with BMI, WC, WHR, WHtR, CRP, IL-6, TNF-α, TG, total cholesterol, and glucose levels (rho= 0.262, 0.331, 0.280, 0.329, 0.147, 0.168, 0.136, 0.915, 0.309, 0.227, respectively; all p-values <0.001), and inversely associated with HDL-cholesterol (rho= -0.395; p <0.001). Even when the analysis was stratified by gender these correlations remained significant for both men and women *(data not shown).*

3.2 Baseline characteristics of study participants by VAI tertiles

Baseline characteristics of the study sample, according to the 10-year CVD status, are presented in ***Table 1***. Compared to CVD-free participants, the group of participants who developed CVD during the 10-year follow-up consisted mainly of older men, heavier smokers, and had higher BMI, WC, WHR, WHtR and TG/HDL ratio values. Furthermore, this group also exhibited higher fasting lipid and glucose levels, higher BP, as well as worse Mediterranean Diet adherence (*i.e.*, lower

MedDietScore) (all p-values <0.001). Participants with a CVD event during the 10-year study follow-up had 29% higher VAI baseline values compared to those without (p <0.001).

For the purposes of this study, the 2020 study participants with complete 10-year CVD incidence data were further grouped in VAI tertiles (<2.4; 2.4-4.5; >4.5) based on their baseline VAI scores as presented in ***Table 2***. Overall, the age of participants and the baseline prevalence of hypertension, diabetes, hypercholesterolemia and MetS increased significantly across VAI tertiles. Of note, study participants in the 1st VAI tertile (reflecting a less dysfunctional visceral adipose tissue profile) were younger, mostly women, lighter smokers, more physically active, and with lower total and central obesity, and higher Mediterranean diet adherence (*i.e.,* higher mean MedDietScore: 28 out of 55) compared to those in the 3rd VAI tertile (highest VAI values). Furthermore, the participants in the lower VAI tertile also exhibited decreased baseline prevalence of various established cardiometabolic risk factors (*i.e.*, hypertension, diabetes, hypercholesterolemia and MetS; all p-values <0.001). As expected, these participants in the 1st VAI tertile exhibited the lowest 10-year CVD incidence.

3.3 Ten-year CVD incidence and VAI

The aforementioned comparisons may be prone to residual confounding due to various potential factors; therefore, a multi-adjusted analysis was also performed, controlling for multiple CVD covariates. Importantly, variables being part of the VAI formulas (*i.e.*, BMI, WC, TG and HDL) were not included in the models with VAI due to multicollinearity. ***Table 3*** presents the results from the multi-adjusted analyses

in the context of this study, evaluating VAI and the risk of having a CVD event (fatal or non-fatal) within the 10-year follow-up period. Our study *Full Model 1* included common demographic factors (age and male sex), clinical characteristics (*i.e.*, hypertension, diabetes, hypercholesterolemia) and certain factors (*i.e.*, smoking, physical activity, educational level, and Mediterranean diet adherence) that generally predict the 10-year CVD incidence. When VAI was included in the analyses, binary logistic regression models showed that it was independently associated with the 10-year CVD risk. Indeed, VAI was associated with higher 10-year CVD incidence in the age-sex adjusted analysis (*Model 2*, ***Table 3***). This association remained even after adjusting for smoking, physical activity educational level and the MedDietScore, as well as for other established CVD risk factors (*i.e.*, hypertension, diabetes, and hypercholesterolemia) (*Model 3*, ***Table 3***). *Model 3* was further adjusted for certain circulating inflammatory biomarkers, *i.e.* CRP, IL-6 and TNF-α, to explore the potential mediating role of pro-inflammatory processes in the association between adiposity and CVD. Thus, CRP, IL-6 and TNF-α, were entered consecutively and separately in *Model 3,* as presented in *Model 4, 5,* and *6*, respectively. According to these latter models, CRP, IL-6 and TNF-α appeared to similarly impact on VAI when entered in *Model 3*, since the VAI-CVD association shifted borderline towards significance, suggesting possible mediating effects. Overall, the presented binary logistic regression models showed that VAI is an index/indicator that independently predicts 10-year CVD risk within our study cohort. Notably, as seen in *Model 3*, a 2.5-unit increase of the VAI score, which is approximately the difference in the means between the CVD event-free study group and the CVD group, was associated with 13% increase of the 10-year CVD event risk.

Furthermore, to compare the predictive value of VAI on the 10-year CVD risk against more simple anthropometric and lipidemic variables/ratios (some of which are included in the VAI formulas), additional analyses were applied including models which instead of the VAI included BMI, WC, WHR, WHtR and TG/HDL-cholesterol, respectively. ***Table 4*** presents the HR and 95% CI of these models, whilst the Hosmer-Lemeshow test was used to compare head-to-head the CVD predictive value of VAI against the rest of these variables. As presented in ***Table 4***, VAI had a better CVD prognostic value (HR= 1.05, 95%CI: 1.01, 1.10, -2Loglikelihood= 517) than BMI (HR= 1.03, 95%CI: 0.99-1.08, -2Loglikelihood= 701). In addition, VAI proved to be superior than all the other anthropometric indices, including WC (HR= 1.01, 95%CI: 0.99, 1.02, -2Loglikelihood= 692), WHR (HR= 1.33, 95%CI: 0.18, 9.98, -2Loglikelihood= 687), and WHtR (HR= 2.71, 95%CI: 0.21, 5.45, -2Loglikelihood= 690) and the TG/HDL ratio (HR= 1.07, 95%CI: 1.01, 1.14, -2Loglikelihood= 523).

To test how much the VAI adds to the predictive and discriminant power of the HellenicSCORE [18] (a tool that evaluates 10-year risk of fatal CVD events using age, sex, smoking, total cholesterol and blood pressure levels, according to the ESC SCORE project), an additive logistic model was developed. It was observed that inclusion of VAI on the model contained HellenicSCORE improved models' predictive ability by 2% (-2log Likelihood of the crude model 1106 vs. the model that included VAI, 1084) and the "discriminant power" from 18.5% to 20.7% (Nagelkerke R-square).

Finally, taking into account that VAI is a sex-specific index, the analysis described in Model 3 (**Table 3**) was repeated separately in men and women. These results showed that VAI remained significantly predictive of CVD in men alone (HR=

1.06, 95%CI: 1.00, 1.11,) but not in women (HR= 1.06, 95%CI: 0.96, 1.10). Furthermore, the VAI had better predictive ability against 10-year CVD incidence when examined in men alone (HR= 1.06, 95%CI: 1.00, 1.11,-2Loglikelihood= 128) compared to the whole study sample (HR= 1.05, 95%CI: 1.01, 1.10, -2Loglikelihood= 517).

4. Discussion

Our study presents novel data from a large cohort of prospectively followed Caucasian (Mediterranean/Greek) adults without previous CVD, showing that higher VAI scores (indicating accumulated dysfunctional visceral adipose tissue [9]) exhibit a significant and independent association with 10-year CVD incidence, particularly in men. Interestingly, this positive association remained even after taking into account a wide range of potential confounders, including lifestyle factors and clinical/biochemical variables. Indeed, in our fully adjusted model only VAI was a significant indicator of the 10-year CVD risk, independently of other established CVD factors. Thus, it could be hypothesized that because VAI takes into account both anthropometric (BMI and WC) and metabolic/lipidemic (TG and HDL) variables of obesity (particularly central obesity), it may reflect more directly the array of potential pro-atherogenic processes that in the long-term result in higher CVD risk (*e.g.* visceral and ectopic fat deposition, dysregulated adipokine production, and increased adipose tissue lipolysis and free fatty acid efflux) [19-21].

The mechanisms by which VAI may be associated with CVD risk are not fully understood. Of note, when inflammation (as expressed by circulating CRP, IL-6 and TNF-a) was tested in this study in a mediation analysis, as a possible explanatory

factor of the association between VAI and 10-year CVD risk, the effect of VAI baseline values on 10-year CVD incidence remained significant. This could be potentially attributed to a positive association of VAI with higher long-term CVD risk through early pro-inflammatory mechanisms related to dysfunctional visceral adiposity (*e.g.* altered adipokine profile, including amplified secretion of pro-inflammatory adipokines and cytokines, such as IL-6 and TNF-a), which are not always directly reflected in the clinical evaluation of CVD risk [10].

Additionally, sex-specific analysis in our study revealed a positive association of VAI on CVD risk in men, but not in women. This could be attributed to the fact that men - in general - as well as in our study, had greater BMI, higher WC and lower HDL levels. Considering that men are more susceptible to visceral adiposity, this could also suggest greater visceral adipose tissue dysfunction (*e.g.* greater changes in expression/secretion of multiple adipokines such as visfatin, resistin, leptin, omentin, and adiponectin). Thus, VAI, as an empirical mathematical model/index of adipose distribution and function that indirectly expresses cardio-metabolic risk [9, 10], appears to have higher accuracy in linking visceral obesity dysfunction and CVD risk in men compared to women. Nevertheless, VAI importance in predicting future CVD candidates should be considered irrespective of individuals' gender, since visceral adiposity has shown its crucial role in the development and progression of atherosclerotic disease in both men and women.

Importantly, our findings indicate that the predictive effect of VAI for the 10-year CVD incidence in this study cohort was better when compared to typically used anthropometric and lipidemic variables, *i.e.* BMI, WC, WHR, WHtR and TG/HDL ratio. This is significant also for the clinical practice, since it suggests that the

calculation of VAI based on the simple measurements of BMI, WC, TG and HDL-cholesterol offers a stronger CVD indicator compared to its individual components and to other simpler ratios (*e.g.* WHR, WHtR, TG/HDL ratio).

Over the last few decades there has been compelling evidence showing that adipose tissue acts as an endocrine organ with pleiotropic effects, due to the dynamic secretory function of adipocytes and resident macrophages [22]. These effects may lead to a chronic, low-grade pro-inflammatory state in obesity (particularly in visceral/central/android obesity) that is further associated with insulin resistance and CVD [22]. Indeed, sustained weight increase and adipose tissue accumulation induces significant changes inside the various fat depots, resulting in dysregulated function with increased lipolysis, insulin resistance and altered adipokine production (*e.g.*, increased production of pro-inflammatory adipokines, such as leptin, and cytokines such as TNF-α and IL-6; and decreased production of anti-inflammatory adipokines, such as adiponectin and omentin) [22]. These changes are considered responsible for the overall adipose tissue dysfunction, playing a vital role in the obesity-related cardiometabolic sequelae [22-24]. Visceral adiposity appears to be a critical factor in the underlying pathophysiological mechanisms [6], whilst it is also hypothesized that subcutaneous fat has a limited capacity to safely increase its mass [25]. As such, emphasis has been placed in identifying simple indices which can be reliably applied in everyday clinical practice as surrogate markers of visceral adiposity and as indicators of increased cardiometabolic risk. In 2010 Amato *et al.* proposed VAI as a novel index with such attributes in the context of the retrospective AlkaMeSy study [9]. In the 1498 Caucasian adults of this study, VAI was shown to correlate with visceral adipose tissue, whilst exhibited a strong positive association with

cardiovascular events (OR: 2.45, 95% CI: 1.52-3.95) and an inverse association with insulin sensitivity [9]. Moreover, ROC analysis in this study proved VAI to have greater sensitivity and specificity than its individual components regarding cardiovascular events, although the cross-sectional nature of the study did not allow exploring causal inferences between VAI and CVD [9]. Since 2010, the VAI has been studied in various cohorts from general/healthy populations and in specific patient groups at increased cardiometabolic risk (*e.g.*, in obese patients or in women with polycystic ovary syndrome, PCOS), with particular focus on the prediction of type 2 diabetes [26, 27]. However, so far there are limited data from prospective studies exploring VAI as an indicator of long-term CVD risk. It should be also highlighted that, the VAI was modeled and validated on a Caucasian (Italian/Mediterranean) population of adults from the city of Alcamo in Western Sicily, whereas currently existing data on the long-term association between VAI and CVD are mostly from population-based studies conducted in Asia and South America [28, 29]. Indeed, a 9-year follow-up in the context of the Tehran lipid and glucose study demonstrated that the VAI was associated with multivariate-adjusted, increased risk of incident CVD among women [28]. However, the magnitude of risk conferred by VAI in this study was not significantly higher than those conferred by BMI, WHR or WHtR [28]. Additionally, data from a long-term study in Buenos Aires (Argentina) showed that VAI was not able to recognize subjects at high CVD risk compared to the TG/HDL ratio [29]. These results are not in accord with the present findings of the ATTICA study, potentially due to significant differences in the ethnicity and in the age range of the studied cohorts [28, 29], especially since as aforementioned VAI is modeled based on a cohort of Caucasian adults [Caucasian (Italian/Mediterranean) men and women

aged between 19 and 83 years) [9, 30, 31]. Hence, the application of VAI has not been validated in non-Caucasian populations (*e.g.* the study in Tehran studied a population of Persian ancestry) [28], and in adolescents aged <16 years (*e.g.* the study in Buenos Aires studied a population aged ≥15 years) [29].

Compared to available data, the significance of our findings is strengthened by the characteristics of our study design and population, *i.e.,* prospective, long-term design, large sample size, Caucasian/Mediterranean population, and adjustment for multiple anthropometric and clinical parameters. In addition, our data originate from a reliable, prospective follow-up process in a well-characterized population-based sample in which CVD and its risk factors have been assessed with standardized measures/procedures both at baseline and during the follow-up. As such, the ATTICA study extends previous research on the application of VAI, indicating that this relatively simple index may represent a useful predictive marker for men from the general population in whom CVD risk classification based on simple/single variables, such as BMI, is particularly sub-optimal. Indeed, in the fully adjusted analyses, accounting for age, gender, clinical, behavioral, and pro-inflammatory variables, the HR (95% CIs) for 10-year CVD incidence was 1.05 (1.01, 1.10) per 1 unit increase in the baseline VAI score, while including separately the rest of the anthropometric indices (BMI, WC, WHR, WHtR) had no better predictive effect. This is also significant for epidemiological research studies, since additional reliable indices associated to visceral adiposity (android/central obesity) are still required in the effort to predict more accurately the obesity-related long-term cardiometabolic risk without the need for visceral fat assessments by imaging techniques (*i.e.*, CT and MRI).

4.1 Limitations

Despite the aforementioned strengths, certain limitations of the present study should be also acknowledged. Thus, it must be noted that the baseline study examination was performed once and may be susceptible to measurement error. However, the applied methodology within our study protocol was similar to those of other prospective epidemiological studies in Europe and the US, and therefore our results can be considered reliable and comparable. Moreover, taking into consideration that the number of fatal events was too small [17], we decided to present the analyses on VAI and 10-year CVD fatal and not-fatal incidence combined.

4.2 Conclusion

In conclusion, this study offers novel evidence revealing an independent positive association between the VAI and 10-year CVD incidence in men from a cohort of Caucasian adults without previous CVD. Thus, it may be suggested that this index, which reflects visceral adipose tissue accumulation/dysfunction, could be also applied as a relatively simple tool for long-term CVD risk assessment, at least in healthy or apparently healthy men of Caucasian/Mediterranean origin. Additional research is clearly required to further explore these findings in even larger cohorts, in specific patient populations (*e.g.* in non-alcoholic liver disease or PCOS) and in other ethnic groups.

Conflict of interest

The authors declare that they have no conflict of interest.

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Contributors

Georgia-Maria Kouli performed data analyses, interpreted the results and approved the final manuscript version. Demosthenes Panagiotakos and Christos Pitsavos designed the ATTICA study, critically reviewed the manuscript and approved the final manuscript version. Ioannis Kyrou developed the concept of the paper and drafted the manuscript. Ekavi Georgousopoulou, Christina Chrysohoou, Ioannis Kyrou, Constantine Tsigos, and Dimitrios Tousoulis, critically reviewed the manuscript and approved the final manuscript version.

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Table 1. Demographic, behavioral, lifestyle and clinical characteristics of the ATTICA Study participants at baseline and at the 10-year follow-up by cardiovascular disease (CVD) status *(n=2020)*.

		CVD status at 10-year follow-up		*P*
	Baseline	CVD event free	CVD events	
	n = 2020	*n=1703*	*n= 317*	
Age (years, range 18-89)	45 ± 14	43 ± 13	58 ± 13	<0.001
Gender, %male	50.24	47.97	62.46	<0.001
Smoking (pack years)	496 ± 501	441 ± 425	767 ± 705	<0.001
Smoking at baseline or before, %	43	55	57	0.46
Physical Activity, % physically active	41	40	41	0.95
Body Mass Index (kg/m^2)	26 ± 5	26 ± 5	28 ± 5	<0.001
Waist circumference (cm)	90 ± 15	89 ± 15	97 ± 14	<0.001
Waist to hip ratio	0.86 ± 0.1	0.85 ± 0.11	0.92 ± 0.11	<0.001
Waist to height ratio	0.53 ± 0.08	0.52 ± 0.08	0.57 ± 0.07	<0.001
Triglycerides to HDL-cholesterol ratio	2.79 ± 2.89	2.62 ± 2.33	4.32 ± 6.02	<0.001
Obesity, %yes	18	20	30	<0.001
Hypertension, %yes	30	28	51	<0.001
Diabetes Mellitus, %yes	7	5	22	<0.001
Hypercholesterolemia, %yes	39	40	57	<0.001
MedDietScore (range 0-55)	26 ± 7	26 ± 6	23 ± 7	<0.001
Metabolic syndrome, %yes	20	18	41	<0.001
C-reactive protein (mg/L)	1.93 ± 2.4	0.99±0.9	1.42 ±1.15	<0.001
Interleukin-6 (ng/mL)	1.46 ± 0.55	1.43± 0.55	1.65 ± 0.51	<0.001
Tumor necrosis factor-α (pg/mL)	6.21 ± 4.90	6.00 ± 4.70	8.33 ± 5.47	<0.001
VAI	4.4 ± 5	4.1 ± 3	6.6 ± 9	<0.001

Continuous variables are presented as mean values ± standard deviation and categorical variables as frequencies. *P* values for the comparisons between CVD event and event-free group derived using the t-test, while for the comparisons of categorical variables using the chi-square test. VAI: Visceral adiposity index; MedDietScore: score evaluating the adherence to the Mediterranean diet (higher score values indicate better adherence)

Table 2. Cardiovascular disease (CVD) events during the 10-year ATTICA Study follow-up, as well as participants' baseline characteristics (*n=2020*) grouped by tertiles of the Visceral Adipose Index (VAI tertiles: <2.4; 2.4-4.5; >4.5).

	VAI tertiles at baseline			
	1st tertile	**2nd tertile**	**3rd tertile**	***P***
CVD event during 10-yr follow-up, %yes	8	12	24	<0.001
Baseline characteristics				
Age (years)	19 ± 13	45 ± 14	50 ± 13	<0.001
Gender, %male	42	45	58	<0.001
Smoking (pack years)	362 ± 385	422 ± 420	617 ± 534	<0.001
Physical Activity, % physically active	48	43	33	<0.001
Body Mass Index (kg/m^2)	24 ± 3.6	26 ± 4.5	28 ± 4.6	<0.001
Waist circumference (cm)	82 ± 13	90 ± 14	98 ± 14	<0.001
Waist to hip ratio	0.81 ± 0.10	0.86 ± 0.11	0.91 ± 0.12	<0.001
Waist to height ratio	0.48 ± 0.07	0.53 ± 0.08	0.58 ± 0.08	<0.001
Triglycerides to HDL-cholesterol ratio	1.12 ± 0.34	2.12 ± 5.06	5.06 ± 4.05	<0.001
Education (years of school)	13 ± 3.4	12 ± 3.8	11 ± 3.9	<0.001
Hypertension, %yes	21	32	43	<0.001
Diabetes mellitus, %yes	2	4	13	<0.001
Hypercholesterolemia, %yes	22	45	64	<0.001
MedDietScore (range 0-55)	28 ± 7	26 ± 7	24 ± 6	<0.001
Metabolic Syndrome, %yes	3	14	55	<0.001
C-reactive protein (mg/L)	1.36 ± 2.05	2.03 ± 2.53	2.65 ± 2.6	<0.001
Interleukin-6 (ng/mL)	1.35 ± 0.39	1.47 ± 0.40	1.6 ± 0.43	<0.001
Tumor necrosis factor-α (pg/mL)	5.44 ± 3.81	6.41± 4.75	7.22 ± 3.63	<0.001

Continuous variables are presented as mean values ± standard deviation and categorical variables as frequencies. *P* values for the comparisons between the 1st and the 3rd VAI tertile groups derived using the t-test, while for comparisons of categorical variables using the chi-square test. MedDietScore: score evaluating the adherence to the Mediterranean diet (higher score values indicate better adherence).

Table 3. Results from the Cox proportional hazard models applied to evaluate the 10-year risk of having a cardiovascular event (outcome) according to the baseline Visceral Adipose Index (VAI) values.

All participants	Hazard Ratios (HR)*, 95% Confidence Intervals					
	Full Model 1	*Model 2*	*Model 3*	*Model 4*	*Model 5*	*Model 6*
Age (per 1 year)	1.06 (1.04-1.08)	1.08 (1.07-1.10)	1.06 (1.04-1.09)	1.06 (1.04-1.09)	1.05 (1.03-1.08)	1.06 (1.03-1.09)
Men vs. Women	1.66 (1.06-2.61)	2.03 (1.47-2.81)	2.29 (1.32-3.98)	2.07 (1.17-3.67)	1.99 (1.14-3.50)	1.94 (1.01-3.76)
VAI (per 1 unit)	-	1.06 (1.02-1.09)‡	1.05 (1.01-1.10)‡	1.04 (1.00-1.09)‡	1.04 (1.00-1.09)‡	1.05 (1.00-1.11)‡
Smokers vs. non smokers	1.00 (1.00-1.01)	-	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (1.00-1.05)	1.00 (1.00-1.01)
Physically activity vs. inactive	0.74 (0.49-1.11)	-	0.82 (0.51-1.32)	0.87 (0.52-1.43)	0.87 (0.53- 1.43)	0.63 (0.35-1.13)
Education (years of school)	0.98 (0.93-1.04)	-	0.98 (0.93-1.05)	0.97 (0.91-1.03)	0.97 (0.91-1.04)	0.95 (0.88-1.02)
MedDietScore (range 0-55)	0.95 (0.92-0.99)	-	0.96 (0.92-1.01)	0.95 (0.91-1.00)	0.95 (0.91-0.99)	0.95 (0.91-1.00)
Hypertension (y/n)	1.06 (0.71-1.60)	-	1.22 (0.77-1.94)	1.44 (0.89-2.32)	1.43 (0.88-2.31)	1.23 (0.71-2.14)
Diabetes Mellitus (y/n)	2.39 (1.33-4.29)	-	2.35 (1.18-4.70)	2.13 (1.02-4.43)	2.23 (1.08-4.62)	2.21 (0.91-5.39)
Hypercholesterolemia (y/n)	1.33 (0.90-1.97)	-	1.43 (0.90-2.28)	1.32 (0.82-2.14)	1.32 (0.82-2.12)	1.67 (0.97-2.88)
C-reactive protein (mg/L)	-	-	-	1.12 (1.03-1.21)	-	-
Interleukin-6 (ng/mL)	-	-	-	-	1.62 (0.92-2.86)	-
Tumor necrosis factor-α (pg/mL)	-	-	-	-	-	1.10 (1.01-1.20)

* Hazard Ratios derived from semi-parametric Cox proportional hazards models. ‡ $p < 0.05$.

MedDietScore: score evaluating the adherence to the Mediterranean diet (higher score values indicate better adherence). Smoking was analyzed as a continuous variable through pack years.

Table 4. Results from the additive Cox proportional hazards models developed to evaluate the predictive role of VAI on the risk of 10-year cardiovascular disease events (outcome) compared to other anthropometric indices such as the body mass index (BMI), waist circumference, waist to hip ratio, waist to height ratio and triglycerides to HDL-cholesterol ratio.

	Hazard Ratios (HR)[2]	95% Confidence Intervals	Hosmer and Lemeshow Goodness of fit test[3]
Full Model[1]	-	-	704.29
Full Model[1]+ *VAI*	1.05	(1.01-1.10)	516.72
Full Model[1] + *BMI*	1.03	(0.99-1.08)	700.79
Full Model[1]+ *waist circumference*	1.01	(0.99-1.02)	691.80
Full Model[1] + *waist to hip ratio*	1.33	(0.18-9.98)	687.37
Full Model[1] + *waist to height ratio*	2.71	(0.21-5.45)	690.42
Full Model[1] + *triglycerides to HDL-cholesterol ratio*	1.07	(1.01-1.14)	526.94

[1]adjusted for age, sex, smoking (pack years), physical activity (active/inactive), education (years of school), MedDietScore, history of hypertension, diabetes mellitus, hypercholesterolemia

[2]Hazard Ratios derived from semiparametric Cox proportional hazards models.

[3]The Hosmer–Lemeshow statistical test was used for goodness of fit for the logistic regression models.