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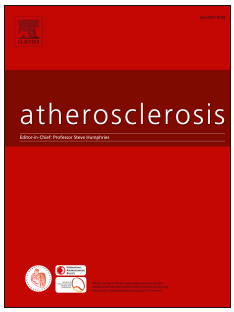

Lipid accumulation product in relation to 10-year cardiovascular disease incidence in Caucasian adults: The ATTICA study

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Lipid accumulation product in relation to 10-year cardiovascular disease incidence in Caucasian adults; the ATTICA Study

The ATTICA epidemiological cohort study

3042 adults without pre-existing CVD, from the Greek general population, aged 18 -89 years, 1514 men

LAP values at baseline were calculated using WC (cm) and TG (mmol/l) values; (i) LAP for men= [WC (cm) - 65] x [TG concentration (mmol/L)]; and (ii) LAP for women= [WC (cm) - 58] x [TG concentration (mmol/L)].

10-year follow-up

2583 of the 3042 initial participants were re-assessed during this follow-up

Incidence of Cardiovascular Disease

LAP was positively associated with with 10-year CVD incidence, even after adjusting for hypertension, diabetes, hypercholesterolemia, smoking, physical activity, Mediterranean diet adherence, and key pro-inflammatory biomarkers (Hazard Ratios per 10 cm·mmol/L of LAP ranging from 1.1 to 1.21, p=0.04).

LAP predicted CVD risk better than common obesity indices (BMI, WC, waist-to-hip, waist-to-height ratio).

LAP is considered to expand on the concept of the hypertriglyceridemic waist as regards C VD risk

An independent positive association between LAP and long-term CVD incidence in CVD-free Caucasian adults from the general population was revealed.

Lipid accumulation product in relation to 10-year cardiovascular disease incidence in Caucasian adults: The ATTICA Study

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Abstract

Background and aims: The lipid accumulation product (LAP) is an index describing lipid over-accumulation based on waist circumference (WC) and fasting triglycerides, and can outperform the body mass index (BMI) in recognizing cardiovascular disease (CVD) risk. We aimed to explore the association of LAP with long-term CVD risk and compare its CVD-predictive value against common anthropometric indices/ratios of obesity.

Methods: ATTICA is a prospective, population-based cohort that recruited 3042 adults without pre-existing CVD from the Greek general population (age 18 -89 years; 1514 men). The 10-year study follow-up (2011-2012) captured the fatal/non-fatal CVD incidence in 2020 participants (50% men). Baseline LAP (cm·mmol/L) was calculated and analyzed in relation to the 10-year CVD incidence.

Results: In total, 317 CVD cases (15.7%) were documented during the follow-up. Baseline LAP showed a significant positive association with the 10-year CVD incidence, even after adjusting for hypertension, diabetes, hypercholesterolemia, smoking, physical activity, Mediterranean diet adherence, and key pro-inflammatory biomarkers (Hazard Ratios per 10 cm·mmol/L of LAP ranging from 1.1 to 1.21, p=0.04). Moreover, LAP predicted the 10-year CVD study incidence better than common obesity indices (BMI, WC, waist-to-hip, waist-to-height ratio).

Conclusions: These findings support an independent positive association between LAP and long-term CVD incidence in CVD-free Caucasian adults from the general population.

Key words: Lipid accumulation product, LAP, cardiovascular disease, CVD risk, ATTICA study

1. Introduction

Obesity, particularly central (abdominal/visceral), is a key risk factor of cardiovascular disease (CVD), with the abdominal fat mass playing a critical role in this relationship [1-3]. However, the body mass index (BMI), which is used to categorize obesity in clinical practice [4], fails to differentiate between lean and total fat mass, let alone it accounts for the abdominal fat mass [3-5]. As such, BMI has been shown to be a crude predictor of CVD risk [5-7], whilst other surrogate markers of central obesity, such as waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR), are now used in clinical practice and/or epidemiological research as better CVD predictors [3,5-8].

In 2005, using data from the third National Health and Nutrition Examination Survey (NHANES III), Henry Kahn proposed the calculation of the lipid accumulation product (LAP) based on WC (cm) and fasting serum triglycerides (TG; mmol/L) [LAP in men: (WC-65) × TG; LAP in women: (WC-58) × TG] [9]. LAP was proposed as a better continuous marker/index to describe lipid over-accumulation in relationship to central obesity, and was shown to outperform BMI for identifying CVD risk [9]. Indeed, in this NHANES cohort, LAP exhibited better correlations with key CVD risk factors (*i.e.*, heart rate and circulating levels of lipids and uric acid) compared to BMI [9]. Subsequently, LAP has been reported as a reliable marker of CVD risk in women with polycystic ovary syndrome (PCOS) and a useful index for recognizing insulin resistance, non-alcoholic fatty liver (NAFLD) and metabolic syndrome in various cohorts [10-16].

To date, the existing epidemiological data from prospective, community-based studies on the predictive value of LAP for long-term CVD risk are very limited [17,18]. Therefore, the objective of the present work was to assess LAP as a predictor of the 10-year CVD incidence in the ATTICA study cohort of Caucasian adults without previous CVD, and

compare its discriminating ability against BMI and other commonly used anthropometric indices/ratios of central obesity (*i.e.*, WC, WHR and WHtR).

2. Materials and methods

2.1 Study sample

In brief, ATTICA is a prospective, population-based, cohort study performed in Attica (Athens metropolitan region, Greece), which recruited 3042 non-institutionalized adults (Caucasians; women/men: 1528/1514; age: ≥18 years) without previous CVD. Recruitment was conducted during 2001-2002, applying a random sampling protocol and selecting a single participant per household. All participants underwent detailed baseline assessments which included medical history, physical examination and blood sampling for biochemical measurements. Baseline CVD was excluded in all participants by the study physicians. The ATTICA study was approved by our Institutional Ethics Committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki, with all participants providing written informed consent. The methodology details of the ATTICA study have been previously described [19].

2.2 Baseline study assessments

Comprehensive baseline data were collected, including demographic details (*e.g.*, age, gender and years of education), personal/family history of cardio-metabolic disease (*e.g.*, hypertension, diabetes, hypercholesterolemia and CVD), and information about dietary and pertinent lifestyle habits (*e.g.*, physical activity and smoking in pack years), as described previously [19]. Briefly, all participants underwent a detailed baseline dietary evaluation through the EPIC-Greek questionnaire [20], which is a validated semi-quantitative food-frequency questionnaire that was kindly provided by the Unit of Nutrition of Athens Medical School. Moreover, the MedDietScore was used to assess the adherence to the Mediterranean

diet (score range: 0-55; higher values indicate better adherence) [21]. The physical activity level of each participant was also assessed at baseline using the International Physical Activity Questionnaire (IPAQ; participants reporting no physical activities or exercise on the IPAQ were classified as physically inactive) [22].

Standardized measurements were performed by trained study researchers at baseline examination, including body weight and height, as well as WC and hip circumference in order to calculate the BMI (body weight in kilograms divided by the height in meters squared; kg/m^2), WHR and WHtR. Furthermore, resting arterial blood pressure (BP; average of three recordings in sitting position) was also measured at baseline, and individuals exhibiting average BP ≥140/90 mmHg or taking antihypertensive medication(s) were categorized as hypertensive.

At baseline, morning (8-10 am) fasting blood samples were obtained from all participants after overnight fasting (10-12 hours without food/alcohol intake, except for water). TG, total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) levels were measured by a chromatographic enzymatic method using a Technicon automatic analyzer RA-1000 [Dade Behring, Marburg, Germany; corresponding intra- and inter-assay coefficients of variation (CV): <4%, <9%, and <4%, respectively]. Low-density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald formula [23]. Hypercholesterolemia was defined as TC >200 mg/dl or treatment with lipid-lowering drug(s). Moreover, fasting blood glucose (FBG) levels were measured by a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA, USA) and subjects with FBG >125 mg/dl or on antidiabetic treatment were classified as having diabetes. Selected circulating pro-inflammatory biomarkers were also measured, namely high sensitivity C-reactive protein (CRP) by particle-enhanced immunonephelometry (N Latex; Dade-Behring Marburg GmbH, Marburg, Germany; intra- and inter-assay CV: <5%), as well as tumor necrosis factor-alpha (TNF-α)

and interleukin-6 (IL-6) by high-sensitivity enzyme-linked immunosorbent assays (R&D Systems; for both assays intra- and inter-assay CV: <10%), following the corresponding manufacturer's protocols.

Finally, participants were also categorized based on the presence/absence of metabolic syndrome at baseline according to the harmonized metabolic syndrome definition [24], namely based on the presence/absence of three or more of the following criteria: (i) WC ≥88 cm for women or ≥102 cm for men (WC cut-off values for central obesity as proposed by the European Cardiovascular Societies for European populations in the harmonized metabolic syndrome definition); (ii) TG ≥150 mg/dL (or relevant treatment); (iii) HDL-C <50 mg/dL for women or <40 mg/dL for men (or relevant treatment); (iv) systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg (or antihypertensive treatment); (v) FBG ≥100 mg/dL (or antidiabetic treatment).

2.3 Lipid accumulation product (LAP) at baseline

The LAP values at baseline were also calculated for the study participants using the baseline WC (cm) and TG (mmol/l) values and the LAP formulas as proposed by Kahn [9], namely (i) LAP for men= [WC (cm) - 65] x [TG concentration (mmol/L)]; and (ii) LAP for women= [WC (cm) - 58] x [TG concentration (mmol/L)]. Accordingly, the baseline LAP tertiles (<19, 19 – 44, >44 cm·mmol/L) were also extracted.

2.4 Cardiovascular disease incidence at the 10-year follow-up (2002-2012)

The 10-year study follow-up was conducted in 2011-2012 (median follow-up period: 8.41 years), as previously described [25]. Briefly, 2583 of the 3042 initial participants were re-assessed during this follow-up [participation rate: 85%; no difference to the total study sample; baseline age (mean ± standard deviation; SD): 45±14 and 46±14 years for women and men, respectively]. As per protocol at the follow-up, study physicians obtained the medical records/data (WHO-ICD-10) to confirm the CVD status of each participant,

capturing detailed relevant data (*e.g.*, development of myocardial infarction, angina pectoris and other identified forms of ischemia, as well as chronic arrhythmias, heart failure and stroke). For participants who died during this study follow-up, relevant data were acquired from death certificates and living relatives. Through this process at the 10-year study follow-up, accurate CVD status data were obtained for 2020 participants (***Table 1***). In line with the aim of the present study to assess LAP as a potential predictor of long-term CVD risk, herein we present the analysis of the ATTICA study data and the documented 10-year incidence of fatal/non-fatal CVD of these 2020 participants in relation to the LAP at baseline.

2.5 Statistical analysis

Normally distributed continuous variables are presented as mean values ± SD, and categorical variables as frequencies. Normality was tested using the Shapiro-Wilk criterion; the non-normally distributed variables are presented as median and 1^{st}, 3^{rd} tertile. Crude, non-fatal and fatal incidence rates of combined CVD (coronary heart disease or stroke) were calculated as the ratio of new CVD cases to the number of participants at the 10-year follow-up. Associations between categorical variables were tested by the chi-square test, whereas between continuous variables by the Pearson *r* or Spearman's *rho* coefficients for normally distributed and skewed variables, respectively. Continuous variables were tested for normality via P-P plots. For normally distributed variables, comparisons of means between participants who developed CVD and those who remained CVD-free were performed by the Student's t-test, after controlling for equality of variances by the Levene's test. For continuous variables without normal distribution (*e.g.*, TG), comparisons were performed by the non-parametric Mann-Whitney U-test. Cox proportional hazards models were used to estimate the hazard ratios (HR) and the corresponding 95% confidence intervals (CI) of developing a CVD event during the study follow-up according to the baseline characteristics of participants. Kaplan-Meier curves were plotted to illustrate 10-year CVD survival by LAP

tertile (Log rank test was calculated to evaluate between tertiles' comparisons). The -*2logLikelihood* was also calculated to evaluate the goodness-of-fit of the estimated models. Moreover, ROC analysis and the corresponding Area Under the Curve (AUC; 95% CI) was also calculated in order to evaluate the additive value of LAP and other metabolic markers (*e.g.*, TG, waist circumference, BMI) on top of known CVD risk factors (illustrated in Model 1, Table 4). All *p*-values are based on two-sided tests and the corresponding 95% CI. STATA 15 software was used for all analyses (M Psarros and Assoc., Sparti, Greece / Stata Corp LLC, Texas, USA).

3. Results

3.1 Baseline LAP and key cohort characteristics according to 10-year CVD incidence

Table 1 presents key baseline characteristics of the *n*=2020 study participants according to the documented 10-year CVD status. The 10-year fatal/non-fatal CVD event rate was 157 cases/1000 participants [n=317 participants; 119 women (118 cases/1000 participants) and 198 men (195 cases/1000 participants); p for gender difference <0.001]. As expected, the group of participants who developed CVD during the 10-year study follow-up consisted mainly of older men, heavier smokers, and exhibited higher anthropometric indices/ratios of total and central obesity (BMI, WC, WHR, WHtR), compared to those who remained CVD-free. Furthermore, this group had higher BP and FBG levels, lower MedDietScore (*i.e.*, worse Mediterranean diet adherence), as well as worse profiles of circulating pro-inflammatory biomarkers (*i.e.*, CRP, IL-6, TNF-α) and lipids (*i.e.*, TC, TG, HDL-C, LDL-C) (all p-values <0.001; ***Table 1***). The mean LAP value at baseline was 69% higher in the group of participants who developed a CVD event during the 10-year follow-up than in those who remained CVD-free (p <0.001, ***Table 1***).

Key characteristics of the study participants according to the baseline LAP tertiles are presented in ***Table 2***. The age of participants and the prevalence of hypertension, diabetes, hypercholesterolemia and metabolic syndrome significantly increased across the baseline LAP tertiles, with the participants in the lowest LAP tertile being younger and exhibiting lower prevalence rates of these CVD risk factors (all p-values <0.001; ***Table 2***). In addition, participants in the lowest LAP tertile were mostly women, lighter smokers, more physically active, more adherent to the Mediterranean diet (*i.e.*, higher MedDietScore), and had lower BMI, WC, WHR and WHtR (all p-values <0.001; ***Table 2***).

Moreover, LAP at baseline exhibited significant positive correlations to BMI, WC, WHR, WHtR, TG, TC, FBG, CRP, IL-6 and TNF-α levels (rho= 0.488, 0.624, 0.515, 0.075, 0.897, 0.343, 0.281, 0.189, 0.225, and 0.221, respectively; all p-values <0.001), whereas it was inversely associated to HDL-C (rho= -0.318; p <0.001). All these correlations remained significant for both male and female participants following stratification by gender *(data not shown).*

3.2 Baseline LAP in relationship to the 10-year CVD incidence

Figure 1 illustrates the higher survival rate of participants in the 1st tertile of LAP as compared to those in the 2nd and 3rd (*p* for Log-rank test = 0.04; inter-tertile comparisons: $p_{\text{1st vs. 3rd}}$ <0.001, $p_{\text{1st vs. 2nd}}$ <0.001, $p_{\text{2nd vs. 3rd}}$ = 0.77). Moreover, the higher rate in the decay of the 10-year CVD survival was observed from approximately the sixth year of study follow-up, as expected based on the characteristics of the study's sample (relatively young adults without pre-existing CVD).

The association between baseline LAP and 10-year CVD incidence was further evaluated through a multi-adjusted analysis that controlled for multiple CVD-related covariates (***Table 3***). Due to multicollinearity, the two variables used in the LAP formulas (*i.e.*, WC, TG) were not entered together with LAP in these multi-adjusted models. At first,

typical demographic variables (*i.e.*, age, sex, education), as well as certain lifestyle factors (*i.e.*, smoking, physical activity, Mediterranean diet adherence) and key clinical parameters (*i.e.*, hypertension, diabetes, hypercholesterolemia) that hold known associations with long-term CVD risk were entered into *Baseline Model 1* (***Table 3***). In the age-sex only adjusted model (*Model 2*, ***Table 3***) LAP was significantly associated with higher 10-year CVD incidence. Subsequently, LAP was added into the *Baseline Model 1*, showing that baseline LAP had a significant and independent positive association with the 10-year CVD incidence, even after adjusting for established CVD risk factors (*Model 3*, ***Table 3***). In this latter model, a 10-unit increase in the baseline LAP value was associated with an 11% increase of the 10-year risk of developing CVD. Moreover, in order to test for potential mediating effects of key circulating pro-inflammatory biomarkers in this association between LAP and CVD, *Model 3* was additionally adjusted for CRP, IL-6 and TNF-α which were entered separately and consecutively, as presented in *Models 4*, *5*, and *6*, respectively (***Table 3***). The association between LAP and 10-year CVD remained significant in these models, suggesting absence of robust mediating effects from these circulating pro-inflammatory biomarkers.

3.3 Predictive value of LAP on the study 10-year CVD risk against other common anthropometric indices/ratios of obesity

Table 4 presents the hazard ratios (HR and corresponding 95% CI), as well as -2logL and AUC values of the models applied to compare the predictive value of baseline LAP on the 10-year CVD risk against the single components/variables of the LAP formulas (*i.e.*, WC or fasting TG), as well as against the most commonly used anthropometric indices of obesity (*i.e.*, models incorporating BMI, WHR, or WHtR instead of LAP). Based on these models, LAP exhibited better predictive value for the study 10-year CVD incidence as revealed through the -2logLikelihood ratio (the lower the better) and the AUC values (the higher the

better), than its components, *i.e.*, WC and TG. Similarly, baseline LAP was also a better predictor of the 10-year CVD than BMI, WHR and WHtR (***Table 4***).

4. Discussion

In the 2005 study, which introduced LAP as a better index for recognizing CVD risk compared to BMI, Henry Kahn highlighted the need for prospective data in order to assess whether LAP can be a useful CVD predictor in clinical practice and cardiovascular epidemiology [9]. The present study addresses this still unmet need by offering new long-term prospective data, which show that baseline LAP exhibited a significant positive association with the 10-year CVD incidence in a large community-based cohort of CVD-free Caucasian adults. Notably, this positive association remained significant even after adjusting for various CVD risk factors (*i.e.*, Mediterranean diet adherence, physical activity smoking, hypertension, hypercholesterolemia and diabetes). Moreover, in the performed comparisons of the predictive value of LAP on the 10-year CVD incidence, LAP was better not only than each of its individual components (*i.e.*, WC and fasting TG), but also than common anthropometric indices of total and central obesity (*i.e.*, BMI, WHR and WHtR). Thus, the presented findings suggest that LAP may constitute a predictive marker of CVD, better than other classical anthropometric/metabolic CVD risk indices/ratios, but cannot be considered as an additive marker for CVD risk stratification.

Since its introduction in the literature, LAP has been shown to be a powerful index for identifying metabolic syndrome, insulin resistance, and NAFLD in several cohorts, as well as a reliable marker of CVD risk in women with PCOS [10-16]. Furthermore, a retrospective study by Ioachimescu *et al.*, has reported that LAP, but not BMI, can predict the all-cause mortality in high CVD risk, non-diabetic patients attending a preventive cardiology clinic [26]. Recent studies from China have also indicated that LAP is associated with increased

risk of intracranial atherosclerotic stenosis in middle-aged and elderly Chinese females [27], whilst it may be a valuable index in predicting the risk of hypertension in the Chinese population [28]. Moreover, in a cross-sectional study with 191 adults and elderly participants LAP exhibited high accuracy in visceral obesity discrimination, as assessed by the area of visceral adipose tissue identified by computed tomography [29]. Notably, LAP has been also shown to discriminate between patients with and without steatosis, although it could not quantitatively predict liver fat as determined quantitatively by proton magnetic resonance spectroscopy [30]. Further research is still needed to specifically explore the correlation between LAP and quantitative measures/markers of adiposity.

Despite the increasing evidence on the direct correlation between LAP and multiple CVD risk factors, so far there is a paucity of data from prospective, community-based studies on the predictive value of LAP for long-term CVD incidence [17,18]. In this context, data from the prospective Tehran Lipid and Glucose Study have showed that LAP is an independent predictor of CVD (median follow-up: 10.1 years; 160 CVD cases during follow-up) in 2378 CVD-free adults (57% men) with normal BMI [18]. This is in accord with our results on the association of LAP to the 10-year CVD incidence. However, in this Iranian study LAP was not superior for predicting CVD over BMI, WC, WHR and WHtR [18]. This apparent inconsistency with our findings may be attributed to the fact that this community-based study included only adults with BMI <25 kg/m^2, whilst other significant differences in the cohort characteristics of this Iranian study are also present (*e.g.*, different ethnic background and higher minimum age at recruitment, since these participants were of Persian ancestry and at least 30 years old) [18].

It is noteworthy that, Després *et al.*, first introduced the concept of the "*hypertriglyceridemic waist*" as a dichotomous cardiometabolic risk marker/indicator, which proved to be a good predictor of CVD outcomes and mortality [31-35]. However, obesity is a

continuous process rather than dichotomous, whilst both circulating TG and WC as single continuous variables are associated with CVD risk [9,35-37]. Thus, LAP is considered to expand on the concept of the hypertriglyceridemic waist by providing a continuous risk marker which better indicates the lipid over-accumulation in central obesity and the related cardiometabolic risk [9]. This related risk reflects the underlying continuous process which follows the excessive accumulation of visceral adipose tissue, progressively promoting metabolic dysregulation, atherosclerosis and low-grade inflammation [38-43]. Indeed, several pro-inflammatory adipokines/cytokines secreted mainly by the abdominal fat depots exert detrimental effects on metabolism and the cardiovascular function [41-44]. Of note, the significant positive association between LAP and CVD in the present study persisted even when the analysis was adjusted for CRP, IL-6 and TNF-α, suggesting lack of significant mediating effects from these circulating biomarkers which are implicated in obesity-related inflammation. Overall, the median CRP values observed in the ATTICA study cohort were similar to those generally noted in other general populations from European regions [45]. Further studies are required to fully elucidate the spectrum of underlying mechanisms/factors (*e.g.*, dysregulated adipokine secretion and ectopic fat accumulation in the liver, skeletal muscles and heart) which may be implicated in the documented association between LAP and CVD.

4.1 Limitations

The present study is not without limitations. As such, it should be acknowledged that the baseline/entry study examination was conducted once and, hence, may be susceptible to a certain degree of measurement error. However, the applied study methodology is similar to that in other large, prospective, CVD-outcome epidemiological studies, and followed standardized and validated protocols/methods for collecting all study data. Thus, our findings can be considered reliable and generally comparable to those from studies with similar

cohorts. Moreover, the study cohort consisted only of Caucasians, hence the present results cannot be extrapolated fully to other ethnicities. Finally, due to lack of an intervention arm in our protocol/design, the present study cannot provide insight as to whether it is useful to monitor LAP as an indicator of intervention effectiveness.

4.2 Conclusion

The present study adds to the emerging evidence suggesting that LAP may constitute a simple and accurate prognostic marker of CVD risk as compared to other obesity-related indices/markers. Indeed, the present findings offer new prospective data suggesting that LAP exhibits a positive association with the 10-year CVD incidence in Caucasian (Mediterranean/European) adults from the general population without pre-existing CVD. LAP appears to be a better predictor of the long-term CVD risk than the commonly used anthropometric indices/ratios of total and central obesity. Future studies are still required to further evaluate the association between LAP and CVD in different ethnic and patient populations and explore whether monitoring LAP over time can be a useful indicator of the effectiveness of interventions against CVD.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions:

Demosthenes Panagiotakos, Ioannis Kyrou, and Georgia-Maria Kouli had the concept of the paper, performed data analyses and interpreted the results. Ekavi Georgousopoulou, Christina Chrysohoou, Constantine Tsigos, Dimitrios Tousoulis, and Christos Pitsavos contributed by providing comments on the design of the study, and critically reviewed the paper. All authors approved the final version.

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References

[1] Bhupathiraju SN, Hu FB. Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. Circ Res. 2016 May 27;118(11):1723-35.

[2] Kyrou I, Randeva HS, Tsigos C, Kaltsas G, Weickert M. Clinical Problems Caused by Obesity. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000- Updated 2018 Jan 11. Available from: www.ncbi.nlm.nih.gov/books/NBK278973/

[3] Klein S, Allison DB, Heymsfield SB, et al. Waist Circumference and Cardiometabolic Risk: a Consensus Statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. Obesity (Silver Spring). 2007 May;15(5):1061-7.

[4] World Health Organization: Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation on Obesity. Geneva, World Health Organization, 1998.

[5] Franzosi MG. Should we continue to use BMI as a cardiovascular risk factor? Lancet. 2006 Aug 19;368(9536):624-5.

[6] Romero-Corral A, Montori VM, Somers VK, et al. Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. Lancet. 2006 Aug 19;368(9536):666-78.

[7] Yusuf S, Hawken S, Ounpuu S, et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet. 2005 Nov 5;366(9497):1640-9.

[8] Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. Obes Rev. 2012 Mar;13(3):275-86.

[9] Kahn HS. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. BMC Cardiovasc Disord. 2005 Sep 8;5:26.

[10] Wiltgen D, Benedetto IG, Mastella LS, Spritzer PM. Lipid accumulation product index: a reliable marker of cardiovascular risk in polycystic ovary syndrome. Hum Reprod. 2009 Jul;24(7):1726-31.

[11] Macut D, Božić Antić I, Bjekić-Macut J, et al. Lipid accumulation product is associated with metabolic syndrome in women with polycystic ovary syndrome. Hormones (Athens). 2016 Jan-Mar;15(1):35-44.

[12] Xia C, Li R, Zhang S, et al. Lipid accumulation product is a powerful index for recognizing insulin resistance in non-diabetic individuals. Eur J Clin Nutr. 2012 Sep;66(9):1035-8.

[13] Kahn HS. The lipid accumulation product is better than BMI for identifying diabetes: a population-based comparison. Diabetes Care. 2006 Jan;29(1):151-3.

[14] Dai H, Wang W, Chen R, Chen Z, Lu Y, Yuan H. Lipid accumulation product is a powerful tool to predict non-alcoholic fatty liver disease in Chinese adults. Nutr Metab (Lond). 2017 Aug 1;14:49.

[15] Taverna MJ, Martínez-Larrad MT, Frechtel GD, Serrano-Ríos M. Lipid accumulation product: a powerful marker of metabolic syndrome in healthy population. Eur J Endocrinol. 2011 Apr;164(4):559-67.

[16] Nascimento-Ferreira MV, Rendo-Urteaga T, Vilanova-Campelo RC, et al. The lipid accumulation product is a powerful tool to predict metabolic syndrome in undiagnosed Brazilian adults. Clin Nutr. 2017 Dec;36(6):1693-1700.

[17] Bozorgmanesh M, Hadaegh F, Azizi F. Predictive performances of lipid accumulation product vs. adiposity measures for cardiovascular diseases and all-cause mortality, 8.6-year follow-up: Tehran lipid and glucose study. Lipids Health Dis. 2010 Sep 16;9:100.

[18] Hosseinpanah F, Barzin M, Mirbolouk M, Abtahi H, Cheraghi L, Azizi F. Lipid accumulation product and incident cardiovascular events in a normal weight population: Tehran Lipid and Glucose Study. Eur J Prev Cardiol. 2016 Jan;23(2):187-93.

[19] Pitsavos C, Panagiotakos DB, Chrysohoou C, Stefanadis C. Epidemiology of Cardiovascular risk factors in Greece; aims, design and baseline characteristics of the ATTICA study. BMC Public Health 2003;3:32:1-9.

[20] Katsouyanni K, Rimm EB, Gnardellis C, Trichopoulos D, Polychronopoulos E, Trichopoulou A. Reproducibility and relative validity of an extensive semi-quantitative food frequency questionnaire using dietary records and biochemical markers among Greek schoolteachers. Int J Epidemiol. 1997;26: S118-27.

[21] Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis. 2006; 16:559-68.

[22] Papathanasiou G, Georgoudis G, Papandreou M, et al. Reliability measures of the short International Physical Activity Questionnaire (IPAQ) in Greek young adults. Hellenic J Cardiol. 2009 Jul-Aug;50(4):283-94.

[23] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 Jun;18(6):499-502.

[24] Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009 Oct 20;120(16):1640-5.

[25] Panagiotakos DB, Georgousopoulou EN, Pitsavos C, et al. Ten-year (2002-2012) cardiovascular disease incidence and all-cause mortality, in urban Greek population: the ATTICA Study. Int J Cardiol. 2015 Feb 1;180:178-84.

[26] Ioachimescu AG, Brennan DM, Hoar BM, Hoogwerf BJ. The lipid accumulation product and all-cause mortality in patients at high cardiovascular risk: a PreCIS database study. Obesity (Silver Spring). 2010 Sep;18(9):1836-44.

[27] Li R, Li Q, Cui M, et al. Visceral adiposity index, lipid accumulation product and intracranial atherosclerotic stenosis in middle-aged and elderly Chinese. Sci Rep. 2017 Aug 11;7(1):7951.

[28] Wang H, Chen Y, Sun G, Jia P, Qian H, Sun Y. Validity of cardiometabolic index, lipid accumulation product, and body adiposity index in predicting the risk of hypertension in Chinese population. Postgrad Med. 2018 Apr;130(3):325-333.

[29] Roriz AK, Passos LC, de Oliveira CC, Eickemberg M, Moreira Pde A, Sampaio LR. Evaluation of the accuracy of anthropometric clinical indicators of visceral fat in adults and elderly. PLoS One. 2014 Jul 31;9(7):e103499.

[30] Cuthbertson DJ, Weickert MO, Lythgoe D, et al. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. Eur J Endocrinol. 2014 Nov;171(5):561-9.

[31] Lemieux I, Pascot A, Couillard C, et al. Hypertriglyceridemic waist: A marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? Circulation. 2000 Jul 11;102(2):179-84.

[32] LaMonte MJ, Ainsworth BE, DuBose KD, et al. The hypertriglyceridemic waist phenotype among women. Atherosclerosis. 2003 Nov;171(1):123-30.

[33] Kahn HS, Valdez R. Metabolic risks identified by the combination of enlarged waist and elevated triacylglycerol concentration. Am J Clin Nutr. 2003 Nov;78(5):928-34.

[34] Tankó LB, Bagger YZ, Qin G, Alexandersen P, Larsen PJ, Christiansen C. Enlarged waist combined with elevated triglycerides is a strong predictor of accelerated atherogenesis and related cardiovascular mortality in postmenopausal women. Circulation. 2005 Apr 19;111(15):1883-90.

[35] Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. Am J Clin Nutr. 2004 Mar;79(3):379-84.

[36] Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am J Cardiol. 1998 Feb 26;81(4A):7B-12B.

[37] Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Triglyceride concentration and ischemic heart disease: an eight-year follow-up in the Copenhagen Male Study. Circulation. 1998 Mar 24;97(11):1029-36.

[38] Le Lay S, Krief S, Farnier C, et al. Cholesterol, a cell size-dependent signal that regulates glucose metabolism and gene expression in adipocytes. J Biol Chem. 2001 May 18;276(20):16904-10.

[39] Matsuzawa Y. The metabolic syndrome and adipocytokines. FEBS Lett. 2006 May 22;580(12):2917-21.

[40] Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol. 2010 Mar 25;316(2):129-39.

[41] Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. Ann N Y Acad Sci. 2010 Nov;1212:E1-E19.

[42] Matsuzawa Y, Funahashi T, Nakamura T. The concept of metabolic syndrome: contribution of visceral fat accumulation and its molecular mechanism. J Atheroscler Thromb. 2011;18(8):629-39.

[43] Kyrou I, Mattu HS, Chatha K, Randeva HS. Chapter 7 - Fat Hormones, Adipokines. In Schisler JC, Lang CH, Willis MS (eds) Endocrinology of the Heart in health and disease. pp 167-205. Academic Press, 2017.

[44] Yang X, Smith U. Adipose tissue distribution and risk of metabolic disease: does thiazolidinedione-induced adipose tissue redistribution provide a clue to the answer? Diabetologia. 2007 Jun;50(6):1127-39.

[45] Arcari A, Zito F, Di Castelnuovo A, et al; European Collaborative Group of Immidiet Project. C reactive protein and its determinants in healthy men and women from European regions at different risk of coronary disease: the IMMIDIET Project. J Thromb Haemost. 2008 Mar;6(3):436-43.

Table 1. Demographic, lifestyle, behavioral and clinical characteristics of the ATTICA study participants at baseline based on the10-year follow-up by cardiovascular disease (CVD) status *(n= 2020).*

Baseline variable	**Total** *(n= 2020)*	**CVD status at 10-year follow-up**		*p*
		CVD-free *(n=1703)*	**CVD** *(n= 317)*	
Age (years)	45 ± 14	43 ± 13	58 ±13	<0.001
Gender, % male	50	48	62	<0.001
Smoking (pack years)	496 ± 501	441 ± 425	767 ± 705	<0.001
Physical Activity, % physically active	41	40	41	0.95
MedDietScore (range: 0-55)	26 ± 7	26 ± 6	23 ± 7	<0.001
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
Metabolic syndrome, %yes	20	18	41	<0.001
Hypertension, %yes	30	28	51	<0.001
Diabetes Mellitus, %yes	7	5	22	<0.001
Hypercholesterolemia, %yes	39	40	57	<0.001
Total cholesterol (mmol/L)	4.99±1.09	4.99±1.06	5.33±1.09	<0.001
HDL-cholesterol (mmol/L)	1.24±0.39	1.27±0.39	1.16±0.31	<0.001
LDL-cholesterol (mmol/L)	3.15±0.96	3.13±0.96	3.39±1.03	<0.001
Triglycerides (mmol/L), median, tertiles	1.10, 0.77-1.60	1.09, 0.77-1.61	1.11, 0.78-1.73	<0.001
C-reactive protein (mg/L), median, tertiles	1.02, 0.45-2.30	0.98, 0.44-2.23	1.50, 0.66-2.95	<0.001
Interleukin-6 (pg/mL)	1.46 ± 0.55	1.43± 0.55	1.65 ± 0.51	<0.001
Tumor necrosis factor-α (pg/mL), median, tertiles	5.77, 3.38-7.67	5.52, 3.22-7.49	7.49, 5.88-9.86	<0.001
LAP (cm·mmol/L), median, tertiles	31, 19-44	30, 14-54	35, 20-61	<0.001

Normally and non-normally distributed continuous variables are presented as mean values ± standard deviation, or median and 1st, 3rd tertile, respectively, and categorical variables as frequencies. *p*-values for the comparisons between the 10-year CVD-free and CVD group derived using the t-test, while for comparisons of categorical variables by the chi-square test or Mann-Whitney non-parametric test for variables without normal distribution (*i.e.*, triglycerides, CRP and LAP). LAP: Lipid Accumulation Product; MedDietScore: validated score evaluating the Mediterranean diet adherence (higher score indicates better adherence)

Table 2. Cardiovascular disease (CVD) cases during the ATTICA study 10-year follow-up and key baseline characteristics of the participants (n=2020) when categorized by baseline tertiles of the Lipid Accumulation Product (LAP tertiles: <19; 19-44; >44cm·mmol/L)

	Baseline LAP tertiles (cm·mmol/L; participants)			
	1st tertile (<19; n=660)	**2nd tertile** (19–44; n=680)	**3rd tertile** (>44; n=680)	***P***
CVD during 10-year follow-up, %yes	5	14*	25*	<0.001
Baseline characteristics				
Age (years)	36 ± 11	46 ± 13*	50 ± 13*	<0.001
Gender, % male	27	53*	67*	<0.001
Smoking (pack years)	304 ± 316	482 ± 487*	644 ± 550*	<0.001
Physical Activity, % physically active	49	40*	36*	<0.001
MedDietScore (range: 0-55)	29 ± 7.2	25 ± 5.8*	24 ± 5.9*	<0.001
Education (years of school)	13 ± 3.2	12 ± 3.7	11 ± 4.0*	<0.001
Body Mass Index (kg/m^2)	23 ± 2.7	26 ± 3.4	30 ± 4.4*	<0.001
Waist circumference (cm)	76 ± 8.6	92 ± 9.1*	103 ± 13*	<0.001
Waist-to-hip ratio	0.78 ± 0.08	0.87 ± 0.08*	0.93 ± 0.10*	<0.001
Waist-to-height ratio	0.52 ± 0.05	0.54 ± 0.05	0.60 ± 0.07*	0.007
Metabolic syndrome, %yes	1	12*	53*	<0.001
Hypertension, %yes	11	31*	46*	<0.001
Diabetes mellitus, %yes	1	3*	14*	<0.001
Hypercholesterolemia, %yes	18	43*	65*	<0.001
C-reactive protein (mg/L), median, tertiles	1.01, 1.08-2.56	1.08, 1.05-2.69*	1.80, 1.10-2.80*	<0.001
Interleukin-6 (pg/mL)	1.3 ± 0.36	1.5 ± 0.4*	1.6 ± 0.4*	<0.001
Tumor necrosis factor-α (pg/mL), median, tertiles	5.9, 2.9-4.6	6.1, 3.0-7.0*	7.8, 2.6-8.1*	<0.001

Normally and non-normally distributed continuous variables are presented as mean values ± standard deviation, or median and 1st, 3rd tertile, respectively, and categorical variables as frequencies. *p<0.01; p values for the between 1st *vs.* 2nd, 3rd tertile comparisons derived using the t-test, while for comparisons of categorical variables using the chi-square test, after correcting for the inflation of type-I error using the Bonferroni rule. MedDietScore: validated score evaluating the adherence to the Mediterranean diet (higher score indicates better adherence).

Table 3. Results from the applied Cox proportional hazard models evaluating the 10-year risk of developing a cardiovascular disease event (outcome) in relation to the baseline values of Lipid Accumulation Product (LAP).

All participants	**Hazard Ratios (HR)*, 95% Confidence Intervals**					
	Baseline Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Age (per 1 year)	1.06 (1.04-1.08)	1.08 (1.07-1.09)	1.06 (1.04-1.08)	1.06 (1.04-1.08)	1.05 (1.03-1.08)	1.06 (1.03-1.08)
Men *vs.* women	1.66 (1.06-2.61)	1.86 (1.36-2.55)	1.81 (1.10-3.01)	1.70 (1.01-2.88)	1.65 (0.98-2.77)	1.38 (0.76-2.52)
LAP (per 10 cm·mmol/L)	-	1.04 (1.00-1.08)‡	1.10 (1.05-1.15)‡	1.11 (1.04-1.19)‡	1.18 (1.09-1.27)‡	1.21 (1.11-1.31)‡
Pack-years of smoking	1.00 (1.00-1.01)	-	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (1.00-1.01)
Physically active *vs.* inactive	0.74 (0.49-1.11)	-	0.75 (0.48-1.18)	0.79 (0.49-1.26)	0.80 (0.50- 1.28)	0.63 (0.36-1.08)
Education (years of school)	0.98 (0.93-1.04)	-	0.98 (0.93-1.04)	0.97 (0.91-1.03)	0.97 (0.91-1.03)	0.95 (0.88-1.02)
MedDietScore (range: 0-55)	0.95 (0.92-0.99)	-	0.97 (0.93-1.01)	0.96 (0.92-1.00)	0.96 (0.92-0.99)	0.97 (0.92-1.02)
Hypertension (y/n)	1.06 (0.71-1.60)	-	1.17 (0.75-1.81)	1.36 (0.86-2.15)	1.34 (0.85-2.11)	1.23 (0.72-2.08)
Diabetes mellitus (y/n)	2.39 (1.33-4.29)	-	2.15 (1.11-4.18)	1.84 (0.91-3.74)	1.96 (0.97-3.93)	2.01 (0.87-4.67)
Hypercholesterolemia (y/n)	1.33 (0.90-1.97)	-	1.43 (0.92-2.22)	1.34 (0.84-2.13)	1.35 (0.85-2.12)	1.45 (0.86-2.42)
C-reactive protein (mg/L)	-	-	-	1.10 (1.02-1.19)	-	-
Interleukin-6 (pg/mL)	-	-	-	-	1.49 (0.86-2.59)	-
Tumor necrosis factor-α (pg/mL)	-	-	-	-	-	1.13 (1.05-1.22)

* Hazard Ratios derived from semi-parametric Cox proportional hazards models. ‡ p <0.05. MedDietScore: validated score evaluating the adherence to the Mediterranean diet (higher score indicates better adherence).

Table 4. Results from the nested Cox proportional hazards models evaluating the predictive value of the Lipid Accumulation Product (LAP) at baseline, as well as waist circumference, fasting triglyceride levels and other commonly used anthropometric indices of obesity, on the 10-year cardiovascular disease event risk (outcome).

	Hazard Ratios (HR) [b]	95% Confidence Intervals (CI)	-2logL[c]	AUC (95%CI)
Baseline Model 1[a]	-	-	704	0.80 (0.77-0.83)
Baseline Model 1[a] + LAP (per 1 cm·mmol/L)	1.01	(1.00-1.01)	584	0.84 (0.78-0.89)
Baseline Model 1[a] + Waist circumference (per 1 cm)	1.01	(0.99-1.02)	692	0.81 (0.76-0.85)
Baseline Model 1[a] + Fasting triglycerides (per 1 mg/dL)	1.00	(1.00-1.01)	592	0.82 (0.78-0.85)
Baseline Model 1[a] + Body mass index (per 1 kg/m^2)	1.03	(0.99-1.08)	701	0.80 (0.76-0.86)
Baseline Model 1[a] + Waist-to-hip ratio (per 1 unit)	1.33	(0.18-9.98)	687	0.80 (0.74-0.86)
Baseline Model 1[a] + Waist-to-height ratio (per 1 unit)	2.71	(0.21-5.45)	690	0.80 (0.76-0.83)

[a] Baseline Model 1 included age, sex, smoking (pack years), physical activity (active/inactive), years of education, Mediterranean diet adherence (MedDietScore: higher score indicates better adherence), and history of hypertension, diabetes mellitus, and hypercholesterolemia.

[b] Semi-parametric Cox proportional hazards models were used to calculate the hazard ratios.

[c] Goodness of fit for the logistic regression models was assessed with the -2logLikelihood (the lower the better).

AUC: Area Under the ROC Curve

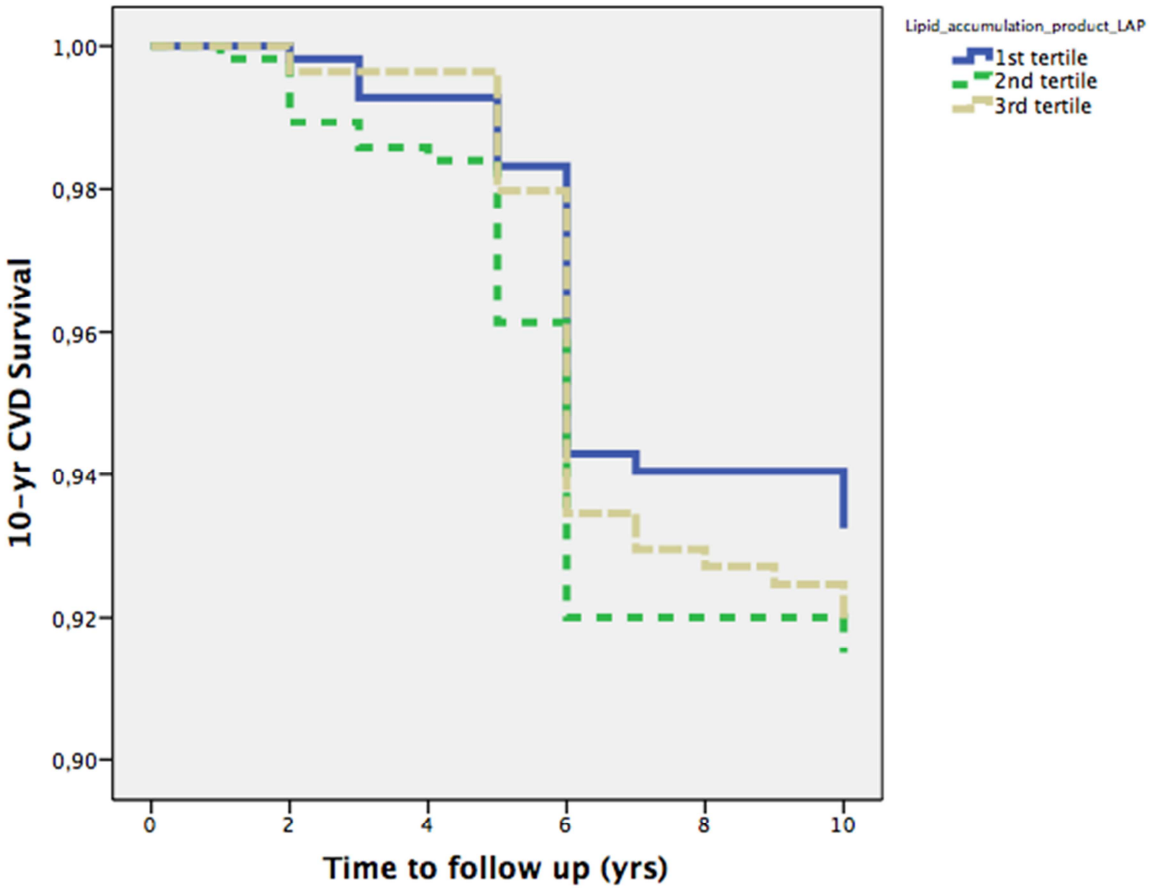

Figure 1. Survival curves derived from Cox proportional hazard models evaluating 10-year cardiovascular disease (CVD) risk by baseline Lipid Accumulation Product (LAP) tertile, in n=2020 participants of the ATTICA Study (*p* for Log-rank test = 0.04; inter-tertile comparisons: $p_{\text{1st vs. 3rd}} < 0.001$, $p_{\text{1st vs. 2nd}} < 0.001$, $p_{\text{2nd vs. 3rd}} = 0.77$).

Highlights

- LAP correlates to the 10-year CVD incidence in adults without pre-existing CVD
- LAP is associated to long-term CVD risk independently of other CVD risk factors
- LAP is a better predictor of 10-year CVD incidence than BMI, WC, WHR and WHtR