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Visceral Adiposity Index (VAI) outperforms common anthropometric indices in predicting 10-year diabetes risk: results from the ATTICA study.

Visceral adiposity index and diabetes

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/dmrr.3161

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Abstract

Aims: Visceral adiposity index (VAI) is a novel marker of visceral adipose tissue accumulation and dysfunction. The study aim was to explore the association of VAI with the 10-year type 2 diabetes mellitus (T2DM) incidence in apparently healthy individuals, and compare its T2DM predictive ability against common anthropometric indices.

Materials and Methods: in 2001-02, the ATTICA study (Greece) recruited a random sample of 1514 and 1528 CVD-free men (18-87 years old) and women (18-89 years old), respectively. Socio-demographic, lifestyle, clinical, and biochemical characteristics of participants were measured at baseline, and the 10-year follow-up was performed during 2011-2012. After excluding participants with diabetes at baseline and participants without complete follow-up information regarding diabetes status and/or baseline VAI values, the working sample consisted of 1049 participants. In this sample, the predictive value of baseline VAI value was studied in relation to 10-year diabetes incidence.

Results: 133 incident cases of diabetes were documented (10-year incidence: 12.7%). In the fully adjusted model, VAI significantly increased diabetes risk by 22% (OR per 1-unit increase

=1.22; 95%CI: 1.09, 1.37). Markers of oxidative stress and inflammation were found to, at least partly, mediate this relationship. Also, a moderating effect of menstruation status was revealed among women. VAI showed the highest predictive ability and contributed the most, along with waist-to-height ratio, to the correct classification of participants who developed diabetes.

Conclusions: the present findings suggest that VAI may be a useful index for predicting long-term diabetes development, and may exhibit better predictive ability to commonly used anthropometric indices.

Keywords: Visceral Adiposity Index; VAI; type 2 diabetes; anthropometric indices; prognostic markers

INTRODUCTION

The Visceral Adiposity Index (VAI) is a sex-specific surrogate marker of visceral adiposity accumulation and dysfunction, which is calculated from both common anthropometric [body mass index (BMI) and waist circumference (WC)] and lipidemic [triglycerides (TG) and high-density lipoprotein (HDL) cholesterol] parameters, and is independently associated with cardiometabolic risk [1]. In 2010 Amato *et al.* first introduced this novel index showing that it exhibited a strong and independent correlation with insulin resistance and the incidence of cardiovascular disease (CVD) in Caucasian adults of the Alkam Metabolic Syndrome Study (AlkaMeSy retrospective study in the city of Alcamo, Western Sicily, Italy) [1]. Similarly, in relation to diabetes development, there is a positive association between VAI and the risk of type 2 diabetes mellitus (T2DM) [2-9], in line with the evidence that links visceral fat accumulation to the development of insulin resistance and dysglycemia[10-13]. Of note, prospective data by Zhang *et al.* have shown that, among Chinese adults, men and women in the highest VAI tertile had a 2.8- and 3.5-fold higher 4-year T2DM risk, respectively,

compared to those in the lowest VAI tertile [5]. Another prospective study by Wang *et al.* has also shown that VAI could independently predict T2DM in the studied Chinese population, with increasing VAI values being associated to higher 15-year T2DM risk [6]. Moreover, VAI has been proven superior for diagnosing metabolic syndrome compared to many anthropometric indices in Chinese adults [14], although according to a study by Wander et al. VAI may not be the best estimator of visceral adipose tissue area, compared to existing adiposity surrogates [15]. However, it must be noted that, although VAI has been developed and validated based on data from Caucasian (Italian) adults [1], the emerging evidence on the association between VAI and T2DM incidence comes mostly from studies in Asia (*e.g.*, cohorts from China and Iran) [5-9], whereas there is a paucity of such prospective studies in Caucasian populations.

Furthermore, it should be also highlighted that, depending on the studied cohort, the existing evidence is conflicting on whether VAI constitutes a better T2DM prognostic indicator compared to its components (*e.g.*, BMI and WC) and other more easily calculated anthropometric indices, such as the waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR). Indeed, Amato *et al.* have initially shown that VAI is a better indicator for incident diabetes compared to its individual components [1], which can also outperform the T2DM predictive value of BMI and WC in women with polycystic ovary syndrome (PCOS) [3]. Moreover, Chen *et al.* have shown that VAI is a better surrogate marker than BMI, WHtR, and WC for identifying T2DM risk in Chinese adults [7]. However, other studies have shown that the T2DM predictive value of VAI is not higher than that of the most common anthropometric indices (*i.e.*, BMI, WC, WHR and WHtR) [5,6,8,9,16]

Accordingly, the aim of the present work was to perform secondary analyses in the context of the prospective ATTICA study in order to evaluate the association of VAI with the 10-year T2DM incidence among non-diabetic, CVD-free, Caucasian adults from the general

population, and also compare its T2DM predictive value against that of other common anthropometric indices.

MATERIALS AND METHODS

ATTICA study cohort

The ATTICA study is a large-scale, population-based, prospective survey which was initiated in 2001-2002 in the metropolitan area of Athens (Attica, Greece), as previously described [17]. In brief, the ATTICA study was designed to enroll one CVD-free adult per household using a multistage and random process according to the local age/gender distribution (2001 National Census). In total, 3042 eligible adults (men/women: 1514/1528; age: 46±13 and 45±13 years, respectively) consented to participate in this study. The ATTICA study was approved by the ethics committee of the First Cardiology Department of the University of Athens and was conducted according to the guidelines of the Declaration of Helsinki. Informed consent was obtained from each participant prior to any study procedures.

Baseline assessments (2001-2002)

At the baseline ATTICA study examination, standardized questionnaires and calibrated devices were utilized by trained personnel (*i.e.*, cardiologists, general practitioners, nurses and dietitians) to collect all the information/data required by the study protocol [17]. Socio-demographic data (*e.g.*, age, sex, education status) and medical history details were obtained from each participant (*e.g.*, smoking habits, used medications, self-reported menstruation status and family medical history). In the context of this study, smokers were defined as those participants who smoked at least one cigarette per day or had quitted smoking within the previous year, while the rest were defined as non-smokers. Furthermore, the International Physical Activity Questionnaire (IPAQ; a validated index of weekly energy expenditure) was used to evaluate the level of physical activity of each participant (participants not reporting any

physical activity were classified as physically inactive) [18]. The evaluation of the dietary habits was based on a validated semi-quantitative food-frequency questionnaire, namely the EPIC-Greek questionnaire, which was kindly provided by the Unit of Nutrition of Athens Medical School [19]. Mediterranean diet adherence was also evaluated using the validated *MedDietScore* (score range: 0–55; higher score values indicate greater adherence) [20].

Body weight (in kilograms; kg), height (in meters; m), waist (in centimeter; cm) and hip (in cm) circumference were measured using standardized procedures, and commonly used anthropometric indices, *i.e.*, BMI (kg/m^2), WHR and WHtR, were accordingly calculated. Resting arterial blood pressure (BP) was also measured (right arm; mean of three recordings) at the end of the physical examination with the participant in a sitting position for at least 30 min. Based on these BP measurements, hypertension was defined as average BP ≥140/90 mmHg (or use of antihypertensive medication).

Biochemical measurements were performed in the same laboratory which followed the criteria of the World Health Organization (WHO) Lipid Reference Laboratories. All study blood samples were collected from the antecubital vein at 8-10 a.m. in a sitting position after 12 hours of fasting and alcohol abstinence. Serum total cholesterol, HDL cholesterol, and TG concentrations were measured using a chromatographic enzymatic method in a Technicon automatic analyzer RA-1000 (Dade Behring, Marburg, Germany). Hypercholesterolemia was defined as total cholesterol serum concentrations >200 mg/dL or use of lipid-lowering agents. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [21]. Serum insulin concentrations were assayed by radioimmunoassay (RIA100, Pharmacia Co., Erlangen, Germany). Serum glucose concentrations were measured with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA, USA) and diabetes diagnosis was based on the American Diabetes Association criteria (*i.e.,* fasting plasma glucose ≥126 mg/dL or use of antidiabetic medication). Insulin resistance was assessed by calculating the Homeostatic Model

Assessment for Insulin Resistance (HOMA-IR) based on the following formula: HOMA-IR= fasting glucose (mmol/l) x fasting insulin (μU/mL) /22.5, as previously described [22]. Selected circulating pro-inflammatory markers were also measured, including high-sensitivity C-reactive protein (CRP) and serum amyloid-A (SAA) by particle-enhanced immunonephelometry (N Latex; Dade Behring Marburg GmbH, Marburg, Germany); interleukin-6 (IL-6) by a high-sensitivity enzyme-linked immunoassay (ELISA; R&D Systems Europe Ltd, Abingdon, United Kingdom); and tumor necrosis factor-α (TNF-α) by ELISA (Quantikine HS/human TNF-α, R&D Systems, Inc. Minneapolis, MN). Homocysteine was measured using a pulsar fluorescence method (Axsym Abbott Inc., Inning, TX), and fibrinogen by BNII Dade Behring automatic nephelometry. Finally, serum total antioxidant capacity (TAC) was measured with a colorimetric test (ImAnOx; Immunodiagnostik AG, Bensheim, Germany) and plasma oxidized LDL-cholesterol by ELISA (Mercodia AB, Uppsala, Sweden).

Visceral adiposity index (VAI) assessment

For each participant at the baseline study examination, the VAI was also calculated based on the following sex-specific formulas described by Amato *et al.* [1]:

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

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

Both TG and HDL serum concentrations are expressed in mmol/l, whilst VAI=1 is assumed in healthy non-obese adults with normal adipose distribution and normal HDL and TG levels [1].

For the aims of the present study, VAI tertiles (*i.e.*, <1.02, 1.03 to 1.87, >1.88) at baseline were also calculated for the study sample.

10-year follow up assessment (2011-2012) and study sample size

The 10-year ATTICA study follow-up was performed during 2011-2012, capturing the data of 2583 enrolled participants (85% participation rate). From these participants, after excluding participants with diabetes at baseline (n=210) and also participants without complete/confirmed follow-up information regarding their 10-year diabetes status and/or baseline VAI values, a working sample of 1049 subjects was extracted for the objectives and analyses of the present study (***Table 1***). This study sample size was adequate to achieve 95% statistical power to evaluate a relative risk (RR) of 1.20, between the null and alternative two-sided hypotheses, when the exposure variable was increased by 1-unit of VAI and with a significance level (alpha) of 0.05. In the context of the ATTICA study protocol, at the 10-year follow-up assessment there were no precise data captured on the exact onset of T2DM for each study participant, but only the presence or absence of a confirmed T2DM diagnosis based on the American Diabetes Association criteria during the 10-year study follow-up. The complete details of the 10-year ATTICA study follow-up have been presented elsewhere [23].

Statistical analysis

Incidence of T2DM was calculated as the ratio of new cases to the total number of participants in the 10-year follow-up (*n*=1049). Descriptive statistics were utilized to compare the baseline characteristics of the study participants across the baseline VAI tertiles. Analysis of variance (ANOVA) was performed to compare the mean values of normally distributed variables by VAI tertile. Post-hoc analyses using the Bonferroni rule were performed to account for the inflation of the probability of type-I error. For non-normally distributed variables, the Kruskal-Wallis test was applied, and next the Mann-Whitney test was performed between every two groups to detect significant mean differences. Continuous variables were tested for normality with the Kolmogorov-Smirnov test. The relative risk of developing diabetes during the 10-year study follow-up period according to the baseline characteristics of participants was estimated through the odds ratios (OR) and the corresponding 95% confidence intervals (CI), as derived

from logistic regression models. This type of analysis was preferred, since no detailed data were captured on the exact onset of T2DM for each study participant, but only the presence or absence of a confirmed T2DM diagnosis during the 10-year study follow-up. However, as previously demonstrated, the estimation of the OR approximated the relative risk given an infrequent disease occurrence [24]. Known confounders were also included in the tested models after testing for collinearity. Interactions with VAI were checked in all steps, and when significant sub-group analyses were performed. The predictive ability of VAI versus other common anthropometric/metabolic indices/ratios was ranked, calculating the -2log-likelihood of each model (lower values indicating better predictive ability). In addition, correct classification rates (before and after the inclusion of the aforementioned indices) were calculated and used to evaluate models' goodness-of-fit. To determine the appropriate cut-off point for VAI, the score with the highest Youden's index (sensitivity+specificity-1) was considered optimal for classifying incident cases from the rest. All reported P-values were based on two-sided tests. STATA 15 software was used for all analyses (M Psarros and Assoc., Sparti, Greece / Stata Corp LLC, Texas, USA).

RESULTS

10-year diabetes incidence

Among the n=1049 participants of the present study sample, 133 participants were classified as having diabetes during the 10-year study follow-up period; yielding a crude diabetes incidence of 12.7%.

Baseline characteristics of study participants by baseline VAI tertiles

Selected study-relevant baseline socio-demographic, lifestyle, clinical and biochemical characteristics of the participants by baseline VAI tertiles are presented in ***Table 1***. As baseline

VAI values increased, participants were more likely to be men, older, smokers, less educated, less physically active and less adherent to the Mediterranean diet, as well as to have higher mean BMI and WC. Hypertension and hypercholesterolemia were also more frequent across the baseline VAI tertiles. Total cholesterol, LDL-cholesterol and TG mean concentrations were higher with increasing baseline VAI, whereas HDL-cholesterol was lower. The mean fasting glucose, insulin and HOMA-IR levels also increased across the baseline VAI tertiles. Moreover, participants in the highest VAI tertile had the highest mean ox-LDL, IL-6, TNF-a, CRP, homocysteine and fibrinogen levels. On the other hand, the family history of diabetes, as well as TAC and SAA mean concentrations did not differ significantly between the three baseline VAI tertiles.

VAI and 10-year diabetes incidence

The 10-year incidence of diabetes noted across the baseline VAI tertiles in this study was n=24 cases (6.9%); n=34 cases (9.7%) and n=75 cases (21.5%), respectively ***(Table 1)***. Participants who did not develop diabetes (n=916) within the 10-year study follow-up period were almost equally distributed among the three VAI groups (35.6%, 34.5% and 29.9%, respectively), whereas in the group of participants who developed diabetes at the 10-year follow up (n=133) the majority belonged in the higher baseline VAI tertile (18%, 25.6% and 56.4%, respectively).

In order to control for residual confounding, multi-adjusted logistic regression analysis was also performed through nested models ***(Table 2)***. In the age-sex adjusted model (***Table 2, Model 1***), 1-unit increase in VAI at baseline was found to increase the 10-year diabetes risk by 29% (OR=1.29, 95%CI: 1.16, 1.44), with this association being evident for participants in the 3rd VAI tertile (OR= 2.33, 95%CI: 1.39, 3.88) compared to the 1st VAI tertile. These findings remained significant even after controlling for years of education, physical activity and adherence to the Mediterranean diet (***Table 2, Model 2***), as well as baseline smoking,

hypertension and hypercholesterolemia status (***Table 2, Model 3***). In the model that was further adjusted for family history of diabetes (***Table 2, Model 4***), VAI increase per 1-unit was associated with a 22% increase in the 10-year diabetes risk (OR=1.22, 95%CI: 1.09, 1.37).

To investigate potential mediating mechanisms underlying the noted positive association between VAI and the 10-year diabetes incidence, selected biomarkers of oxidative stress (*i.e.*, ox-LDL and TAC) and inflammation (*i.e.*, CRP, IL-6, TNF-α, SAA, homocysteine, fibrinogen), as well as fasting glucose and insulin were sequentially, and separately, entered to the fully adjusted Model 4 (with VAI as a constant variable). For the tested oxidative stress biomarkers, this analysis revealed a mediating effect only for TAC (OR=0.82; 95%CI: 0.50, 1.33). The tested pro-inflammatory markers were also found to mediate this association, but partially, as all these *P-values* for VAI remained <0.05, but with decreased ORs (data not shown), while no mediation was observed when fasting glucose and insulin were entered in the model.

No significant interactions were observed between age, sex, and other covariates used in the estimated models (***Table 2***) and VAI; nevertheless, based on clinical relevance, subgroup analyses were performed by sex, family history of diabetes and fasting glycaemia. As such, for participants with family history of diabetes 1-unit increase in baseline VAI was found to increase the 10-year diabetes risk by 36% (OR=1.36; 95%CI: 1.10, 1.68), whereas for participants without family history of diabetes this risk was still increased, but to a lower degree (17% increase; OR=1.17; 95%CI: 1.01-1.35). Stratification by sex and fasting glucose values (using 110 mg/dL as a cut-off point) revealed that increasing baseline VAI increased the 10-year diabetes risk only for male participants (OR=1.28; 95%CI: 1.11, 1.48) and participants with fasting glucose levels <110 mg/dl (OR=1.16; 95%CI: 1.01-1.34), whereas among women and individuals with baseline fasting glucose ≥110 mg/dl results were not significant (data not shown).

It should also be noted that a significant interaction was detected between VAI and menstruation status (*p* for interaction=0.003); thus, subgroup analysis in women was repeated by menstruation status. A significant positive association between VAI and 10-year diabetes development was found among women at menstruation (OR=1.52; 95%CI: 1.03, 2.27), whereas for postmenopausal women no such significant association was detected (OR=0.81; 95%CI: 0.50, 1.30).

Predictive value of baseline VAI on the 10-year diabetes incidence against other common anthropometric indices

In order to assess the predictive value of VAI on the 10-year diabetes incidence against the variables included in the VAI formulas, as well as other common anthropometric/metabolic indices (*i.e.*, BMI, WC, TG/HDL, WHR, and WHtR), multiple logistic regression was repeated replacing VAI with each of these indices separately, and -2Loglikelihood values were compared (*i.e.*, lowest values indicating better predictive ability). The -2Loglikelihood of the model that included VAI as predictor of 10-year diabetes incidence was 556. Of the other tested indices/ratios, the one with the best predictive ability for the 10-year diabetes incidence was TG/HDL (-2Loglikelihood=633), followed by WHtR, WC, WHR, and BMI (-2Loglikelihood values: 668, 681, 690 and 796, respectively). In order to further quantify the contribution of VAI in the 10-year diabetes incidence, correct classification rates were calculated before and after the inclusion of VAI in the models. It was found that VAI added significantly in the correct classification of the diabetes incident cases during the 10-year follow-up. Particularly, in the fully-adjusted model, 4.4% of the participants that developed diabetes were correctly classified, while the correct classification increased to 13.8% after adjustment for VAI, yielding a 213% increase in the correct classification rate. Similar contribution was found for WHtR, for which the correct classification rate was calculated equal to 14%. The respective correct classification rates for BMI, WC, WHR and TG/HDL were found lower (*i.e.*, 8.9%, 10.9%, 9.5% and 8.8%,

respectively). Furthermore, based on the sensitivity-specificity analysis, VAI value ≥ 1.61 was considered as the optimal threshold for classifying incident from diabetes-free cases (sensitivity 67%).

DISCUSSION

Visceral obesity is now recognized as a major determinant of cardiometabolic disease, including atherosclerosis, hypertension and T2DM [25-27], and there is a need in clinical practice and research for simple indices that precisely reflect this association. Accordingly, VAI has been introduced as a novel, relatively simple, index of visceral adipose tissue accumulation and dysfunction in Caucasians, which appears to accurately reflect the related cardiometabolic risk [1]. To our knowledge, in this work, for the first time, VAI was prospectively studied in relation to 10-year diabetes development among non-diabetic, CVD-free Caucasian adults from the general population. In the fully adjusted model of this study, VAI was found to increase by 22% the 10-year diabetes risk per 1-unit of VAI increase at baseline (in a linear way). The related risk was even higher for male participants, those with family history of diabetes and participants with fasting glucose levels <110 mg/dl, while a significant moderating effect of menstruation status was revealed among women. Furthermore, TAC and certain markers of inflammation (*i.e.*, CRP, IL-6, TNF-α, SAA, homocysteine, fibrinogen) were found to, at least partly, mediate this association. Of note, VAI showed the best predictive ability over other commonly used anthropometric indices (*i.e.*, WC, WHR, BMI, WHtR) and TG/HDL, and its inclusion in the fully-adjusted diabetes prediction model in this study contributed the most (along with WHtR) in the correct classification of individuals who developed diabetes during the 10-year study follow-up.

The noted mediating effect of oxidative stress and pro-inflammatory biomarkers on the VAI-diabetes association within this study may be attributed, at least in part, to the complex

interplay between oxidative stress, inflammation and glucose metabolism in obesity. Indeed, the aggravating effect of excess adipose tissue (particularly visceral) on oxidative stress, inflammatory status, and insulin resistance pathways is now established [28]. In obesity (particularly central/visceral), lipid accumulation in hypertrophic adipocytes initiates a state of cellular stress (increased reactive oxygen species and endoplasmic reticulum stress), implicated in the activation of two important insulin resistance pathways, namely (i) the c-Jun N-terminal kinase (JNK) pathway, which induces serine *vs.* tyrosine phosphorylation in the insulin receptor substrate (IRS)-1, blocking normal insulin receptor signaling; and (ii) the IkB kinase b (IKKb)/nuclear factor-kB (NF-kB) pathway that leads to production of pro-inflammatory cytokines, such as TNF-α and IL-6, resulting in diminished glucose uptake, FFA esterification and storage [29,30]. Notably, the activation of pro-inflammatory pathways has been shown to independently increase the diabetes risk [31,32].

It is also important to highlight the better diabetes predictive ability of VAI against the common anthropometric indices (*i.e.*, BMI, WC, WHR, WHtR) in this study population. BMI is a crude marker of overall obesity and, hence, does not precisely reflect central/visceral fat distribution which is a well-established key contributor to insulin resistance [33]. Compared with WC, as well as WHR and WHtR, which better reflect abdominal fat accumulation (both subcutaneous and visceral), VAI appears to reflect more accurately the accumulation and dysfunction of visceral adipose tissue [1]. This may be important for identifying the related cardiometabolic risk since, compared to subcutaneous, visceral adipose tissue promotes more vigorously obesity-related inflammation, through higher release of pro-inflammatory adipokines and other factors (*e.g.*, monocyte chemoattractant protein-1 and colony-stimulating factor-1) [34]. Indeed, Vasan et al., by measuring total and regional adiposity by dual energy X-ray absorptiometry in 4950 apparently healthy participants, found that among upper adiposity depots, visceral adipose tissue showed stronger odds ratios for T2DM risk compared with WC,

and concluded that typically used anthropometry may underestimate the associations of visceral adiposity with T2DM risk [35]. Similarly, using ultrasonography in a population at high T2DM risk, visceral fat was found to significantly correlate with glucose intolerance and insulin resistance, even after adjustment for WC [36].

Finally, a moderating effect of the menstruation status was found on the association between VAI and 10-year diabetes incidence in the women of this study. Body fat distribution varies significantly with sex hormone levels, with increased visceral fat accumulation following the physiologic decrease in estrogen levels among women going through the menopausal transition [37]. However, in the women participants of this study increased premenopausal VAI values appear to be an important predictor of developing diabetes within the next 10-years. It could be speculated that within this timeframe menopause would occur for those women close to this transition, further adding to the T2DM risk compared to women already at menopause; however, the mean age of the subgroup of premenopausal women in our study was relatively young (37 years), hence not old enough to fully account for this finding based on a possible menopause transition. Factors/variables not accounted in the context of this study (*e.g.*, genetic factors, hyperandrogenaemia in women) may play a role in the noted moderating effect of the menstruation status. Further research is clearly needed to confirm these findings on the association between VAI and 10-year diabetes in women.

Limitations and strengths

This study has some limitations that should not be disregarded. Since the exact time of diabetes onset was not known in all cases, hazard ratios were estimated through odds ratios which may have over- estimated the true association. However, for low frequency diseases, odds ratio is suggested to be an accurate estimate (converges) of the relative risk. Furthermore, considering that according to the study protocol only CVD-free individuals were included at baseline, there may have been an underestimation of the long-term diabetes incidence. In

addition, diabetes diagnosis was set using only a single fasting glucose measurement (or antidiabetic medication); thus, underestimation of diabetes cannot be ruled out. Moreover, the menstruation status of women in this study was self-reported, whilst there are various factors/variables (*e.g.* genetic factors, hyperandrogenaemia) which may have had an impact on the noted moderating effect of the menstruation status and were not accounted for in the context of this study. Menopausal transition details were also not precisely captured in this study. In addition, the present study included only Caucasian men and women, therefore the present findings cannot be directly extrapolated to other ethnic groups. Indeed, the construction of ethnicity-related versions of VAI is now considered, such as the Chinese visceral adiposity index (CVAI) which has been found to be superior to VAI in predicting pre-diabetes and T2DM in Chinese adults [38]. Finally, as noted in such long-term prospective studies, the fact that some participants might have changed their lifestyle behaviors (*e.g.*, diet, physical activity, smoking) during the 10-year study follow-up should also be acknowledged.

Despite these limitations, the large and representative study sample from the general population, the prospective study design with long-term follow-up, as well as the detailed and standardized assessment/measurement of multiple lifestyle, clinical and biochemical data, ensure that the present work is of robust quality and included adjustment for multiple known confounders.

Conclusions

The present study demonstrated the independent association of VAI to long-term diabetes development among CVD-free Caucasian adults from the general population. This association between VAI and long-term diabetes incidence was evident among men and premenopausal women, and particularly in those with family history of diabetes. When assessing such individuals, VAI, which constitutes a novel index of visceral adiposity accumulation/dysfunction, could be utilized as an additional surrogate marker of diabetes risk,

since it appears to both increase the correct diabetes classification rate and be better than other common anthropometric indices. Future studies are needed to determine optimal cut-off values for VAI in relationship to diabetes development, as well as to assess the performance of VAI in relationship to diabetes risk estimation models both in Caucasians and other ethnic groups.

ACKNOWLEDGEMENTS

The authors would like to thank the investigators of the ATTICA Study: Natassa Katinioti, Labros Papadimitriou, Constantina Masoura, Spiros Vellas, Yannis Lentzas, Manolis Kambaxis, Konstadina Palliou, Vassiliki Metaxa, Agathi Ntzouvani, Dimitris Mpougatsas, Nikolaos Skourlis, Christina Papanikolaou, Georgia-Maria Kouli, Aimilia Christou, Adella Zana, Maria Ntertimani, Aikaterini Kalogeropoulou, Evangelia Pitaraki, Alexandros Laskaris, Mihail Hatzigeorgiou and Athanasios Grekas for their assistance in the initial physical examination and follow-up evaluation; Efi Tsetsekou for her assistance in psychological evaluation; as well as the laboratory team: Carmen Vassiliadou and George Dedoussis (genetic analysis), Marina Toutouza-Giotsa, Constadina Tselika and Sia Poulopoulou (biochemical analysis) and Maria Toutouza for the database management. In addition, the authors would also like to thank all the participants of the ATTICA Study.

Conflict of interest. None to declare

Funding. The ATTICA Study has received grants from the Hellenic Cardiology Society (HCS2002) and the Hellenic Atherosclerosis Society (HAS2003). Demosthenes Panagiotakos and Ekavi Georgousopoulou have also received research grants by Coca-Cola SA (10.9.2013). Demosthenes Panagiotakos and Efi Koloverou have been funded from ATHLOS project to study trajectories of healthy aging (European Union's Horizon 2020 research and innovation program, grant agreement No 635316).

Contribution statement. EK analyzed the data and wrote the paper. DP, CP, CC contributed to the conception and design of the ATTICA study and interpretation of data. DP, IK conceived the research hypothesis and reviewed the paper. EG, DT and IS contributed to the acquisition of data and design of the study. All authors contributed to the critical revision of the manuscript and approved its final version.

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Table 1. Distribution of selected study-relevant baseline socio-demographic, lifestyle, clinical and biochemical characteristics of the study sample (n=1049) by baseline tertiles of the Visceral Adiposity Index (VAI).

Baseline characteristics	Total Sample (n=1049)	VAI Tertile-1 (<1.02) (n=350)	VAI Tertile-2 (1.03 – 1.87) (n=350)	VAI Tertile-3 (>1.88) (n=349)	***p***
Diabetes 10-year incidence, n (%)	133 (12.7)	24 (6.9)	34 (9.7)	75 (21.5)	<0.001
Age, years	43.4±12.8	38.4±12.1	44.7±12.6**	47.2±12.0**	<0.001
Male sex, n (%)	502 (47.9)	141 (40.3)	164 (46.9)	197 (56.4)	<0.001
Education, years of school	12.6±3.6	13.3±3.2	12.7±3.5*	11.9±3.8**	<0.001
BMI, kg/m^2	26.1±4.5	24.1±3.7	26.1±4.2**	27.9±4.7**	<0.001
Waist circumference, cm	89.3±14.7	81.6±12.8	89.5±12.9**	96.9±14.1**	<0.001
Diabetes family history, n (%)	230 (23.9)	64 (19.6)	80 (24.8)	86 (27.6)	0.2
Hypertension, n (%)	296 (29.7)	65 (19.6)	111 (33.1)	120 (36.3)	<0.001
Hypercholesterolemia, n (%)	450 (42.9)	70 (20)	153 (43.7)	227 (65)	<0.001
Total cholesterol, mg/dL	194.0±40.1	173.2±34.1	195.1±39.0**	212.0±36.9**	<0.001
HDL-cholesterol, mg/dL	49.2±15.3	58.4±18.3	49.5±10.7**	39.8±9.2**	<0.001
LDL-cholesterol, mg/dL	122.1±36.7	103.2±31.0	126.0±34.9**	137.1±35.7**	<0.001
Triglycerides, mg/dL	112.1±69.2	60.0±16.4	98.4±24.0**	179.0±78.5**	<0.001
Current smokers, n (%)	584 (55.7)	177 (50.6)	179 (51.1)	228 (65.3)	<0.001
Physically active, n (%)	460 (43.9)	171 (48.9)	161 (46.0)	128 (36.7)	<0.05
MedDietScore (range 0-55)	26.2±6.7	28.0±7.3	25.8 ±6.5**	24.9±5.7**	<0.001
Fasting glucose, mg/dL	90.0±12.3	89.1±12.3	89.3±11.7	91.6±12.7*	0.01
Fasting insulin, μU/mL	12.6±1.2	12.3±1.6	12.5±1.4	13.0±1.5*	<0.001
HOMA-IR	2.8±0.7	2.7±0.7	2.8±0.6	3.0±0.7*	<.001
TAC, μmol/L	241.1±45.2	242.3±44.2	234.2±49.3	245.0±42.2	0.2
ox-LDL, mg/dL	55.5±26.1	53.0±27.8	52.7±24.2	60.1±25.8**	<0.001
IL-6, pg/mL	1.5±0.4	1.3±0.4	1.8±0.4**	1.5±0.4**	<0.001
TNF-α, pg/mL	6.3±3.7	5.6±4.0	6.3±3.5**	7.1±3.5**	<0.001
CRP, mg/L	1.9±2.4	1.2±1.8	2.1±2.7**	2.4±2.5**	<0.001
Homocysteine, μmol/L	11.9±6.6	11.5±6.7	11.8±7.0	12.7±5.9**	<0.001
SAA, mg/dL	4.9±4.5	3.9±4.3	4.6±5.2	4.5±4.0	0.1
Fibrinogen, mg/dL	308.2±67.2	285.3±62.6	315.1±68.0**	323.0±65.0**	<0.001

Data are presented as mean values and standard deviations or absolute and relative frequencies. P-values derived from ANOVA for the normally distributed variables, which fulfilled the assumption of homogeneity of variances of the variable across VAI tertiles (blood glucose and insulin levels) and Kruskal-Wallis test for the rest, non-normally distributed, variables or chi-square test for the categorical variables. The mean difference is significant at the 0.05 level.

*p<0.05 and **p<0.001 from post-hoc analyses, using the Bonferroni rule, for between VAI Tertile 3 and 2 vs. Tertile 1 as the reference category.

BMI: body mass index; HDL: high-density lipoprotein; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LDL: low-density lipoprotein; TAC: total antioxidant capacity; ox-LDL: oxidized LDL; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; CRP: C-reactive protein; SAA:

serum amyloid-A; *MedDietScore*: score evaluating the adherence to the Mediterranean diet (higher score values indicate better adherence).

Table 2. Results from multiple logistic regression models presented with odds ratios and corresponding 95% confidence intervals (ORs; 95% CIs) that evaluated the association of the Visceral Adiposity Index (VAI) at baseline with the 10-year incidence of diabetes among the study participants (n=1049).

	Per 1-unit increase in VAI	VAI Tertile-1 (<1.02) (n=350)	VAI Tertile-2 (1.03 – 1.87) (n=350)	VAI Tertile-3 (>1.88) (n=349)
Model 1	1.29; 1.16 – 1.44	1 (referent)	0.94; 0.53 – 1.67	2.33; 1.39 – 3.88
Model 2	1.27; 1.14 – 1.42	1 (referent)	0.93; 0.52 – 1.66	2.19; 1.31 – 3.68
Model 3	1.25; 1.12 – 1.40	1 (referent)	0.86; 0.47 – 1.57	2.00; 1.15 – 3.48
Model 4	1.22; 1.09 – 1.37	1 (referent)	0.82; 0.43 – 1.53	1.61; 0.89 – 2.92

Model 1 is adjusted for age and sex;
Model 2: model 1 plus adjustment for years of school, physical activity and adherence to the Mediterranean diet (*MedDietScore*);
Model 3: model 2 plus adjustment for smoking, hypertension and hypercholesterolemia;
Model 4: model 3 plus adjustment for family history of diabetes