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► To cite this version:

Stéphanie Debette, Nathalie Leone, Dominique Courbon, Jérôme Gariépy, Christophe Tzourio, et al.. Calf circumference is inversely associated with carotid plaques.. Stroke, American Heart Association, 2008, 39 (11), pp.2958-65. <10.1161/STROKEAHA.108.520106>. <inserm-00271521>

HAL Id: inserm-00271521

<http://www.hal.inserm.fr/inserm-00271521>

Submitted on 11 May 2009

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Calf circumference is inversely associated with carotid plaques

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Acknowledgments

The 3C Study has been conducted under a partnership agreement between the Institut National de la Santé et de la Recherche Médicale (INSERM), the Victor Segalen – Bordeaux II University, and Sanofi-Aventis. The Fondation pour la Recherche Médicale funded the preparation and initiation of the study.

The 3C-Study is also supported by the Caisse Nationale Maladie des Travailleurs Salariés, Direction Générale de la Santé, MGEN, Institut de la Longévité, Conseils Régionaux of Aquitaine and Bourgogne, Fondation de France, the Institut de Santé Publique, d'Épidémiologie et de Développement (ISPED, Bordeaux), Ministry of Research - INSERM Programme "Cohortes et collections de données biologiques".

Mahmoud Zureik was a recipient of a "Contrat d'Interface" grant from INSERM - Centre Hospitalier Universitaire de Lille

Summary

Background: The association of carotid atherosclerosis with body composition and fat distribution is poorly understood. We aimed to test the cross-sectional association of carotid plaques and common carotid artery intima-media thickness (CCA-IMT) with calf circumference (CC), representing peripheral fat and lean mass, and with waist circumference (WC) and waist-to-hip ratio (WHR), two markers of abdominal obesity.

Methods: The study was performed on 6265 subjects aged ≥ 65 years, recruited prospectively from the electoral rolls of three French cities. Ultrasound examination and anthropometric measures were performed according to a standardized protocol.

Findings: Carotid plaques were less frequent with increasing CC, the odds ratios for the second, third and fourth quartile of CC compared to the first quartile being 0.98 (95%CI:0.84-1.15), 0.85 (95%CI:0.72-1.01), and 0.71 (95%CI:0.58-0.86) (p for trend = 0.0002), independently of age, gender, body mass index (BMI), and other vascular risk factors. There was an opposite and multiplicative effect of CC and WHR on the frequency of carotid plaques (55.1% of individuals in the fourth WHR quartile and the first CC quartile had carotid plaques, against 31.8% in the first WHR and the fourth CC quartile). Mean CCA-IMT was larger with increasing WC, WHR and CC, but the association with CC disappeared after adjusting for BMI.

Interpretation: The present study shows, for the first time, an inverse relationship between carotid plaques and calf circumference. Although this needs to be confirmed in other populations, it may suggest an antiatherogenic effect of large calf circumference.

Introduction

There is growing evidence that body composition and fat distribution are of major importance in determining vascular risk, while global body mass may not be such a good predictor of atherosclerosis and vascular events ^{1, 2}. Coronary heart disease and vascular events such as stroke, myocardial infarction (MI) and cardiac death appear to be strongly associated with abdominal obesity and upper versus lower fat localization ³⁻⁵. More recently, peripheral adiposity was found to be negatively associated with markers of atherosclerosis such as aortic calcifications ⁶, coronary angiography score ⁷ and arterial stiffness ⁸, and it has been suggested that peripheral fat mass may exhibit antiatherogenic effects.

Carotid plaques and elevated carotid intima-media thickness (IMT) are powerful and independent predictors of ischemic stroke, coronary events and vascular mortality ⁹⁻¹², that can easily and non-invasively be assessed in large population samples and can be an interesting tool for the follow-up of prevention strategies. The association of these carotid parameters with markers of body composition and fat distribution has not been fully explored yet. Indeed, several studies have demonstrated an association between carotid IMT and anthropometric markers of abdominal obesity (waist circumference, waist-to-hip ratio, sagittal/transverse ratio) ¹³⁻²¹, but very little data is available on the association of those markers with carotid plaques ²¹, a more advanced marker of carotid atherosclerosis than IMT. It is also poorly understood whether markers of body composition other than abdominal obesity, such as calf circumference ²², are associated with carotid atherosclerosis.

The aim of the present study was to explore more in detail the association of carotid atherosclerosis with several anthropometric markers of body composition in a large population-based sample of elderly subjects. Our two major interests were (i) to assess the relationship of carotid plaques with two common markers of abdominal obesity, waist circumference (WC) and waist-to-hip ratio (WHR) ²³⁻²⁵, as published data on this subject is insufficient, and (ii) to test whether calf circumference (CC), a

rarely used anthropometric marker representing lean mass and peripheral fat, is associated with carotid plaques or IMT, which has never been evaluated before.

Materials and methods

Study population and design

This study was performed within the Three-City (3C) Study, a three-center prospective cohort study, whose design has been described in detail elsewhere ²⁶. To be eligible for the study, persons had to be (i) living in the French cities of Bordeaux, Dijon, Montpellier or their suburbs and registered on the electoral rolls, (ii) aged 65 years or over, and (iii) non institutionalized. Twenty-four percent of the eligible persons selected on the electoral rolls (n=34922) could not be reached; among those contacted, the acceptance rate was 37%, which is similar to the acceptance rate of most population-based prospective cohort studies. A total of 9,693 persons were included between March 1999 and March 2001. Three-hundred and ninety-nine persons were subsequently excluded, seven because they were aged less than 65 years and 392 because they refused to participate in the interview. A baseline ultrasound examination of the carotid arteries was proposed to participants under the age of 85 who were able to come to the examination centers. Due to logistic concerns, the ultrasound examination was not offered to persons included during the last 4 months of subject recruitment. Overall, 73.7% of the participants younger than 85 years (n=6631) had carotid ultrasound measures. As expected, compared with subjects who did not undergo ultrasound examinations, subjects who were able to come to the examination centers to undergo ultrasound examinations had lower means of age (73.5 ± 4.9 versus 74.5 ± 5.1 years; $P<0.001$), body mass index (25.6 ± 4.2 versus 25.9 ± 4.0 kg/m²; $P=0.01$), systolic blood pressure (145.2 ± 21.1 versus 151.0 ± 23.0 mm Hg; $P<0.001$), and total cholesterol (5.77 ± 0.97 versus 5.96 ± 1.03 mmol/L; $P<0.01$). Due to a few missing data for the variables of interest, the present study was performed in 6265 subjects.

Ultrasound examination

For the ultrasound examination, the B-mode system (Ultramark 9 High Definition Imaging) with a 5- to 10-MHz sounding was used at each of the three centers, and a centralized reading was performed according to a standardized protocol. The procedure has been described in detail elsewhere²⁷. The examination involved scanning of the common carotid arteries, the carotid bifurcations, and the origin of the internal carotid arteries. The near and far walls of these arterial segments were scanned longitudinally and transversally to assess, at the time of the examination, the presence of plaques. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the distance between the media-adventitia interface and the internal side of the lesion was ≥ 1 mm, on the common carotid arteries, the carotid bifurcations and the internal carotid arteries. For IMT measurement, far and near walls of the right and the left common carotid arteries (CCAs) 2 to 3 cm proximal to the bifurcation were imaged. For each side, at least one optimal longitudinal image was frozen in end-diastole by electrocardiogram R-triggering. All frozen images were transferred to a computer system (IôTEC) and digitized into 640x580 peak cells with 256 gray levels²⁸. They were stored on CD-ROMs that were sent to the reference center weekly. The IMT was measured at a site free of any discrete plaques along a 10-mm-long segment of the far wall of the CCA as the distance between the lumen-intima interface and the media-adventitia interface. On average, 75 measurements were automatically performed on each image and on each side and a mean CCA-IMT value was computed for each side. The CCA-IMT value used in the analyses was a mean of right and left mean values. Lumen diameter was defined as the average of the distances between the 2 leading edges of far wall and near wall lumen-intima interfaces along at least 0.5 cm of length using a computerized validated program, as described previously²⁷.

All ultrasounds were read at a Reference Reading Center (Hôpital Broussais, Paris) by one trained reader. To ensure reliability and validity of these measurements, programs of centralized training and regular quality-control were implemented for the sonographers (n=7) and the reader. Besides, a

reproducibility study was performed ²⁷. One hundred fourteen subjects underwent 2 ultrasound examinations performed blindly by 2 different sonographers during the same visit. The mean absolute difference and correlation coefficient between repeated examinations of CCA-IMT were, respectively, 0.06 mm and 0.71. For carotid plaques, the Kappa coefficient for agreement between the 2 examinations was 0.78.

Medical history and standard biological parameters

Information about demographic background, medical history and personal habits was collected during a face-to-face interview using a standardized questionnaire administered by trained nurses.

Personal history of vascular disease was defined as a history of stroke, myocardial infarction, angina pectoris, coronary surgery or angioplasty. Physical activity was defined as a three-class variable: *light* physical activity for subjects practicing no sport (hiking, aerobics, swimming...) and walking less than 1 hour per day, *mild* physical activity for subjects practicing sport regularly but less than once a week or for subjects practicing no sport and walking at least 2 hours per day, and *moderate to high* physical activity for subjects practicing sport at least once a week or subjects practicing some sport but less than once a week and walking at least 2 hours per day. Information on physical activity was available for 2 of the 3 centers only, in 4612 subjects. Baseline blood pressure was measured twice in a sitting position using a digital tensiometer (OMRON M4).

Centralized measurements of biological parameters were performed. Fasting total cholesterol, HDL-cholesterol, triglyceride and glucose levels were measured at baseline. LDL-cholesterol was calculated according to the Friedewald formula ($LDL = \text{total cholesterol} - HDL - [\text{triglycerides}/2.2]$) and was considered as missing for triglyceride values > 4.5 mmol/l. For the statistical analyses a log-transformation of the triglyceride levels was performed. Hypertension was defined as a systolic blood pressure ≥ 160 mmHg and/or a diastolic blood pressure ≥ 95 mmHg and/or a blood pressure lowering

therapy, hypercholesterolemia as a total cholesterol ≥ 6.20 mmol/l and/or a cholesterol lowering therapy, diabetes mellitus as a fasting glucose > 7 mmol/l and/or an antidiabetic therapy.

Baseline body-mass index (BMI) was calculated as the ratio of weight (kg) to the square of height (m^2). Anthropometric measures were performed at baseline using a non-elastic but flexible plastic tape. Calf circumference was measured on the left leg (or the right leg for left-handed persons) in a sitting position with the knee and ankle at a right angle and feet resting on the floor. The CC was measured at the point of greatest circumference. Waist circumference was measured midway between the last rib and the top of the iliac crest. Hip circumference was measured at the level of the trochanter major. Waist to hip ratio was calculated as the ratio of waist to hip circumferences.

The metabolic syndrome was defined according to the NCEP III criteria ²⁹ which require the presence of three or more alterations among the following: large waist circumference (>88 cm in women and >102 cm in men), elevated triglycerides (≥ 150 mg/dl), low HDL cholesterol (men < 40 and women < 50 mg/dl), elevated fasting glucose (≥ 110 mg/dl) and elevated systolic (≥ 130 mmHg) or diastolic blood pressure (≥ 85 mmHg) or use of anti-hypertensive medications.

Statistics

Data were analyzed with the SAS 8.02 software package (SAS Institute Inc., Cary, North Carolina, USA). Gender-specific quartiles of CC, WC and WHR were used for the statistical analyses. For CC values the cutoff points were 34.0, 36.0 and 38.0 cm for men and 33.0, 34.5 and 36.5 cm for women. For WHR values the cutoff points were 0.91, 0.95 and 0.98 for men and 0.79, 0.83 and 0.87 for women. For WC values the cut-off points were 89.0, 95.5 and 102.0 cm for men and 75.5, 82.5 and 90.5 cm for women. The main dependent variables, carotid plaques and CCA-IMT were studied as a qualitative and a quantitative variable respectively. For qualitative variables, comparisons between groups were made by means of a chi-square test. Pearson's correlation coefficient (r) was used to calculate correlations between variables. To estimate the odds ratios (OR) a logistic regression was

performed. For continuous variables, analysis of variance and analysis of covariance were used. Multivariable analyses were adjusted for age, gender, smoking habits, alcohol consumption, systolic blood pressure, antihypertensive treatment, HDL-cholesterol, LDL-cholesterol, triglycerides (log-transformed value), lipid lowering drugs, diabetes, and history of vascular disease.

Results

The unadjusted associations of population characteristics with gender-specific quartiles of CC are presented in table 1. Increasing CC was significantly associated with hypertension and metabolic syndrome, with increasing triglycerides and with decreasing age and HDL-cholesterol. History of vascular disease was significantly less frequent with increasing CC.

In a univariate analysis, increasing WHR and WC were both significantly associated with hypertension, diabetes and metabolic syndrome, with increasing age, BMI, tobacco consumption, alcohol consumption, triglycerides and total cholesterol, and with decreasing HDL-cholesterol. History of vascular disease was significantly more frequent with increasing WHR and WC (data not shown).

Increasing age, male gender, vascular risk factors and history of vascular disease were significantly associated with both increasing frequency of carotid plaques and increasing CCA-IMT (data not shown). Physical activity was inversely associated with carotid plaques (frequency of carotid plaques = 49.5%, 43.1% and 37.6% in individuals performing light, mild and moderate to high physical activity respectively, $p < 0.0001$). Increasing BMI was significantly associated with increasing CCA-IMT and increasing frequency of carotid plaques, but for carotid plaques the association disappeared after adjustment for age, gender, vascular risk factors and history of vascular disease (supplemental table 1, available online).

The correlation coefficients between the three anthropometric measures under study were 0.75 ($p < 0.0001$) between WHR and WC, 0.27 ($p < 0.0001$) between WHR and CC, and 0.54 ($p < 0.0001$)

between WC and CC. BMI was correlated with WHR ($r=0.35$, $p<0.0001$), WC ($r=0.77$, $p<0.0001$) and CC ($r=0.64$, $p<0.0001$).

Association of calf circumference with carotid plaques, CCA-IMT and carotid diameter

Carotid plaques were significantly less frequent with increasing CC, even after adjusting for age, gender, vascular risk factors and history of vascular disease. Additionally adjusting for BMI did not modify the results and even strengthened the association (table 2). When adding physical activity to the latter model, results were also unchanged, the ORs for the second, third and fourth quartile of CC compared to the first quartile being 0.97 (95%CI: 0.81-1.16), 0.74 (95%CI: 0.60-0.91), and 0.63 (95%CI: 0.50-0.80) (p for trend < 0.0001). Adjusting for WC instead of BMI yielded similar results (data not shown). Excluding subjects with overt cardiovascular disease and subjects with a metabolic syndrome did not modify the results (supplemental table 2, available online). The association of carotid plaques with CC was substantially unchanged after stratifying on gender, BMI, and physical activity, even though it was only borderline significant in men (supplemental table 2, available online), and there was no significant interaction between CC and gender, BMI or physical activity. We didn't find any significant interaction either between CC and WHR or WC. There was however an opposite and multiplicative relationship of CC and WHR with carotid plaques, as depicted on figure 1. Individuals with the smallest quartile of WHR and the largest quartile of CC had the lowest frequency of carotid plaques (31.8%), while those with the largest quartile of WHR and the smallest quartile of CC group had the highest frequency of carotid plaques (55.1%) (Figure 1).

There was also a significant linear relationship between CC quartiles and the number of sites with plaques. The frequency of subjects with a CC in the fourth quartile was 25.3%, 22.4% and 21.7% among subjects with no plaque, 1 site with plaques and 2 or more sites with plaques, while the frequency of subjects with a CC in the first quartile was 23.8%, 25.5% and 26.5% among subjects with no plaque, 1 site with plaques and 2 or more sites with plaques (p for linear association = 0.001).

CCA-IMT was higher with increasing CC, even after adjusting for age, gender, vascular risk factors and history of vascular disease, but the association disappeared after adjusting additionally for BMI or WC (table 2). Similarly, CCA diameter was higher with increasing CC, but the relationship was largely attenuated after adjusting for BMI (data not shown).

Association of WHR and WC with carotid plaques and CCA-IMT

WHR and WC quartiles were associated with increasing frequency of carotid plaques, but this association was diminished after adjusting for vascular risk factors (table 3). The association was stronger again after adjusting for CC quartiles ($p=0.04$ for WC and $p=0.05$ for WHR).

Both WHR and WC quartiles were significantly associated with increasing CCA-IMT, even after adjusting for age, gender, vascular risk factors and history of vascular disease (table 3). Additionally adjusting for calf circumference did not modify these results (data not shown). All these results were substantially unchanged after adjusting additionally for physical activity. The association of WHR or WC with CCA-IMT was the same after stratifying on the presence of carotid plaques (data not shown).

Discussion

In this population-based study on 6265 subjects aged 65 years or more we demonstrated for the first time that carotid plaques were significantly less frequent with increasing calf circumference, independently of age, gender, vascular risk factors, BMI, abdominal obesity, and physical activity. There was an opposite and multiplicative relationship of CC and WHR with the frequency of carotid plaques, the highest frequency being found in subjects with the highest quartile of WHR and the lowest quartile of CC. Mean CCA-IMT was larger with increasing WHR, WC and CC, but in the case of CC the association disappeared after adjusting for BMI.

Measures of CCA-IMT and carotid plaques have been validated previously and have a high reproducibility³⁰. Of course, replication of our results in independent populations is required. It should also be noted that our population is not perfectly representative of the French general population aged 65 years or more, since only non-institutionalized individuals who accepted to take part in a follow-up study and who were able to come to the study examination centers were included. Persons who participate in a follow-up study are likely to be more health conscious and might have less risk factors and diseases than nonparticipants. This limitation is common to most large population-based prospective studies, whatever the sampling procedure used. There are however no French national data which would permit to determine precisely how different the 3C Study participants are and whether they had better medical follow-up and health care than the general elderly population. Among the 3C Study participants, those who were able to come to the examination centers to undergo ultrasound examinations were younger and had lower BMI, systolic blood pressure and total cholesterol than those who did not undergo ultrasound examinations.

Association of calf circumference with carotid plaques and CCA-IMT

Our study is the first to test the association of calf circumference with carotid parameters. Several arguments suggest that our main finding, the inverse association between calf circumference and carotid plaques, is genuine: (i) the large size of the population, (ii) the fact that the inverse association between carotid plaques and CC was graded, (iii) that it was independent of age, gender, vascular risk factors, BMI, physical activity, and (iv) that stratifying on gender, BMI, and physical activity, or excluding subjects with a metabolic syndrome or a history of vascular event, did not modify the results. The graded inverse association of calf circumference with prevalent vascular events is also in line with our main finding.

The fact that calf circumference was associated with carotid plaques and not with CCA-IMT in a multivariable model may suggest that CC could be involved in later stages of atherosclerosis ³¹. Furthermore, elevated CCA-IMT may in some instances reflect a nonatherosclerotic thickening ³², and the ultrasound technique is unable to differentiate an atherosclerotic from a nonatherosclerotic cause of arterial wall thickening. As a matter of fact, since the association between CCA-IMT and CC disappeared after adjusting for BMI, it may just reflect an arterial wall thickening in response to increased global body mass. Increased body mass requires increased blood supply, which implies size adaptation of the arteries ^{33, 34}, and in response to increased arterial diameter, thickening of the arterial wall may occur in order to maintain normal wall stress ³⁵. Accordingly, animal studies have shown that the overall wall thickness, mainly reflecting the number of smooth muscle cell layers, reflects the size of the animal and the diameter of the vessel ³⁶. Indeed, in the present study we found that increasing CC quartiles were also associated with increasing CCA diameter and this relationship was attenuated after adjusting for BMI. A limitation here is that, using ultrasound, it is not possible to distinguish between intima and media thickening. Therefore, we were unable to determine whether increased IMT in our population reflected intimal atherosclerosis or medial adaptive response to hemodynamic alterations ³².

Calf circumference is a surrogate marker of lean mass and peripheral subcutaneous fat, the relative importance of each component depending on the nutritional status, and the global body mass ²².

³⁷⁻³⁹. If the inverse association we observed between calf circumference and carotid plaques is true, and if it is causal, there are several potential underlying mechanisms. One explanation could be a protective effect of peripheral subcutaneous fat on the occurrence of atherosclerosis. Indeed, recent studies found peripheral adiposity to be negatively associated with type 2 diabetes ^{40, 41} and with several markers of atherosclerosis such as aortic calcifications ⁶, coronary angiography score ⁷ and arterial stiffness ⁸. Peripheral subcutaneous fat, compared to abdominal and in particular visceral fat, has a low rate of lipolysis ^{42, 43}, and is therefore more likely to take up free fatty acids from the circulation and less likely to release them ⁴⁴. Increased peripheral fat stores may protect the liver and other organs from high free fatty acid exposure and thus from insulin resistance ^{45, 46}. Furthermore, differences in gene expression between visceral and subcutaneous adipocytes may also play a role, with a more proatherogenic pattern of gene expression in visceral fat ⁴⁷⁻⁴⁹. Alternatively, the inverse relationship between carotid plaques and increasing CC may also reflect a protective effect of large amounts of lean mass from calf muscles. Two studies ^{8, 50} have assessed the relationship between body composition (using dual-energy X-ray absorptiometry [DEXA]) and IMT, showing a non significant trend of increasing IMT with increasing peripheral lean mass and no association with peripheral or trunk fat mass in the first study ⁸, and no association at all in the second study ⁵⁰. These studies were however performed on smaller samples (336 and 648 participants respectively) of younger individuals, and did not include an evaluation of carotid plaques. Further studies on larger and older populations, implementing imaging devices such as DEXA, or computed tomography and magnetic resonance imaging to measure the muscle and fat component within the calf, could improve our understanding of the respective roles played by calf muscle and fat mass in association with carotid plaques.

Although we cannot rule out a participation of increased physical activity in our results, we believe that it is unlikely to be the only explanation. Firstly, we did not observe a linear increase in physical activity with increasing CC quartiles (table 1), and even though calf circumference was found to be well correlated with total muscle in the malnourished elderly ²², this association is less clear in

healthier elderly subjects ³⁹. Secondly, the association between calf circumference and carotid plaques was unchanged after adjusting for and stratifying on physical activity. **It should be mentioned that the variable we used to quantify physical activity has not been formally validated.**

Association of WHR and WC with carotid plaques and CCA-IMT

Several studies had already shown that CCA-IMT was significantly associated with anthropometric markers of abdominal obesity ¹³⁻²¹, but failed to show any association of these markers with carotid plaques ²¹, even though very little data is available on the latter association. In the present study, supporting previous results, we confirmed the positive association of CCA-IMT with WHR and WC. We also found an association of WHR (and WC) with carotid plaques, which became borderline or non significant after adjusting for vascular risk factors and history of vascular disease. Interestingly, the strength of the association increased after adjusting for CC, suggesting a confounding effect of the latter. CC was indeed strongly correlated with WHR (and WC) and inversely related with carotid plaques. A positive association of abdominal fat markers with carotid plaques may thus be masked if CC is not adjusted for. The multiplicative and opposite effect of CC and WHR on the frequency of carotid plaques suggests that both measures should be taken into account jointly when assessing vascular risk profiles.

To conclude, in a large population-based study on subjects aged 65 years or more, we found that carotid plaques were significantly less frequent in subjects with a large calf circumference, independently of vascular risk factors, global body mass, and physical activity. There was an opposite and multiplicative effect of WHR and CC, subjects with the smallest quartile of CC and the largest quartile of WHR showing the highest frequency of carotid plaques. Our results suggest that calf circumference may be a new anthropometric marker to take into account when assessing **the risk of carotid atherosclerosis**. Validation of this finding in independent populations is required. Future studies

should also test the association of calf circumference with plaque composition (using more detailed measures than ultrasound, such as magnetic resonance imaging), with plaque progression and with vascular events. Besides, research focusing on the underlying mechanisms and in particular the respective role of lean mass and peripheral fat mass is warranted.

References

1. Romero-Corral A, Montori VM, Somers VK, Korinek J, Thomas RJ, Allison TG, Mookadam F, Lopez-Jimenez F. Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: A systematic review of cohort studies. *Lancet*. 2006;368:666-678
2. Franzosi MG. Should we continue to use bmi as a cardiovascular risk factor? *Lancet*. 2006;368:624-625
3. Suk SH, Sacco RL, Boden-Albala B, Cheun JF, Pittman JG, Elkind MS, Paik MC. Abdominal obesity and risk of ischemic stroke: The northern manhattan stroke study. *Stroke*. 2003;34:1586-1592
4. Empana JP, Ducimetiere P, Charles MA, Jouven X. Sagittal abdominal diameter and risk of sudden death in asymptomatic middle-aged men: The paris prospective study i. *Circulation*. 2004;110:2781-2785
5. Ducimetiere P, Richard JL. The relationship between subsets of anthropometric upper versus lower body measurements and coronary heart disease risk in middle-aged men. The paris prospective study. I. *Int J Obes*. 1989;13:111-121
6. Tanko LB, Bagger YZ, Alexandersen P, Larsen PJ, Christiansen C. Peripheral adiposity exhibits an independent dominant antiatherogenic effect in elderly women. *Circulation*. 2003;107:1626-1631
7. Hara M, Saikawa T, Kurokawa M, Sakata T, Yoshimatsu H. Leg fat percentage correlates negatively with coronary atherosclerosis. *Circ J*. 2004;68:1173-1178
8. Ferreira I, Snijder MB, Twisk JW, van Mechelen W, Kemper HC, Seidell JC, Stehouwer CD. Central fat mass versus peripheral fat and lean mass: Opposite (adverse versus favorable) associations with arterial stiffness? The amsterdam growth and health longitudinal study. *J Clin Endocrinol Metab*. 2004;89:2632-2639

9. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The rotterdam study. *Circulation*. 1997;96:1432-1437
10. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular health study collaborative research group. *N Engl J Med*. 1999;340:14-22
11. Hollander M, Bots ML, Del Sol AI, Koudstaal PJ, Witteman JC, Grobbee DE, Hofman A, Breteler MM. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: The rotterdam study. *Circulation*. 2002;105:2872-2877
12. 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;115:459-467
13. Reed D, Dwyer KM, Dwyer JH. Abdominal obesity and carotid artery wall thickness. The los angeles atherosclerosis study. *Int J Obes Relat Metab Disord*. 2003;27:1546-1551
14. Yamamoto M, Egusa G, Hara H, Yamakido M. Association of intraabdominal fat and carotid atherosclerosis in non-obese middle-aged men with normal glucose tolerance. *Int J Obes Relat Metab Disord*. 1997;21:948-951
15. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo 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;146:483-494
16. Folsom AR, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW, Cram KB, Hutchinson RG. Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis risk in communities (aric) study investigators. *Stroke*. 1994;25:66-73

17. Stevens J, Tyroler HA, Cai J, Paton CC, Folsom AR, Tell GS, Schreiner PJ, Chambless LE. Body weight change and carotid artery wall thickness. The atherosclerosis risk in communities (aric) study. *Am J Epidemiol.* 1998;147:563-573
18. De Michele M, Panico S, Iannuzzi A, Celentano E, Ciardullo AV, Galasso R, Sacchetti L, Zarrilli F, Bond MG, Rubba P. Association of obesity and central fat distribution with carotid artery wall thickening in middle-aged women. *Stroke.* 2002;33:2923-2928
19. Takami R, Takeda N, Hayashi M, Sasaki A, Kawachi S, Yoshino K, Takami K, Nakashima K, Akai A, Yamakita N, Yasuda K. Body fatness and fat distribution as predictors of metabolic abnormalities and early carotid atherosclerosis. *Diabetes Care.* 2001;24:1248-1252
20. Lakka TA, Lakka HM, Salonen R, Kaplan GA, Salonen JT. Abdominal obesity is associated with accelerated progression of carotid atherosclerosis in men. *Atherosclerosis.* 2001;154:497-504
21. Czernichow S, Bertrais S, Oppert JM, Galan P, Blacher J, Ducimetiere P, Hercberg S, Zureik M. Body composition and fat repartition in relation to structure and function of large arteries in middle-aged adults (the su.Vi.Max study). *Int J Obes (Lond).* 2005;29:826-832
22. Jauffret M, Jusot JF, Bonnefoy M. Marqueurs anthropométriques et malnutrition de la personne âgée: Intérêt de la circonférence du mollet. *Age nutr.* 1999;10:163-169
23. Williams SR, Jones E, Bell W, Davies B, Bourne MW. Body habitus and coronary heart disease in men. A review with reference to methods of body habitus assessment. *Eur Heart J.* 1997;18:376-393
24. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanus F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the interheart study): Case-control study. *Lancet.* 2004;364:937-952
25. Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, Nadeau A, Lupien PJ. Waist circumference and abdominal sagittal diameter: Best simple anthropometric indexes

- of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol.* 1994;73:460-468
26. 3C-Study-Group. Vascular factors and risk of dementia: Design of the three-city study and baseline characteristics of the study population. *Neuroepidemiology.* 2003;22:316-325
 27. Zureik M, Gariépy J, Courbon D, Dartigues JF, Ritchie K, Tzourio C, Alperovitch A, Simon A, Ducimetiere P. Alcohol consumption and carotid artery structure in older french adults: The three-city study. *Stroke.* 2004;35:2770-2775
 28. Gariépy J, Salomon J, Denarie N, Laskri F, Megnien JL, Levenson J, Simon A. Sex and topographic differences in associations between large-artery wall thickness and coronary risk profile in a french working cohort: The axa study. *Arterioscler Thromb Vasc Biol.* 1998;18:584-590
 29. Executive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). *Jama.* 2001;285:2486-2497
 30. Zureik M, Ducimetiere P, Touboul PJ, Courbon D, Bonithon-Kopp C, Berr C, Magne C. Common carotid intima-media thickness predicts occurrence of carotid atherosclerotic plaques: Longitudinal results from the aging vascular study (eva) study. *Arterioscler Thromb Vasc Biol.* 2000;20:1622-1629
 31. Javid H. Development of carotid plaque. *Am J Surg.* 1979;138:224-227
 32. Bots ML, Hofman A, Grobbee DE. Increased common carotid intima-media thickness. Adaptive response or a reflection of atherosclerosis? Findings from the rotterdam study. *Stroke.* 1997;28:2442-2447
 33. Ferreira I, Twisk JW, Van Mechelen W, Kemper HC, Stehouwer CD. Current and adolescent levels of cardiopulmonary fitness are related to large artery properties at age 36: The amsterdam growth and health longitudinal study. *Eur J Clin Invest.* 2002;32:723-731

34. Huonker M, Halle M, Keul J. Structural and functional adaptations of the cardiovascular system by training. *Int J Sports Med.* 1996;17 Suppl 3:S164-172
35. Mayet J, Stanton AV, Chapman N, Foale RA, Hughes AD, Thom SA. Is carotid artery intima-media thickening a reliable marker of early atherosclerosis? *J Cardiovasc Risk.* 2002;9:77-81
36. Safar ME, Blacher J, Mourad JJ, London GM. Stiffness of carotid artery wall material and blood pressure in humans: Application to antihypertensive therapy and stroke prevention. *Stroke.* 2000;31:782-790
37. Martin AD, Spent LF, Drinkwater DT, Clarys JP. Anthropometric estimation of muscle mass in men. *Med Sci Sports Exerc.* 1990;22:729-733
38. Chumlea WC, Guo SS, Vellas B, Guigoz Y. Techniques of assessing muscle mass and function (sarcopenia) for epidemiological studies of the elderly. *J Gerontol A Biol Sci Med Sci.* 1995;50 Spec No:45-51
39. Rolland Y, Lauwers-Cances V, Cournot M, Nourhashemi F, Reynish W, Riviere D, Vellas B, Grandjean H. Sarcopenia, calf circumference, and physical function of elderly women: A cross-sectional study. *J Am Geriatr Soc.* 2003;51:1120-1124
40. Seidell JC, Han TS, Feskens EJ, Lean ME. Narrow hips and broad waist circumferences independently contribute to increased risk of non-insulin-dependent diabetes mellitus. *J Intern Med.* 1997;242:401-406
41. Snijder MB, Dekker JM, Visser M, Bouter LM, Stehouwer CD, Kostense PJ, Yudkin JS, Heine RJ, Nijpels G, Seidell JC. Associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes: The hoorn study. *Am J Clin Nutr.* 2003;77:1192-1197
42. Arner P. Differences in lipolysis between human subcutaneous and omental adipose tissues. *Ann Med.* 1995;27:435-438

43. Snijder MB, Dekker JM, Visser M, Yudkin JS, Stehouwer CD, Bouter LM, Heine RJ, Nijpels G, Seidell JC. Larger thigh and hip circumferences are associated with better glucose tolerance: The hoorn study. *Obes Res.* 2003;11:104-111
44. Frayn KN. Adipose tissue as a buffer for daily lipid flux. *Diabetologia.* 2002;45:1201-1210
45. Bjorntorp P. Metabolic implications of body fat distribution. *Diabetes Care.* 1991;14:1132-1143
46. Carey DG. Abdominal obesity. *Curr Opin Lipidol.* 1998;9:35-40
47. Lihn AS, Bruun JM, He G, Pedersen SB, Jensen PF, Richelsen B. Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. *Mol Cell Endocrinol.* 2004;219:9-15
48. Vohl MC, Sladek R, Robitaille J, Gurd S, Marceau P, Richard D, Hudson TJ, Tchernof A. A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men. *Obes Res.* 2004;12:1217-1222
49. Fantuzzi G, Mazzone T. Adipose tissue and atherosclerosis: Exploring the connection. *Arterioscler Thromb Vasc Biol.* 2007;27:996-1003
50. Snijder MB, Henry RM, Visser M, Dekker JM, Seidell JC, Ferreira I, Bouter LM, Yudkin JS, Westerhof N, Stehouwer CD. Regional body composition as a determinant of arterial stiffness in the elderly: The hoorn study. *J Hypertens.* 2004;22:2339-2347

Tables and Figures

Table 1: Population characteristics according to gender-specific quartiles of calf circumference

	Calf circumference				p for trend
	Quartile 1*	Quartile 2	Quartile 3	Quartile 4	
<i>n</i>	1560	1641	1576	1488	
Women, %	59.6	58.7	59.6	64.6	0.005
Age, years	74.3±5.1	73.6±4.8	73.3±4.9	72.8±4.7	<0.0001
Hypertension, %	72.2	74.1	75.3	79.9	<0.0001
Systolic BP, mmHg	143.4±21.6	144.9±21.1	146.0±21.7	146.1±20.2	0.0001
Diastolic BP, mmHg	80.0±11.0	81.7±10.8	82.6±11.1	83.8±10.9	<0.0001
Antihypertensive treatment, %	43.0	45.0	45.9	54.5	<0.0001
Alcohol consumption, g/day	13.0±14.3	13.7±14.8	14.1±16.0	11.9±15.0	0.10
Smoking habits, %					
Never	61.5	61.1	60.3	62.0	0.33
Former smoker	31.7	33.1	34.5	32.9	
Current smoker	6.8	5.8	5.2	5.1	
Hypercholesterolemia, %	57.2	56.6	55.7	53.9	0.06
Total cholesterol, mmol/l	5.82±0.98	5.78±0.96	5.78±0.95	5.71±0.96	0.004
HDL cholesterol, mmol/l	1.68±0.42	1.64±0.42	1.59±0.40	1.54±0.37	<0.0001
LDL cholesterol, mmol/l	3.60±0.84	3.60±0.85	3.61±0.83	3.56±0.83	0.26
Triglycerides, mmol/l	1.19±0.57	1.22±0.61	1.27±0.64	1.36±0.64	<0.0001†
Lipid lowering drugs intake, %	30.4	30.7	30.8	32.1	0.34
Diabetes, %	9.6	8.1	9.7	11.0	0.08
Metabolic syndrome, %	7.7	11.6	15.3	27.5	<0.0001
History of vascular disease, %	11.1	8.7	8.6	7.9	0.004
Physical activity‡, %					
light	44.9	42.6	39.5	47.9	
mild	36.9	36.5	37.8	35.0	0.55
moderate to high	18.2	20.9	22.7	17.1	
BMI, kg/m ²	22.6±2.9	24.6±2.7	26.2±3.0	29.3±3.9	<0.0001
WHR	0.87±0.09	0.88±0.09	0.88±0.09	0.89±0.08	<0.0001
WC	82.0±0.29	86.8±0.28	89.7±0.29	96.1±0.29	<0.0001

Data are mean ± standard deviation or %, as indicated; BMI = body mass index; BP = blood pressure;

WC = waist circumference; WHR = waist-to-hip ratio; *lowest quartile; †based on log-transformed values;

‡information on physical activity was available in 4612 subjects

Table 2: Association of carotid parameters with calf circumference

	Calf circumference				p for trend
	Quartile 1*	Quartile 2	Quartile 3	Quartile 4	
<i>n</i>	1560	1641	1576	1488	
Carotid plaques, %					
No adjustment (%)	49.2	48.3	46.5	43.6	0.001
Multivariable OR (95%CI)†	1	1.00 (0.86-1.17)	0.91 (0.78-1.06)	0.79 (0.68-0.93)	0.002
Multivariable OR (95%CI)‡	1	0.98 (0.84-1.15)	0.85 (0.72-1.01)	0.71 (0.58-0.86)	0.0002
CCA-IMT (mm), mean±SE					
No adjustment (mm)	0.705±0.003	0.713±0.003	0.720±0.003	0.715±0.003	0.004
Multivariable† (mm)	0.701±0.003	0.713±0.003	0.721±0.003	0.717±0.004	<0.0001
Multivariable‡ (mm)	0.708±0.003	0.715±0.003	0.719±0.003	0.711±0.003	0.47

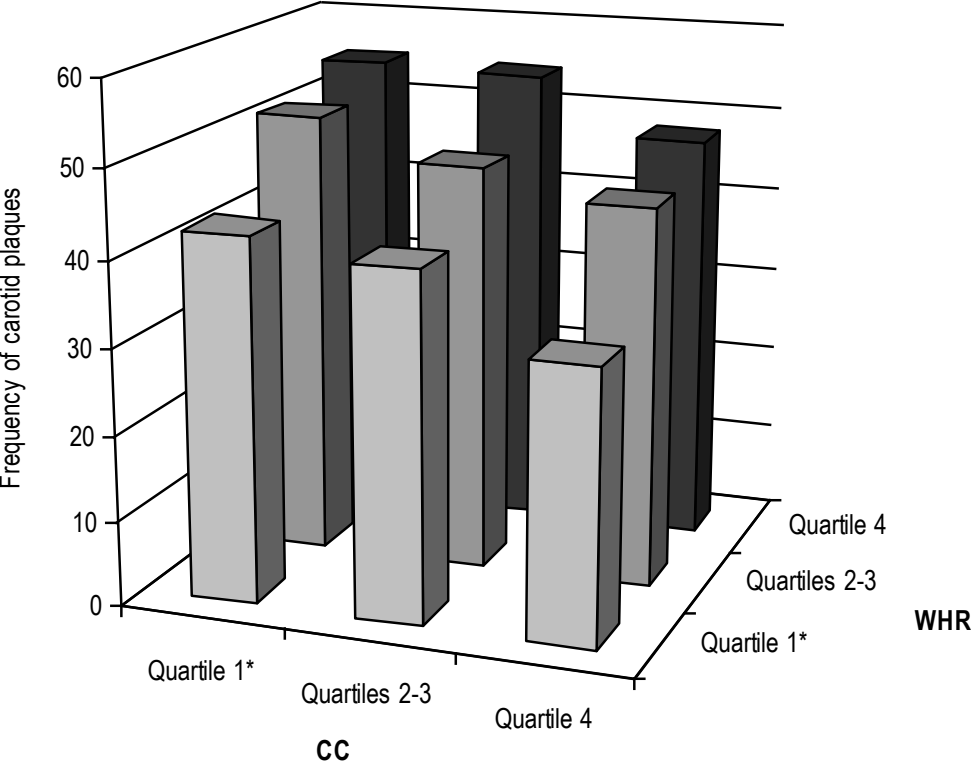
CCA-IMT: Common Carotid Artery - Intima Media Thickness; CI: Confidence Interval; OR: Odds Ratio; SE: Standard Error of Means; *Lowest quartile; †Adjusted for age, gender, smoking habits, alcohol consumption, systolic blood pressure, antihypertensive treatment, HDL-cholesterol, LDL-cholesterol, triglycerides (log-transformed value), lipid lowering drugs, diabetes, history of vascular disease; ‡Like † and additionally adjusted for quartiles of Body Mass Index (BMI)

Table 3: Association of carotid parameters with waist circumference (WC) and waist-to-hip ratio (WHR)

		WC				
		Quartile 1*	Quartile 2	Quartile 3	Quartile 4	p for trend
<i>n</i>		1579	1520	1616	1550	
Carotid plaques, %						
	No adjustment (%)	41.5	44.9	50.2	51.2	<0.0001
	Multivariable OR (95%CI)†	1	1.03 (0.88-1.20)	1.13 (0.97-1.32)	1.01 (0.85-1.19)	0.64
CCA-IMT (mm), mean±SE						
	No adjustment (mm)	0.690±0.003	0.709±0.003	0.719±0.003	0.734±0.003	<0.0001
	Multivariable† (mm)	0.695±0.003	0.712±0.003	0.717±0.003	0.729±0.003	<0.0001
		WHR				
		Quartile 1*	Quartile 2	Quartile 3	Quartile 4	p for trend
<i>n</i>		1564	1563	1572	1566	
Carotid plaques, %						
	No adjustment (%)	39.8	46.5	48.9	52.6	<0.0001
	Multivariable OR (95%CI)†	1	1.16 (1.00-1.36)	1.15 (0.98-1.35)	1.15 (0.98-1.35)	0.13
CCA-IMT (mm), mean±SE						
	No adjustment (mm)	0.695±0.003	0.704±0.003	0.719±0.003	0.733±0.003	<0.0001
	Multivariable† (mm)	0.703±0.003	0.707±0.003	0.716±0.003	0.727±0.003	<0.0001

CCA-IMT: Common Carotid Artery - Intima Media Thickness; CI: Confidence Interval; OR: Odds Ratio; SE: Standard Error of Means; *Lowest quartile; †Adjusted for age, gender, smoking habits, alcohol consumption, systolic blood pressure, antihypertensive treatment, HDL-cholesterol, LDL-cholesterol, triglycerides (log-transformed value), lipid lowering drugs, diabetes, history of vascular disease; ‡Additionally adjusted for quartiles of calf circumference.

Figure 1: Opposite and multiplicative effect of calf circumference (CC) and waist to hip ratio (WHR) on the frequency of carotid plaques



*Lowest quartile

Supplemental table 1: Association of carotid parameters with body mass index (BMI)

	BMI				p for trend
	Quartile 1*	Quartile 2	Quartile 3	Quartile 4	
<i>n</i>	1560	1570	1567	1568	
Carotid plaques, %					
No adjustment (%)	44.4	44.2	48.5	50.7	0.0003
Multivariable OR (95%CI)†	1	0.90 (0.77-1.05)	1.00 (0.86-1.17)	0.95 (0.81-1.12)	0.91
CCA-IMT (mm), mean±SE					
No adjustment (mm)	0.697±0.003	0.709±0.003	0.718±0.003	0.728±0.003	<0.0001
Multivariable† (mm)	0.700±0.003	0.710±0.003	0.717±0.003	0.725±0.003	<0.0001

CCA-IMT: Common Carotid Artery - Intima Media Thickness; CI: Confidence Interval; OR: Odds Ratio;

*Lowest quartile; †Adjusted for age, gender, smoking habits, alcohol consumption, systolic blood pressure, antihypertensive treatment, HDL-cholesterol, LDL-cholesterol, triglycerides (log-transformed value), lipid lowering drugs, diabetes, history of vascular disease.

Supplemental table 2: Association of carotid plaques with calf circumference in population subgroups

	Calf circumference				p for trend
	Quartile 1*	Quartile 2	Quartile 3	Quartile 4	
Men					
<i>n</i>	631	677	637	527	
No adjustment (%)	55.2	53.5	55.1	50.5	0.21
Multivariable OR (95%CI)†	1	1.02 (0.80-1.30)	0.97 (0.74-1.27)	0.77 (0.56-1.06)	0.12
Women					
<i>n</i>	929	964	939	961	
No adjustment (%)	45.2	44.7	40.7	39.8	0.005
Multivariable OR (95%CI)†	1	0.95 (0.77-1.16)	0.77 (0.62-0.96)	0.66 (0.51-0.85)	0.0004
BMI<27 kg/m²					
<i>n</i>	859	1235	1115	1044	
No adjustment (%)	48.2	49.0	41.6	41.1	<0.0001
Multivariable OR (95%CI)†	1	1.10 (0.90-1.33)	0.84 (0.68-1.04)	0.81 (0.65-1.02)	0.008
BMI≥27 kg/m²					
<i>n</i>	521	615	387	489	
No adjustment (%)	56.2	52.5	46.3	48.1	0.002
Multivariable OR (95%CI)†	1	0.88 (0.68-1.14)	0.74 (0.55-0.99)	0.71 (0.52-0.96)	0.02
Light physical activity					
<i>n</i>	471	597	464	479	
No adjustment (%)	53.5	51.9	47.0	44.9	0.002
Multivariable OR (95%CI)†	1	1.03 (0.79-1.34)	0.95 (0.72-1.26)	0.79 (0.59-1.06)	0.16
Mild to high physical activity					
<i>n</i>	483	765	675	678	
No adjustment (%)	47.2	45.0	36.7	37.0	<0.0001
Multivariable OR (95%CI)†	1	0.95 (0.74-1.23)	0.72 (0.55-0.94)	0.69 (0.53-0.90)	0.001
No cardiovascular disease					
<i>n</i>	1393	1492	1439	1370	
No adjustment (%)	46.4	46.4	44.2	42.1	0.01
Multivariable OR (95%CI)†	1	1.00 (0.85-1.18)	0.85 (0.71-1.01)	0.72 (0.59-0.89)	0.0006
No metabolic syndrome					
<i>n</i>	1352	1455	1099	1269	
No adjustment (%)	48.6	47.4	42.4	41.8	<0.0001
Multivariable OR (95%CI)†	1	0.97 (0.83-1.15)	0.81 (0.67-0.97)	0.68 (0.56-0.84)	<0.0001

BMI: Body Mass Index; CI: Confidence Interval; OR: Odds Ratio; *Lowest quartile; †Adjusted for age, gender, smoking habits, alcohol consumption, systolic blood pressure, antihypertensive treatment, HDL-cholesterol, LDL-cholesterol, triglycerides (log-transformed value), lipid lowering drugs, diabetes, history of vascular disease and quartiles of BMI