Carotid Artery Intima-Media Thickness Predicts Major Cardiovascular Events During 7-Year Follow-Up in 64-Year-Old Women Irrespective of Other Glucometabolic Factors

Carotid artery intima-media thickness predicts major cardiovascular events during 7-years of follow-up in 64-year-old women irrespective of other glucometabolic factors

Schmidt C, PhD, Bergström G, MD, PhD

Dept. of Molecular and Clinical Medicine, Wallenberg Laboratory for Cardiovascular and Metabolic Research, University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden

Corresponding author: Caroline Schmidt, Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska Academy at University of Gothenburg, S-413 45 Göteborg, Sweden

E-mail: caroline.schmidt@wlab.gu.se

Telephone: +46 31 342 23 56

Fax: +46 31 82 97 06

E-mail of co-author: goran.bergstrom@hjl.gu.se

ABSTRACT

Cardiovascular (CV) disease (CVD) is a leading cause of morbidity and mortality worldwide. Most CV events are caused by atherosclerosis. Diabetes (DM) and impaired glucose tolerance are associated with greater carotid intima-media thickness (IMT) and increased risk for CVD. The present study examined if common carotid artery (CCA) IMT (ccaIMT) is predictive of CVD irrespective of glucose tolerance category and glycated hemoglobin (HbA1c) in a sample of 639 women with different glucose tolerance categories. During 7-years follow-up, 30 events in the cardiac and 32 events in the cerebral territory were documented. Un-adjusted Cox hazard models showed that ccaIMT, glucose tolerance category and HbA1c were associated with increased risk. An adjusted and extended model, including ccaIMT, glucose tolerance category and HbA1c showed that ccaIMT was still associated with events with an almost unchanged hazard ratio. In conclusion, this study suggests that ccaIMT is predictive of major CV events during 7 years of follow-up, irrespective of glucose tolerance category, HbA1c and other established risk factors in a cohort of 64-year-old women.

Keywords: intima-media thickness, glucose tolerance, HbA1c, cardiovascular disease

INTRODUCTION

Cardiovascular (CV) disease (CVD) is a leading cause of morbidity and mortality worldwide [1]. Most CV events, such as myocardial infarction (MI) and stroke, are caused by atherosclerosis [2]. The intima-media thickness (IMT) in the carotid artery, which can be measured by ultrasound, is considered a biomarker of atherosclerosis [3]. Studies have shown that carotid IMT is associated with increased risk of MI and stroke, independently of conventional CV risk factors [4-6] .

Individuals with diabetes (DM) and those with glucose levels below the diabetic range such as impaired glucose tolerance (IGT) may exhibit an increased carotid IMT [7-12]. In individuals with pre-diabetes who developed DM, internal carotid IMT is significantly higher than in individuals who remained free from DM [13]. Further, DM and IGT have both been shown to be associated with increased risk for CVD [14-17]. The risk of CV events has been shown to increase progressively in men and women in parallel with glucose tolerance raging from normal to newly diagnosed DM [18]. Although women experience relative protection from CVD compared with men, women are known to exhibit an increased risk of atherosclerosis-related CVD after menopause and diabetes blunts the benefit of female gender [19, 20]. Moreover, glycated hemoglobin (HbA1c) has been shown to be associated with increased carotid IMT and CV events in different populations [21-23]. However, little is known about the combined influence of carotid IMT, glucose tolerance category and HbA1c levels on future major CV events.

The objective of the present study was to investigate if common carotid artery (CCA) IMT (ccaIMT) is predictive of CVD irrespective of glucose tolerance category and HbA1c.

RESEARCH DESIGN AND METHODS

The study cohort has previously been described [24]. From a total of 4856, 64-year old women living in Gothenburg, Sweden, a sample of 639 women with different glucose tolerance categories i.e. DM (36.5%), IGT (32.6%) and normal glucose tolerance (NGT) (29.7%) were recruited. Exclusion criteria were recent cancer diagnosis, chronic inflammatory disease, severe mental disorder, other severe illness, drug addiction, or not being able to understand Swedish. Participants were invited to a screening examination including an Oral Glucose Tolerance Test (OGTT). Women with known DM who were treated with oral anti-diabetic drugs or insulin were examined without preceding OGTTs, whereas women with diet treated diabetes and fasting blood glucose (FBG) < 7.5 mmol/L were examined with OGTTs. Women fulfilling the criteria for DM or IGT were re-examined within 2 weeks with a repeated OGTT [24].

All participants received both written and oral information before they gave their consent to take part in the study. The protocol was approved by the Ethics Committee at Sahlgrenska University Hospital.

Measurements

The examinations also included questionnaires regarding previous diseases, current medication, smoking habits and heredity for DM. Anthropometric measurements were performed, and blood pressure and heart rate were recorded. Body weight was measured in light clothing. Waist and hip circumferences were measured according to current guidelines. Blood pressure was measured in the right arm using a cuff of appropriate size after at least 5 min of rest. The mean of two recording was used [24].

Biochemical analysis

At screening capillary blood glucose was measured with a glucose oxidase technique. After inclusion, venous blood samples were drawn, and serum and plasma were frozen in aliquots at -70° within 4 h.

Insulin was assayed at the Department of Clinical Biochemistry Addenbrooke's NHS Trust (Cambridge, UK) [25]

C-reactive protein was measured by a photometric immunoturbidimetric test (Orion Diagnostica, Espoo, Finland). Triglyceride levels were determined by fully enzymatic techniques (Thermo Clinical Labsystems, Espoo, Finland). High density lipoprotein cholesterol (HDL-C) was determined after precipitation of apolipoprotein B (apoB)-containing lipoproteins with magnesium sulfate and dextran sulfate (Thermo Clinical Labsystems) [25]. Apolipoprotein A-I (apoA-I) and apoB were measured on a Konelab 20 Auto-analyzer (Thermo Scientific, Vantaa, Finland) using a turbidimetric method according to the manufacturer's instructions. Using 2 different controls, the between-assay variation for repeated measurements were 5.2 and 5.8% for apoA-I, and 2.5 and 3.2% for apoB, respectively. Corresponding figures for within assay variation were 1.4 and 1.7% for apoA-I, and 1.4 and 1.4% for apoB, respectively [26]. Serum levels of intercellular adhesion molecule (ICAM)-1 were measured using a high-sensitive enzyme-linked immunosorbent assay (ELISA) kit (R&D system, Europe Ldt., Abingdon, UK).

Ultrasound examination

Examinations were performed with an ultrasound scanner equipped with a linear 8L5-MHz transducer (Sequoia 512, Siemens, Mountain View, CA, USA). An electrocardiographic signal (lead II) was simultaneously recorded to synchronize the image capture to the top of the R-wave to minimize variability during the cardiac cycle. Images for measurement of IMT were recorded from 10 mm long sections of the far wall in the right and left CCA. Two images were recorded from each side [27]. An analyzing system based on automatic detection of the echo structures, with the option to make manual corrections by the operator was used for ccaIMT measurements [28]. The average of 4 images was calculated.

CV events

CV events during 7-years of follow-up were defined as CV death or non-fatal MI, non-fatal stroke, or revascularization procedures (PCI or CABG). The events and cause of death were collected searching The Swedish national inpatient register (IPR) after contact with the Centre of Epidemiology at the National Board of Health and Welfare. The IPR has a high external and internal validity for CVD [29].

Statistical analysis

Statistical analyses were performed using PASW Statistics 18 (SPSS Inc., Chicago, Illinois). Results are presented as mean ± standard deviation, unless otherwise indicated. Univariate comparisons between groups were performed using t-test or Chi-square-test. Cox proportional hazard regression model was used to calculate hazard ratios (HR) for major events in relation to ccaIMT (entered as quartiles), to categories of glucose tolerance i.e. NGT, IGT or DM and to HbA1c. Significantly different variables in univariate analyses were entered as co-variates. Variables that were highly correlated ≥ 0.80 were not allowed in the same multivariate analysis. Results from the multivariate analyses are expressed as the β coefficient with 95% confidence intervals (CIs). A two-tailed $p < 0.05$ was considered significant.

RESULTS

In this cohort of 64-year-old women, 62 major CV events (9.7%) were documented during 7-years of follow-up: 15 cases of MI, 15 cases with revascularization procedures and 32 cases of stroke.

Baseline characteristics

Univariate comparisons showed significant differences between the event group and the non-event group (Table 1). The ccaIMT was larger and DM was more common in the event group than in the non-event group. Further, the event group had higher levels of waist-hip-ratio, HbA1c, triglycerides, apoB/apoA-I-ratio, serum-ICAM-1 and cigarette years and lower levels of HDL-C than the non-event group.

Cox proportional hazard regression analysis

Cox proportional hazard regression was used to estimate the risk for major CV events (Table 2). Un-adjusted models showed that ccaIMT, glucose tolerance category and HbA1c were associated with increased risk. In models adjusted for waist-hip-ratio, systolic blood pressure, apoB/apoA-I ratio, triglycerides, HDL-C, serum-ICAM and cigarette years, only ccaIMT remained associated with increased risk for events. Further, an adjusted and extended model, including ccaIMT, glucose tolerance category and HbA1c showed that ccaIMT was still associated with events with an almost unchanged hazard ratio.

DISCUSSION

Little is known about the combined influence of carotid-IMT, glucose tolerance category and HbA1c on major CV events. Our study showed that increased baseline ccaIMT is associated with an increase in MI, revascularization procedures and stroke during 7 years of follow-up after controlling for glucose tolerance category, HbA1c and other risk factors in a cohort of 64-year-old women. The numbers of events occurring in the cardiac and in the cerebral territory were comparable. Univariate analyses showed that the event-group had a more unfavorable CV risk profile with increased waist-hip-ratio, triglycerides, apoB/apoA-I ratio, serum-ICAM, and cigarette-years and lower HDL-C than the non-event group.

We showed that the ccaIMT measured at baseline was associated with CV events irrespective of other risk factors in this group of women with normal glucose tolerance, prediabetes or DM. This is similar with results from the PROG-IMT collaboration, which showed that single-time carotid IMT measurement was positively and robustly associated with CV risk in the general population as well as in people with DM [30, 31].

HbA1c was the only glucose feature that was significantly increased in the event-group. Previously, HbA1c has been shown to be associated with coronary atherosclerosis, MI and stroke both in individuals with and without DM. Further, one study showed that HbA1c was the only glucometabolic factor associated with coronary artery severity in non-diabetic individuals [22, 32, 33]. Abnormalities in the gluco-metabolism have been suggested to progressively worsen CV health and the first step is endothelial dysfunction [34]. Results from two new studies have shown that abnormal gluco-metabolism is associated with increases in ccaIMT in middle-aged individuals with overweight as well as in young patients who are morbidly obese [35, 36]. Also, lipid profile is deranged in individuals with disturbed glucose metabolism even before DM is diagnosed [37]. Together, these factors will have effect on the IMT and an increase in IMT can be seen several years before an event occurs [38].

Results from the present study showed waist-to-hip ratio to be larger in the event group, which is consistent with previous studies [39-41]. We also showed that the event group had significantly lower HDL-C and higher triglyceride levels and apoB/apoA-I ratio which are in agreement with other studies [42-46]. HDL-C has been observed to have greater impact in women and low HDL-C has been associated with MI to a greater extent in women. A recent study showed that increased levels of HDL-C were predictive of absence of carotid atherosclerosis in middle-aged women [47]. Further, we showed that the event-group had more cigarette-years than the non-event group. Several studies have shown that smoking increases the progression of atherosclerosis and is a major cause of CVD [48, 49].

Some limitations need to be addressed: only females in a certain age and from a limited area were studied. Therefore, the results may not translate to other age-groups, areas or males. Also, we only measured IMT in the CCA, which may underestimate the value of IMT, because IMT progresses more rapidly in the carotid bulb [50]. However, the study approach has several strengths: we studied a homogeneous group of women, which rules out confounding factors such as age and gender and the advantage of using IMT in the CCA only is that it is easily visualized in almost all individuals and can be measured with low-variability [51, 52]. Further, measurements of ccaIMT only has been shown to be predictive for prevalence and severity of coronary atherosclerosis in patients without a history of coronary artery disease (CAD) [53] and has been found to be as good as the mean IMT of all carotid segments in prediction of CAD risk [54].

In conclusion, this study suggests that ccaIMT is predictive of major CV events during 7 years of follow-up, irrespective of glucose tolerance category, HbA1c and other established risk factors in a population-based cohort of 64-year-old women. How this finding applies to the general population needs to be confirmed in a larger study with both genders and with a broader age-range.

FUNDING

Grants from the Swedish Heart-Lung Foundation and the Swedish Medical Research Council (12270 and 10880) supported this work. Astra Zeneca, Mölndal, Sweden funded the work without any obligations from our research group.

ACKNOWLEDGEMENT

The authors thank the staff at the Wallenberg Laboratory for Cardiovascular Research (Marie Louise Ekholm, Carita Fagerlund, Magdalena Göthberg, Birgitta Jannemark, Marie Jonasson, Pia Lindén and Ulrica Prahl Abrahamsson) for technical assistance.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

REFERENCES

1) Global status report on non-communicable diseases 2010. Geneva, World Health Organization, 2011.

2) Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation.* 2002;105(9):1135-1143.

3) Stein JH, Korcarz CE, Hurst RT. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: A consensus statement from the American society of Echocardiography carotid intima-media thickness task force. *J Am Soc Echocardiography*. 2008; 21(2): 376-376

4) Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness. A systematic review and meta-analysis. *Circulation.* 2007;115(4):459-467.

5) O'Leary, D.H. Polak, J.F. Intima-media thickness: a tool for atherosclerosis imaging and event prediction. *Am J Cardiol*. 2002; 90(10c): 18L-21L.

6) Ebrahim, S, Papacosta, O, Whincup, P, Wannamethee G, Walker M, Nicolaides AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. *Stroke*. 1999; 30(4): 841-850.

7) Bonora E, Tessari R, Micciolo R, Zenere M, Targher G, Padovani R, et al. Intimal-medial thickness of the carotid artery in non-diabetic and NIDDM patients. Relationship with insulin resistance. *Diabetes Care.* 1997;20(4):627-631.

8) Wagenknecht LE, DÁgostino RB Jr, Haffner SM, Savage PJ, Rewers M. Impaired glucose tolerance, type 2 diabetes, and carotid wall thickness: the Insulin Resistance Atherosclerosis Study. *Diabetes Care.* 1998;21(11):1812-1818.

9) Guvener N, Tutuncu NB, Oto A, Erbas T. Major determinants of the carotid intima-media thickness in type 2 diabetic patients: age and body mass index. *Endocr J.* 2000;47(5):525-533.

10) Sigurdardottir V, Fagerberg B, Hulthe J. Preclinical atherosclerosis and inflammation in 61-year-old men with newly diagnosed diabetes and established diabetes. *Diabetes Care.* 2004;27(4):880-884.

11) Temelkova-Kurktschiev TS, Koehler C, Leonhardt W, Schaper F, Henkel E, Siegert G, et al. Increased intimal-medial thickness in newly detected type 2 diabetes: risk factors. *Diabetes Care.* 1999;22(2):333-338.

12) Henry RM, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Kamp O, et al. Carotid artery remodelling: a maladaptive phenomenon in type 2 diabetes but not impaired glucose metabolism: the Hoorn study. *Stroke.* 2004;35(3):671-676.

13) Hunt KJ, Williams K, Rivera D, O´Leary DH, Haffner SM, Stern MP, et al. Elevated carotid artery intima-media thickness levels in individuals who subsequently develop type 2 diabetes. *Arterioscler Thromb Vasc Biol.* 2003;23(10):1845-1850.

14) Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. *Nature Clin Pract Endocrinol Metab.* 2009;5(3):150-159.

15) Tominaga M, Igarashi K, Eguchi H, Kato T, Manaka H, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funaga diabetes study. *Diabetes Care.* 1999;22(6):920-924.

16) The DECODE study group, on behalf of the European Diabetes Epidemiology Group. Glucose tolerance and mortality: comparison of WHO and American Diabetic Association diagnostic criteria. *Lancet.* 1999;344(9179):1343-1350.

17) Emerging Risk Factors Collaboration. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet.* 2010;375(9733):2215-2222.

18) Anand SS, Dagenais GR, Mohan V, Diaz R, Probstfield J, Freeman R, et al, on behalf of the EpiDream Investigators. Glucose levels are associated with cardiovascular disease and

death in an international cohort of normal glycaemic and dysglycaemic men and women: the EpiDREAM cogort study.*Eur J Prev Cardiol.* 2011;19(4):755-764.

19) Pitha J, Auzký O, Kovár J, Lejsková M, Adámková S, Babková E, et al. Changes in cardiovascular risk profile in women after menopause (Prague Pre and Post Menopausal female study). *Cor Vasa.* 2014;56(2):e113-e117.

20) Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis. Epidemiology, pathophysiology and management. *JAMA.* 202 (19);287:2570-2581.

21) Larsen JR, Brekke M, Bergengen L, Sandvik L, Arnesen H, Hanssen KF, et al. Mean HbA1c over 18 years predicts carotid intima-media thickness in women with type 1 diabetes. *Diabetologia*. 2005;48 (4):776-779.

22) Rivera JJ, Choi EK, Yoon YE, Chun EJ, Choi SI, Nasir K, et al. Association between increasing levels of hemoglobin A1c and coronary atherosclerosis in asymptomatic individuals without diabetes mellitus. *Coron Artery Dis.* 2010;21(3):157-163.

23) EegOlofsson K, Cederholm J, Nilsson P, Zethelius B, Svensson AM, Gudbjörnsdóttir S, et al. New aspects of HbA1c as a risk factor for cardiovascular diseases in type 2 diabetes: an observational study from the Swedish National Diabetes Register (NDR). *JIM.* 2010;268(5):471-482.

24) Brohall G, Behre CJ, Hulthe J, Wikstrand J, Fagerberg B. Prevalence of diabetes and impaired glucose tolerance in 64-year-old Swedish women: experiences of using repeated oral glucose tolerance tests. *Diabetes Care.* 2006;29(2):363-367.

25) Englund Ögge L, Brohall G, Behre CJ, Schmidt C, Fagerberg B. Alcohol consumption in relation to metabolic regulation, inflammation, and adiponectin in 64-year-old Caucasian

women: a population-based study with a focus on impaired glucose regulation. *Diabetes Care.* 2006;29(4):908-913.

26) Wallenfeldt K, Bokemark L, Wikstrand J, Hulthe J, Fagerberg B. Apolipoprotein B/apolipoprotein A-I in relation to the metabolic syndrome and change in carotid artery intima-media thickness during 3 years in middle-aged men. *Stroke.* 2004;35(10):2248-2252.

27) Prahl U, Wikstrand J, Bergström GM, Behre CJ, Hulthe J, Fagerberg B. Slightly elevated high-sensitivity C-reactive protein (hsCRP) concentrations are associated with carotid atherosclerosis in women with varying degrees of glucose tolerance. *Angiology.* 2010;61(8): 793-801.

28) Wendelhag I, Liang Q, Gustavsson T, Wikstrand J. A new automated computerized analyzing system simplifies readings and reduces the variability in ultrasound measurements of intima-media thickness. *Stroke.* 1997;28(11):2195-2200.

29) Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health.* 2001;11:450-455.

30) Lorenz MW, Polak JF, Kavousi M, Mathisesen EB, Völzke H, Toumainen T-P, et al on behalf of the PROG-IMT Study Group. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. *Lancet.* 2012;379(9831**)**:2053-2062.

31) Lorenz MW, Price JF, Robertson C, Bots ML, Polak JF, Poppert H, et al. Carotid intima-media thickness progression and risk of vascular events in people with diabetes: results from the PROG-IMT Collaboration. *Diabetes Care.* 2015;38(10**)**:1921-1929.

32) Deo RK, Karki P, Sharma SK, Achatya P. Association of cardiovascular events with glycosylated haemoglobin in diabetic patients. *Katmandu Univ Med J.* 2008;6(24):476-485.

33) Arbel Y, Zlotnik M, Halkin A, Havakuk O, Berliner S, Herz I, Rabinovich I, Keren G, Bazan S, Finkelstein A, Banai S. Admission glucose, fasting glucose, HbA1c levels and the SYNTAX score in non-diabetic patients undergoing coronary angiography. *Clin Res Cardiol.* 2014;103(3):223-227.

34) Avogaro A, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes. The role of reparatory mechanisms. *Diabetes Care.* 2011;34:s285-s290.

35) Altin C, Sade LE, Gezmis E, Ozen N, Duzceker O, Bozbas H, et al. Assessment of subclinical atherosclerosis by carotid intima-media thickness and epicardial adipose tissue thickness in prediabetes. *Angiology.* 2016; Apr 10. pii: 0003319716643669.

36) Sirbu A, Nicolae H, Martin S, Barbu C, Copaescu C, Florea S, et al. IGF-I and insulin resistance are major determinants of common carotid artery thickness in morbidly obese young patients. *Angiology.* 2016;67(3):259-265.

37) Calanna S, Scicali R, Di Pino A, Knop FK, Piro S, Rabuazzo AM, et al. Lipid and liver abnormalities in haemoglobin A1c-defined prediabetes and type 2 diabetes. *Nutr Metab Cardiovasc Dis.* 2014;24(6):670-676.

38) van den Oord SCH, Sijbrands EJG, ten Kate GL, van Klaveren D, van Domburg RT, van der Steen AF, et al. Carotid intima-media thickness for cardiovascular risk assessment: Systematic review and meta-analysis. *Atherosclerosis.* 2013;228(1):1-11.

39) de Koning L, Merchant AT, Pouge J, Anand SS. Waist circumference and waist to hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *Eur Heart J.* 2007;28(7):850-856.

40) Zhang C, Rexrode KM, van Dam RM, Li TY, Hu FB. Abdominal obesity and the risk of all-cause, cardiovascular, and cancer mortality. Sixteen years of follow-up in US women. *Circulation.* 2008;117(13):1658-1667.

41) Gelber RP, Gaziano JM, Orav EJ, Manson JE, Buring JE, Kurth T. Measures of obesity and cardiovascular risk among men and women. *JACC.* 2008;52(8):605-615.

42) Miller M. Dyslipidemia and cardiovascular risk: the importance of early prevention. *QJ Med.* 2009;102(9):657-667.

43) Toth PP, Barter PJ, Rosenson RS, Boden WE, Chapman MJ, Cuchel M, et al. High-density lipoproteins: a consensus statement from the National Lipid Association. *J Clin Lipidol.* 2013;7(5):484-525.

44) Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation.* 2014;129(25) (suppl 2):S49-S73.

45) Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA.* 2007;298(3):299-308.

46) Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA.* 2008;300(18):2142-2152.

47) Triantafyllidi H, Pavlidis G, Trivilou P, Ikonomidis I, Tzortzis S, Xenogiannis I, et al. The association of elevated HDL levels with carotid atherosclerosis in middle-aged women with untreated essential hypertension. *Angiology.* 2015;66(10):904-910.

48) Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, et al. Endothelial function and dysfunction. Part II: association with cardiovascular risk factors and diseases. A statement by the Working Group on endothelins and endothelial factors of the European society of hypertension. *J Hypertens.* 2005;23(2):233-246.

49) Burn DM. Epidemiology of smoking-induced cardiovascular disease. *Progr Cardiovasc Dis.* 2003;46(1):11-29.

50) Onut R, Balanescu S, Constantinescu D, Calmac L, Marinescu M, Dorobantu M. Imaging atherosclerosis by carotid intima-media thickness in vivo: How to, where and in whom? *Maedica.*2012;7(2):153-162

51) Bauer M, Caviezel S, Teynor A, Erbel R, Mahabadi AA, Schmidt-Trucksäss A. Carotid intima-media thickness as a biomarker of subclinical atherosclerosis. *Swiss Med Wkly.* 2012;142:w13705.

52) Chambless LE, Heiss G, Folsom AR, Rosamond W, Szloko M, Sharrett AR, Clegg LX. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) study. 1987-1993. *Am J Epidemiol.* 1997;146(6):483-494.

53) Jeevarethinam A, Venuraju S, Weymouth M, Atwal S, Lahiri A. Carotid intimal thickness and plaque predict prevalence and severity of coronary atherosclerosis: a pilot study. *Angiology.* 2015;66(1):65-69.

54) Nambi V, Chambless L, He M, Folsom AR, Mosley T, Boerwinkle E et al. Common carotid artery intima-media thickness is as good as carotid intima-media thickness of all carotid segments in improving prediction of coronary heart disease risk in the Atherosclerosis Risk in Communities (ARIC) study. *Eur Heart J.* 201;33:183-190.

Table 1. Characteristics of the women included in the study according to group

	No event (n=577)	**Event (n=62)**	**p-value**
Body Mass Index (kg/m^2)	27.6 ± 4.7	28.1 ± 4.5	0.428
Waist-hip-ratio	0.87 ± .06	0.90 ± 0.06	0.003
Systolic Blood Pressure (mmHg)	139 ± 19	143 ± 21	0.138
Diastolic Blood Pressure (mmHg)	78 ± 9	77 ± 9	0.765
Heart Rate (bpm)	65 ± 10	64 ± 9	0.612
Fasting Plasma Glucose (mmol/L)	6.2 ± 1.7	6.5 ± 1.6	0.213
Plasma Glucose 2-h post-OGTT (mmol/L)	9.4 ± 2.8	10.0 ± 2.9	0.283
HbA1c (%)	5.0 ± 1.0	5.6 ± 1.4	<0.001
Total cholesterol (mmol/L)	5.8 ± 1.1	5.8 ± 1.1	0.601
LDL-cholesterol (mmol/L)	3.5 ± 1.0	3.5 ± 1.1	0.826
HDL-cholesterol (mmol/L)	1.6 ± 0.4	1.5 ± 0.4	0.017
Triglycerides (mmol/L)*	1.3 ± 0.8	1.5 ± 0.9	0.047
Apolipoprotein B (g/L)	1.1 ± 0.3	1.2 ± 0.3	0.084
Apolipoprotein A-I (g/L)	1.6 ± 0.3	1.5 ± 0.3	0.316
ApoB/ApoA-I ratio	0.74 ± 0.21	0.81 ± 0.26	0.019
Serum-ICAM-1 (ng/ml)	271 ± 93	299 ± 100	0.023
Diabetes Mellitus n, (%)	196 (34)	37 (60)	<0.001
Impaired Glucose Tolerance n, (%)	194 (34)	14 (23)	0.622
Normal Glucose Tolerance n, (%)	187 (32)	11 (18)	0.622
Smoking (yes) n (%)	106 (18.4)	17 (28.3)	0.138
Cigarette years	18.0 ± 15.8	24.5 ± 15.0	0.024
Common Carotid Intima-Media Thickness (mm)	0.86 ± 0.17	0.94 ± 0.19	0.001

*Geometric mean

Table 2. Cox proportional hazard regression analysis of cardiovascular events during 7-year of follow-up in 64-year old women.

	Model 1 β (95% CI)	**Model 2** β (95% CI)	**Model 3** β (95% CI)
ccaIMT p value	1.6 (1.2 to 2.0) p<0.001	1.5 (1.1 to 2.1) p=0.025	1.48 (1.04 to 2.20) p=0.033
Glucose Tolerance Category p value	2.0 (1.4 to 2.7) p<0.001	1.7 (1.0 to 3.2) p=0.074	
HbA1c P value	1.5 (1.2 to 1.8) p<0.001	1.3 (0.9 to 1.8) p=0.12	

Model 1: un-adjusted.

Model 2: adjusted for waist-hip-ratio, systolic blood pressure, apoB/apoA-I ratio, triglycerides, HDL-C, serum-ICAM-1, and cigarette years

Model 3: adjusted for waist-hip-ratio, systolic blood pressure, apoB/ apoA-I ratio, triglycerides, HDL-C, serum-ICAM-1, cigarette years, HbA1c and glucose tolerance category

Abbreviations: Apolipoprotein B (apoB), Apolipoprotein A-I (apoA-I), High density lipoprotein-cholesterol (HDL-C), Intercellular adhesion molecule (ICAM), Glycated hemoglobin (HbA1c)