Faculty of Health Science, UiT The Arctic University of Norway

Association between physical activity and carotid atherosclerosis

The Tromsø Study

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Lisa Gullhav Hansen

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Preface

The aim of the master thesis was to investigate the association between physical activity and atherosclerosis of the carotid artery, using data from The Tromsø Study. Previous studies have shown results, but many of them are quite small. The Tromsø Study is a large study with many available data on this topic, which gives a good opportunity to investigate this association.

The timeline of working with this master thesis started some years back. Even though I did not know the exact theme for this thesis, I knew that I would want to have a project related to the carotid ultrasound data. When the 7^{th} survey of the Tromsø Study was ongoing, I visited the station for the carotid ultrasound scanning to get insight into how the data is gathered. This gave me a different and better insight and understanding of the data used in this thesis.

I started searching for and reading literature on the topic this last few months. I wrote the background section when going through the existing literature, and also completed the GRADE evaluations when doing this. In parallel, I did statistical analyses and had to learn the syntaxes and procedures for this in STATA.

Firstly, I would like to thank my main supervisor, Ellisiv B. Mathiesen, for all the help that she has given me through this process and for always being available when I needed guidance. It has been of valuable and irreplaceable help to me. I would also like to thank my co-supervisor Stein Harald Johnsen for all the support.

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Abstract

Background/aim: Physical activity reduces the risk of cardiovascular disease (CVD). Atherosclerosis is an important common underlying cause of CVD. It is not clear whether the beneficial effect of physical activity on CVD is mediated through effect on atherosclerosis, and previous studies have shown diverging results. The aim of this study was to investigate the association between level of physical activity and atherosclerosis.

Method and materials: We included 10,894 participants of the Tromsø Study (1994-2008) who was measured with ultrasound of the carotid artery. Atherosclerosis was assessed as intima-media thickness (IMT), plaque presence (yes/no) and total plaque area (TPA). Linear regression models were used to assess the association between physical activity and intima-media thickness and total plaque area. Logistic regression models were used to assess the association between physical activity across quartiles of IMT and categories of plaque.

Results: We found a significant trend toward lower plaque prevalence and smaller TPA with increasing degree of physical activity. In age-and sex-adjusted models, the OR for plaque presence was 0.74% (95% CI 0.68-0.81) for moderate physical activity and -0.04 (95% CI -0.06 - -0.02) compared to low physical activity. Similar trends were found in age-and-sex-adjusted linear regression analyses or the association between physical activity and TPA. There was an interaction for sex between physical activity and IMT, with a significant inverse association for moderate physical activity (beta-coefficient -0.01 (95% CI -0.02 - -0.001, p=0.02)) and a borderline significance in those with high physical activity (beta-coefficient -0.01 (95% CI -0.03 – 0.000, p=0.06) in men, but not in women.

Conclusion: Physical activity was significantly associated with carotid IMT in men in age-adjusted models and with plaque in age-and-sex-adjusted models. Adjustment for traditional CVD risk factors attenuated these associations. This may imply that the effect of physical activity on atherosclerosis is mediated through effecting these risk factors.

Abbreviations

BMI	Body mass index
CCA	Common carotid artery
CI	Confidence interval
Coef.	Coefficient
CVD	Cardiovascular disease
HDL	High-density lipoprotein
ICA	Internal carotid artery
IMT	Intima-media thickness
LDL	Low-density lipoprotein
OR	Odds ratio
P-value	Probability value
SD	Standard deviation
TPA	Total plaque area

1 Introduction

Physical activity is considered as any bodily movement that is induces by the skeletal muscles, and in which results in consuming energy (1). It is well known to improve our health in many ways by lowering risk of developing cardiovascular diseases (CVD) and thereby improving the health-related quality of life. Ischemic heart disease and ischemic stroke are to two leading causes of death according to World Health Organization, accounting for over 15 million deaths as of 2016 (2). Although it is well known that being physically active improve our health, it is not thoroughly understood how it decreases the risk of disease and mortality (3). Regular physical activity is associated with a lower risk of coronary heart disease (4) stroke (5), and all-cause mortality (6). Additionally, physical activity has also been shown to be beneficiary and lower the risk for patients with established vascular disease or risk factors (3, 7).

Atherosclerosis is a common underlying pathological cause of CVD, and it seems reasonable to expect that higher levels of physical activity would have a protective effect on development of atherosclerosis. In a large population-based study conducted by Stein et al., they found that higher levels of physical activity were independently associated with lower odds of atherosclerosis in both carotid arteries as well as the lower extremities (8). However, previous studies have shown divergent relationship between physical activities and less advanced, subclinical atherosclerosis. The Tromsø Study found that physical activity was associated with a decrease in IMT in men only (9). On the other hand, The ARIC Study did not find an association between physical activity and arterial distensibility (10). It has a direct relationship between higher level of leisure-time physical activity and a lower risk of carotid artery stenosis as well as smaller end-diastolic lumen diameter of the carotid artery (3). One study found that patients with light physical activity had achieved less stiffness of the carotid artery, whereas there were no improvement of stiffness for patients who had a higher level of physical activity (3).

1.1 Atherosclerosis

Atherosclerosis is a pathological condition inside the arteries of the body characterized by a progressive inflammatory disease by accumulation of lipids, causing a vascular disease (11).

It has been recognized as an existing condition for more than 500 years, and as a pathological condition for more than 150 years. Nevertheless, the understanding of atherosclerotic vascular disease has evolved dramatically over the last more than 20 years (12). The condition itself can be morbid because it may impair the blood flow by increasing the lumen diameter, and it can also cause a condition of thromboembolism resulting in cardiovascular and cerebrovascular disease and mortality. Atherosclerosis is the leading cause of vascular disease worldwide with the major clinical manifestations being ischemic heart disease, ischemic stroke and peripheral vascular disease (13). Thus, it has been studied diligently. The word "athero" is best translated as a porridge-like structure and the word "sclerosis" means hard, meaning that atherosclerosis in its direct meaning is a hard porridge-like structure (14). Thus, a hardening and thickening of the artery wall. This pathological condition is a result of a cascade of mechanisms happening in the artery wall.

The mechanisms resulting in formation of atherosclerosis are numerous involving endovascular factors and a cascade of events. The formation occurs by the accumulation of low-density lipoproteins (LDL) in the sub-endothelial space of the arterial wall. Here, the LDL gradually undergo oxidative modifications to form oxidized LDL, which induces inflammation and in turn induce an overexpression of adhesion molecules, among others. The adhesion molecules promote the infiltration of blood-carried monocytes into the inflamed arterial wall, and after the monocytes differentiate to macrophages, they ingest the oxidized LDL. This results in the formation of foam cells which contribute to development of plaque by continuing to secrete mediators of the inflammation in the artery wall (11).

1.1.1 Risk factors

Age, male sex, hypertension, cholesterol and high-density lipoprotein (HDL) cholesterol levels, diabetes and smoking are well established risk factors for both atherosclerosis and CVD, and are often referred to as traditional cardiovascular risk factors (15).

1.2 Physical activity

Physical activity can take place in different situations or occasions, and this is known as the domain of physical activity. Traditionally, there are four different domains for physical activity being occupational (including school), during leisure-time, during transportation, and domestic (house and/or yard work) (16, 17). Physical activity is also regarded is different dimensions as to type of activity, frequency, duration and intensity as well as total energy expenditure. When considering the total energy expenditure, it is helpful to refer to metabolic equivalents. One metabolic equivalent corresponds to the amount of oxygen expended while resting and is equal to 3.5 ml O_2 per kilogram body weight times minutes. It is a simple and practical tool for expression the energy cost of physical activities as a multiple of the resting metabolic rate. The energy that has been expended can be calculated through the oxygen expenditure for the physical activity (18).

1.2.1 Assessment of physical activity in epidemiological studies

The Doubly Labeled Waters is an objective method for measuring energy expenditure and is considered a gold standard for measuring the total energy requirement for humans given normal living conditions. The method uses an indirect calorimetry where the carbon dioxide production in the body provide the basis for calculating the calorie expenditure, and thereby calculating the energy expenditure (19). The Doubly Labeled Waters must be regarded as the best method to measure level of physical activity. Nevertheless, this is an expensive tool and may be time-consuming to implement for epidemiological studies. Wong et al. conducted a study to investigate the scarce existing literature on reproducibility of this method, and found high reproducibility and validity (20).

Accelerometer is another tool for calculation level of physical activity which provide objective measurements. As the name indicate, it is a device that measure accelerations, primarily locomotor activity when worn over the hip. The biggest limitation with this device is that it cannot distinguish whether the person wearing it is carrying any weight, which results in a higher energy expenditure. Additionally, it is an expensive tool. Even though the technological advances have made it easier to conduct large-scale studies using accelerometers, there are still some challenges as to how the data should be handled (21). Montoye et al. conducted a study where participants wore a portable accelerometer while performing physical activity. The results showed that the accelerometer was a better tool of estimating oxygen requirement than a waist movement counter and a wrist movement counter. The study also reported high reproducibility compared to the two latter objective methods for measuring physical activity (22).

In epidemiological studies, questionnaires, which are more objective tools, are commonly used to assess physical activity. The Saltin-Grimby Physical Activity Level Scale is one of the longest existing questionnaires regarding physical activity and was first published in 1968 and have since then been modified to a more user-friendly and practical questionnaire. This is a four-level questionnaire that was originally developed to estimate physical activity both during leisure time and at work. The four levels range from sedentary (being almost completely inactive) to vigorous physical activity (23). The questionnaire has been applied in many countries both with and without minor modifications to the original questionnaire, including The Tromsø Study in Norway for the majority of the conducted surveys (24).

1.3 Physical activity and atherosclerosis

As mentioned above, results from previous studies on the association between physical activity and subclinical atherosclerosis are heterogeneous. A Mexican cross-sectional study on 612 participants found that combined sedentary behaviour and low physical activity was associated with carotid plaque presence (25). Another epidemiological study on 2034 Greek participants found that sedentary behaviour was correlated to a higher prevalence of carotid plaque as well as a higher mean carotid IMT (26). Kozakova et al. found that among young- and middle-aged individuals, baseline measurements of IMT was directly associated with sedentary lifestyle, while vigorous physical activities was associated with less progression of IMT (27). Sedentary lifestyle is believed to have an association to overweight, higher BMI and blood pressure, and this can partly, at least, explain the findings (27). Poor self-efficacy to perform physical activity has shown to be associated with higher incidence of cardiovascular events compared to individuals with good self-efficacy (28).

Normal biological processes may also contribute to plaque presence. Menopause among women has been found to have an influence on atherosclerotic processes due to, among other things, significant increase in serum lipids when entering menopause (29). A previous study suggested that engagement in regular physical activity during midlife was associated with less subclinical atherosclerosis, thus speculation that it was partially mediated by BMI (30).

The protective effect of physical activity against vascular disease may be due to beneficial effect of CVD risk factors. There is a large body of evidence which indicates a positive effect of physical activity towards lower blood pressure levels (31). Physical activity had a favourable effect on lipid levels, it increases HDL cholesterol and lowers triglycerides (32-34). It has been implied that the intensity of physical activity is more important than the length of the activity (32). Exercise up to three times per week has been shown to improve lipid profile, but not more frequent exercise (34). Just to mention a few of the risk factors.

1.4 Carotid ultrasound assessment

An artery consists of three layers in the vessel wall. Looking from the luminal side (the inside), the three layers are tunica intima, tunica media and tunica adventitia. The previously describes atherosclerotic process commences with thickening of the tunica intima and the tunica media layers of the wall and can be detected with ultrasound measurements. The measure of the intima-media thickness (IMT) is the distance from the lumen-intima interface to the media-adventitia interface of the artery wall (35). Ultrasound is not able to distinguish the intima layer of the artery from the media layer, there both layers are measured together as the intima-media complex. An advantage of measuring IMT it that it can be measured in all individuals, considering that it is a normal vessel structure. However, increased intima-media thickness does not necessarily indicate an atherosclerotic process, but many represent adaptive increase in the media layer due to hypertension (36), and can therefore be a marker for beginning of subclinical organ damage (37).

In addition to measuring IMT, total plaque area (TPA) is also a method to assess the atherosclerotic burden. The total plaque area refers to the sum area of all detectable plaques measured by ultrasound (38). Unlike IMT, plaques are clear manifestations of atherosclerosis and are shown as focal protrusions into the vessel lumen on ultrasound (39 , p.14). Plaques can be measured as plaque presence (yes/no), quantitatively as plaque number, plaque thickness and plaque area.

Ultrasound measurements of the carotid arteries are suitable for detection of early atherosclerosis, and it can detect atherosclerosis as an increase in arterial wall thickness before a reduction in lumen diameter occurs (40). Ultrasound is non-invasive and inflicts no risk for the person being examined (40). It is inexpensive, reliable and valid (41), and has shown good reproducibility (40, 42, 43). Traditionally, serum biomarkers have been a widely used tool for assessing risk of developing atherosclerosis. However, measurements of atherosclerosis with ultrasound has the theoretical advantage of directly visualizing the disease itself (44), and may therefore be an advantage considering its positive qualities.

Measuring the total plaque area is a method quantifying the plaque burden and offer a better risk profile than simply measuring presence of plaque or not. Calculation of total plaque area in an arterial region of interest, defined as the sum of all plaque areas in the region, has been shown to be a better predictor of coronary heart disease (45, 46) and stroke (38) than IMT. It has also been shown to be superior to artery stenosis in prediction of stroke, myocardial infarction and death (47).

1.5 Aim of the study

The aim of the study was to investigate the association between physical activity and carotid artery atherosclerosis. We also wanted to study whether the association between physical activity was different for IMT and plaque, and whether the associations were similar in men and women.

2 Material and method

2.1 Study population

The study population included in this study were participants of the Tromsø Study, which is a population-based health survey of the inhabitants of the municipal of Tromsø. The first survey was initiated in 1974, primarily to investigate the high mortality rate of cardiovascular disease in the population of Tromsø. Today, there have been conducted a total of seven surveys. Ultrasound scanning of the carotid arteries were introduced in the 4th survey in 1994-1995, and have continued in the 5th (2001-2002) and 6th (2007-2008) surveys (48).

For the present study, we included all first-time attenders of the carotid ultrasound scanning in either the 4th, 5th or 6th survey. A total of 10,894 subjects attended, of whom 6680 subjects attended for the first time in the 4th survey, 594 subjects in the 5th survey, and 3620 subjects in the 6th survey. Of these, 291 subjects were excluded due to not answering questions about physical activity. All participants had available data on plaque measurements, while, IMT measurements were available for 10,537 subjects.

The surveys were conducted by the University of Tromsø in collaboration with the National Health Screening Service. The Tromsø Study was approved by the Regional Committee for Medical and Health Research Ethics North Norway, the Norwegian Data Inspectorate and the Norwegian Directorate of Health. All participants gave written informed consent.

2.2 Carotid ultrasound measurements

In the 4th and 5th surveys, carotid ultrasound scanning was performed with a high-resolution B-mode ultrasonography with a duplex scanner (Acuson Xp10 128, ART-upgraded) equipped with a 7.5 MHz linear array transducer was used. In the 6th survey, a duplex scanner GE Vivid 7 with a 12 MHz linear transducer was used (39 , p. 25). The measurement protocol was the same in all surveys. In all participants, six different locations of the right carotid artery were scanned and visualized for presence of plaque. The six locations included the far and near wall of the following: right common carotid artery (CCA), the bifurcation, and the internal carotid artery (ICA). A plaque was defined as a localized thickening of the vessel wall of more than 50% compared to the adjacent (normal) intima-media thickness (49). The area for each plaque was outlined and calculated off-line. For subjects with more than one plaque, TPA was calculated as the sum of all plaque areas. A standardized method was used for IMT-recordings in which the images was recorded and captured at the top of the R wave in an ECG signal, this to minimize variability in IMT during the cardiac cycle. Automated measurements of IMT were performed in 10 mm segments. Mean IMT from the images was calculated for each location of the artery (40).

There were 3 to 5 observers who performed the ultrasound scanning in the three surveys. To ensure that the examinations were equal and standardized regarding techniques and measurement procedures, all sonographers completed a 2-month pre-study training protocol (49). The inter- and intra-observer reproducibility as well as the inter-equipment reproducibility was acceptable.

2.3 Physical activity

Physical activity was registered in all Tromsø 4, 5 and 6 surveys, by a self-reporting questionnaire. The wording of the questions regarding physical activity were different from the 4th survey compared to the 5th and 6th survey. In the 5th and 6th surveys, The Saltin-Grimby Physical Activity Level Scale with four categories was used (24).

The wording of the questions was as following: “Describe exercise and physical exertion in your leisure time. If your activity varies much, for example between summer and winter, then give an average. The question refers only to the last year.” Following the four categories to choose from:

“1) Reading, watching TV, or other sedentary activity?

2) Walking, cycling or other forms of exercise at least 4 hours a week? (include walking or cycling to work, Sunday walk/roll, etc.)

3) Participation in recreational sports, heavy gardening etc.? (Note: duration of activity at least 4 hours a week)

4) Participation in hard training or sport competitions, regularly several times a week?".

The 4th survey had a different question regarding leisure time physical activity which was worded like this "How has your physical activity in leisure time been during the last year? Think of your weekly average for the year. Time spent going to work counts as leisure time". For this question, participants gave separate answers for both light activity and hard activity, where light physical activity was defines as "physical activity without sweating or being out of breath" and hard physical activity defined as the opposite "physical activity with sweating or being out of breath". For both these questions, the following four categories were listed:

"1) None

2) <1 hour per week

3) 1-2 hours per week

4) ≥ 3 hours per week"

To be able to compare the three surveys we made a new common variable for physical activity which included all possible answers from the two different wording of question for physical activity. The method we used to make one common variable was the same as the one Vangen-Lønne used in her thesis (24), where physical activity is graded in three categories denoted as low, moderate and high physical activity. The low category included participants who answered category 1 in the Saltin and Grimby question of the 5th and 6th surveys, and those who answered that they belonged to category 1 on the "hard physical activity" question and category 1,2 or 3 on the "light physical activity" question used in the 4th survey. The high physical activity category included participants who answered category 3 or 4 in the Saltin and Grimby questionnaire, and those who answered category 4 for "hard physical activity" in the 4th survey, regardless of what they answered for "light physical activity". Moderate physical activity included participants who answered category 2 to the Saltin-Grimby question. Those who answered categories for "light physical activity" and "hard physical activity" that not included them in any of the new categories for "high physical activity" and "low physical activity" as described above, were also included in the moderate physical activity group (24).

2.4 Cardiovascular risk factors

Information about cardiovascular risk factors was obtained in all three given surveys of the Tromsø study. This was gathered through self- administered questionnaires, fasting blood samples and physical examination of the participants (48). The traditional cardiovascular risk factors age, sex, BMI, systolic blood pressure, serum lipids, diabetes and smoking will be handled in the analysis.

Information on current smoking (yes/no) and diabetes (yes/no) was obtained from self-administered questionnaires. Both weight and height were measured without shoes on, but with light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. Blood pressure, both for systolic and diastolic as well as for hearth rate, was measured using Dinamap Vital Signs Monitor 1846 (Critikon Inc., Tampa, FL, USA). This is a microprocessor-controlled device which uses the oscillometric method and is non-invasive. For each participant, the circumference of the upper right arm was measured to fit the correct size cuff for measuring. The subjects seated for 2 min with the cuff on, and thereafter their blood pressure and heart rate was measured three time, using the mean of the two last measurements as their clinical blood pressure (50).

Serum lipids for each participant was taken by non-fasting blood samples. These were analysed for levels of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides. Analysing method for total cholesterol and triglycerides was an enzymatic colorimetric method with commercially available kits (CHOD-PAP for cholesterol, GPO-PAP for triglycerides; Boehringer-Mannheim), whereas HDL cholesterol was measured after drizzling LDL with heparin and manganese chloride (50).

2.5 Statistical analysis

All statistical analyses were performed using the statistical program STATA (StataMP 15.1 edition, StataCorp LLC Texas, USA).

To compare characteristics of risk profile at baseline between men and women, an independent two-sample t-test was used to compare for continuous variables and a Pearson's Chi-square test was used to compare for categorical variables. For normally distributed variables the mean (standard deviation) was denoted, and median (interquartile range) for variables with skewed distribution.

To explore the distribution of categories of IMT and plaque across levels of physical activity, IMT was divided into quartiles and TPA categorized in categories where 1=no plaque, 2=1.tertile, 3=2.tertile and 4=3.tertile. For IMT a 3x4 table was made, and for TPA a 4x4 table was made. The numbers (percentages for each group was denoted).

All variables that were included in the models were tested for normality by graphical method with histogram declaration. For the variables that were not normally distributed, we had to transform them to obtain the best normally distribution. IMT and TPA were not normally distributed, and IMT was transformed using log transformation, whereas TPA was transformed using square root transformation.

Linear regression model was used when assessing the association between physical activity and the continuous variables IMT and TPA, performed separately for IMT and TPA. For these analyses, IMT and TPA were the dependent variables (outcome) and physical activity an independent variable (predictor). We used three different models, where model 1 was unadjusted, model 2 was adjusted for age and sex, and model 3 was additionally adjusted for body mass index, total cholesterol, HDL cholesterol, systolic blood pressure, diabetes and current smoking. We tested for interaction by multiplying the independent variables with each other, one by one, and including the new interaction variable in the models to consider whether the interaction was significant or not. There was an interaction between sex and physical activity for IMT, and therefore all analyses for IMT were performed for men and women separately. As there were no interactions for TPA, all analyses were performed for both sexes together to increase power.

A logistic multivariate regression models was used when assessing the association between physical activity and plaque presence (yes/no) in three separate models with the same adjustments as describes above.

A two-sided probability value (p-value) <0.05 was regarded as statistically significant, as well as a 95% confidence interval (CI) that did not include the value 1. We also investigated the strength of the association using the odds ratio (OR) for the analyses with logistic regression and the regression coefficient in the analyses performed by linear regression.

2.6 GRADE evaluation

For a number of the articles on the reference list, we have evaluated these by using the GRADE method (51). The present articles have been enclosed at the ending of the thesis in the appendix section.

3 Results

The baseline characteristics of the participants were sex-stratified and listed in table 1. IMT was measured in 4922 men and 5950 women, while TPA was measured in 4917 men and 5977 women at baseline from 1994 until 2008 depending on date of inclusion to the study. At baseline, 50.2% of men and 41.2% of women had plaques in the carotid artery. Men also had larger IMT and TPA compared to women, with median of 87 mm in men and 80 mm in women for IMT, and median of 18.14 mm^2 in men and 13.44 mm^2 in women for TPA. Twice as many men than women reported that they were highly physical active (15.0% for men and 6.7% among women), while there were slightly more women engaging in moderate and low physical activity compared to men (table 1).

In analyses stratified by sex, there was a significant trend for both men and women towards lower plaque prevalence and thinner IMT with higher physical activity level (p for trend <0.001 for both sexes). For TPA, there was also a significant trend for men with decreasing value of TPA along with increasing level of physical activity (p=0.006), but this trend was not significant for women (p=0.065) (table 2).

In analyses with quartiles of IMT, there was a greater proportion of participants with low physical activity (36.3%) in the 4. quartile compared with participants with high physical activity (28.6) for men (p for trend = 0.001). For women there was a greater proportion of participants with high physical activity (36.5%) compared with low physical activity (28.6%) in the 1. quartile (p for trend <0.001) (table 3).

Similarly, we found a significant trend towards higher plaque burden in participants with low physical activity (p for trend <0.001) (table 4).

In age-adjusted sex-stratified linear regression models there was a significant inverse association for moderate physical activity (beta-coefficient -0.01 (95% CI -0.02 to -0.001, p=0.02)) and a borderline significance in those with high physical activity (beta-coefficient -0.01 (95% CI -0.03 – 0.000), p=0.06)) in men, but not in women (table 5). No significant relationship was found in multivariable-adjusted analyses.

In age- and sex-adjusted models, the OR for plaque presence was 0.74 (95% CI 0.68 – 0.81) for moderate physical activity and -0.04 (95% CI -0.06 – -0.02) compared to low physical activity. Similar trends were found in age- and sex-adjusted linear regression analyses or the association between physical activity and total plaque area. The association became insignificant after adjustment for CVD risk factors (table 6).

4 Discussion

In the present study, we found a significant trend toward lower plaque prevalence and smaller total plaque area with increasing degree of physical activity. The relationship between physical activity and IMT was significantly different in men and women, while no significant interactions was found for plaque measurements. We found a significant inverse association between moderate physical activity and IMT (beta-coefficient -0.01 (95% CI -0.02 – -0.001, p=0.002)) and a non-significant relationship with high physical activity (beta-coefficient -0.01 (95% CI -0.03 – 0.000, p=0.06)) in men, but not in women.

Our results are in line with the population-based study on more than 3 million participants in the USA, in which they found an association between higher level of physical activity and lower odds of carotid artery stenosis (8). In another previous study comparing 47 sedentary and 40 active participants found an increasing IMT for the sedentary participants compared with those who were active (52). Additionally, a study of 263 subjects showed an inverse association between degree of physical activity and carotid augmentation index (53). In a previous study on 6408 participants of the 4th survey of the Tromsø Study, IMT was associated with physical activity in men only (9). A study of physical activity and carotid intima-media thickness among patients with type 2 diabetes mellitus found that there was no difference in change of IMT after one year of exercise training (54). Nevertheless, our dividing results from this study may be due to the difference that they studied a population with established disease. The ARIC Study of 10,644 participants did also not find an association between physical activity and arterial distensibility(10). The mean age of the study population in that study was 55-56 years, while the mean age in our study was 59 years. This might be one explanation for the different findings. The relationship between physical activity and plaque has not been assessed previously in the Tromsø Study.

It is not clear why the results showed a sex dependent association for IMT, but not for plaque. A higher proportion of men reported high level of physical activity compared with women. It is possible that men and women comprehend the self-reported questions differently, and that this may have contributed to better discrimination between categories of physical activity in men compared to women. Furthermore, women have less advanced atherosclerosis than men at the same age, and this may have contributed to the differences between men and women, and most for IMT, which represents the early stages of atherosclerosis, while plaque are manifest, overt atherosclerotic lesions.

Adjustment for possible confounders, such as BMI, smoking, blood pressure, diabetes and lipid levels, significantly attenuated the estimates, which were no longer significant. In women, multivariable adjustment even led to reversal of the association between IMT and moderate and (less markedly) high levels of physical activity, which is hard to explain and may have been due to chance. Although the cross-sectional design does not allow for inferences with regard to the temporal relationship between physical activity, CVD risk factors and markers of atherosclerosis, one may speculate that physical activity exert its effect on atherosclerosis through its favourable influence on other risk factors.

Limitations of the study are the cross-sectional design and the use assessment of self-reported questionnaires to assess physical activity. A further limitation is that atherosclerosis was measured only in the right carotid artery. Any such measurement error generally tends to weaken the true associations between the variables of interest. Furthermore, we did not adjust for other possible confounders such as CRP, fibrinogen levels and socioeconomic status.

The strength of the study is the population-based design and the large number of participants.

5 Conclusion

In conclusion, in this cross-sectional study we found that higher levels of physical activity were significantly associated with lower carotid plaque prevalence and smaller total plaque size in age- and sex-adjusted models. For carotid IMT similar associations were present in men only. After adjustment for traditional CVD risk factors, these associations were no longer significant. This may imply that the effect of physical activity on atherosclerosis is mediated through its effect on these risk factors.

The temporal relationship between physical activity, CVD risk factors and atherosclerosis should be further investigated in a prospective design.

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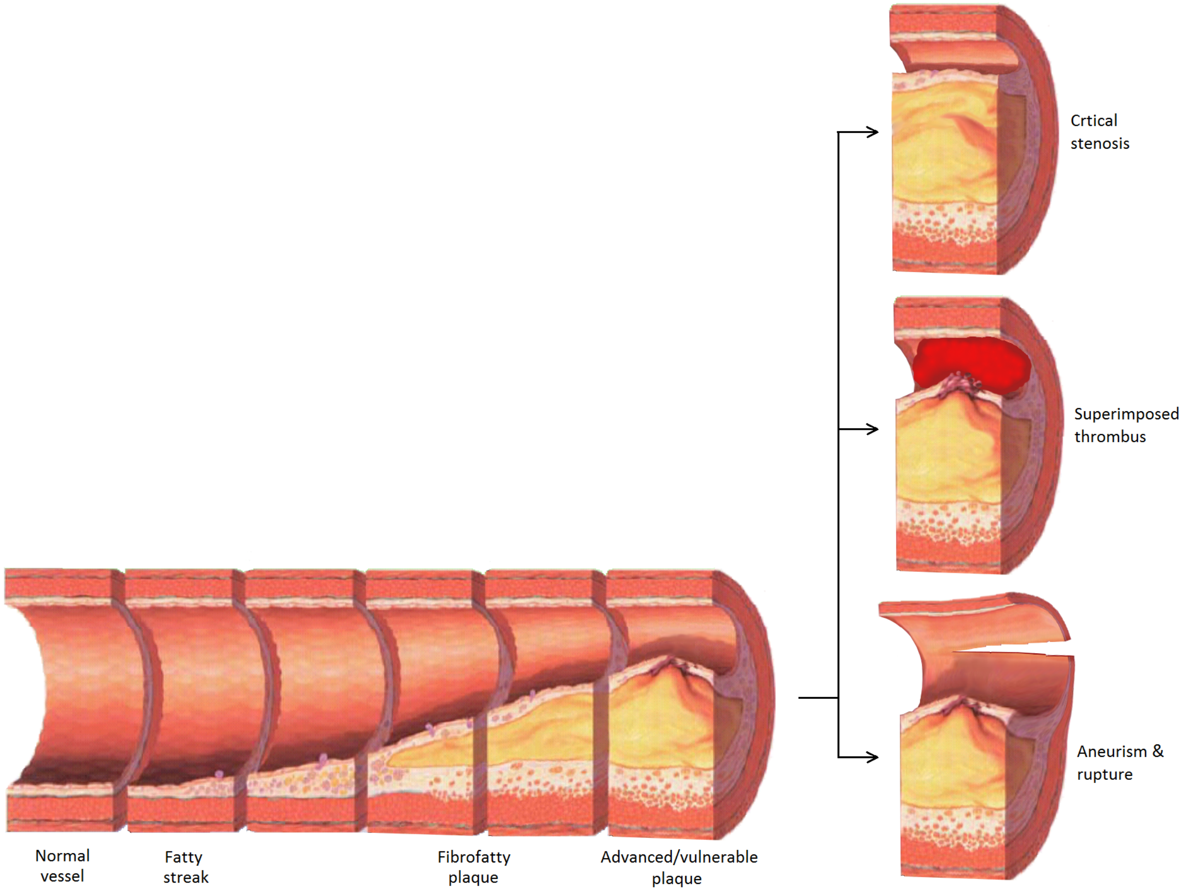
7 Figures

Figure 1. The figure shows the development of atherosclerosis; from a normal healthy artery to a gradually progression of plaque formation, and in the end a condition of critical stenosis (55).



8 Tables

Table 1. Characteristics of study participants, stratified by sex.

	Men (n=4921)	Women (n=5973)	p values
Age, years	59.4 (8.8)	59.9 (8.8)	0.002
BMI, kg/m^2	17.7 (12.5)	17.2 (12.9)	0.02
Total cholesterol, mmol/L	6.38 (1.2)	6.55 (1.4)	<0.001
HDL cholesterol, mmol/L	1.39 (0.4)	1.67 (0.4)	<0.001
Systolic blood pressure, mm Hg	142.7 (20.4)	140.7 (23.9)	<0.001
Diabetes, n (%)	175 (3.6)	195 (3.3)	0.4
Current smoking, n (%)	1446 (29.4)	1656 (27.7)	0.06
Physical activity n (%)			<0.001
High physical activity	727 (15.0)	338 (6.7)	
Medium physical activity	2643 (54.7)	3327 (57.8)	
Low physical activity	1466 (30.3)	2047 (35.5)	
Ultrasound assessments of atherosclerosis			
IMT, mm*	0.87 (0.23)	0.80 (0.20)	<0.001
Plaque presence, n (%)	2471 (50.2)	2462 (41.2)	<0.001
TPA, mm^2 *†	18.14 (20.86)	13.44 (14.88)	<0.001

The values are means (SD) or number (percentage), unless otherwise indicated. BMI indicates body mass index; HDL, high-density lipoprotein; IMT, intima-media thickness; TPA, total plaque area.

*Median (inter-quartile range), n=4816 in men, n=5721 in women

†In participants with plaque only

Table 2. Levels of ultrasound assessed atherosclerosis in participants with low, moderate and high levels of physical activity.

	Plaque presence, %	TPA, mm^{2*}	IMT, mm
Men			
Low physical activity	53.7	19.4	0.88
Moderate physical activity	49.9	17.6	0.87
High physical activity	44.3	17.5	0.86
p for trend	<0.001	0.006	<0.001
Women			
Low physical activity	47.9	13.6	0.81
Moderate physical activity	37.2	13.3	0.79
High physical activity	35.6	13.3	0.78
p for trend	<0.001	0.065	<0.001

The values are medians or percentages.

*In participants with plaque only

Table 3. Distribution of level of physical activity within quartiles of IMT.

	1.quartile IMT	**2.quartile IMT**	**3.quartile IMT**	**4.quartile IMT**
Men				
Low physical activity	254 (17.4)	273 (18.7)	402 (27.6)	530 (36.3)
Moderate physical activity	476 (18.1)	598 (22.7)	730 (27.7)	828 (31.5)
High physical activity	129 (17.8)	180 (24.8)	209 (28.8)	207 (28.6)
p for trend = 0.001				
Women				
Low physical activity	579 (28.6)	514 (25.4)	473 (23.4)	458 (22.6)
Moderate physical activity	1084 (32.7)	954 (28.8)	720 (21.8)	553 (16.7)
High physical activity	141 (36.5)	112 (29.0)	75 (19.4)	58 (15.1)
p for trend <0.001				

The values are number (percentage).

Table 4. Distribution of level of physical activity by subjects with no presence of plaque and by tertials of TPA.

	No plaque (%)	**1.tertile TPA**	**2.tertile TPA**	**3.tertile TPA**
Low physical activity	1747 (49.9)	571 (16.3)	559 (16.0)	625 (17.8)
Moderate physical activity	3406 (57.2)	868 (14.6)	874 (14.7)	802 (13.5)
High physical activity	655 (59.1)	147 (13.3)	148 (13.4)	157 (14.2)

p for trend <0.001

Table 5. Association between physical activity and IMT, stratified by sex.

	Model 1		**Model 2**		**Model 3**	
	Beta-coefficient (95% CI)	p-value	Beta-coefficient (95% CI)	p-value	Beta-coefficient (95% CI)	p-value
Men						
Low physical activity	Ref.		Ref.		Ref.	
Moderate physical activity	-0.02 (-0.04 – -0.01)	0.001	-0.01 (-0.02 – - 0.001)	0.02	-0.007 (-0.017 – 0.004)	0.2
High physical activity	-0.04 (-0.06 – -0.02)	0.000	-0.01 (-0.03 – 0.000)	0.06	0.001 (-0.014 – 0.016)	0.9
Women						
Low physical activity	Ref.		Ref.		Ref.	
Moderate physical activity	-0.03 (-0.04 – -0.02)	<0.001	-0.01 (-0.015 – 0.003)	0.2	0.013 (0.003 – 0.022)	0.008
High physical activity	-0.05 (-0.07 – -0.02)	<0.001	-0.005 (-0.02 – 0.01)	0.6	0.016 (-0.003 - 0.035)	0.09

*Model 1: unadjusted, Model 2: adjusted for age, Model 3: adjusted for age, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes and current smoking.

Table 6. Association between level of physical activity and carotid plaque measurements.

	Model 1		Model 2		Model 3	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Plaque (yes/no)						
Low physical activity	Ref.		Ref.		Ref.	
Moderate physical activity	0.74 (0.68 – 0.81)	<0.001	0.82 (0.75 – 0.89)	<0.001	0.93 (0.85 – 1.02)	0.139
High physical activity	0.69 (0.61 – 0.79)	<0.001	0.77 (0.66 – 0.89)	<0.001	0.92 (0.79 – 1.08)	0.302
	Beta-coefficient (95% CI)	p-value	Beta-coefficient (95% CI)	p-value	Beta-coefficient (95% CI)	p-value
TPA						
Low physical activity	Ref.		Ref.		Ref.	
Moderate physical activity	-0.38 (-0.48 – -0.28)	<0.001	-0.25 (-0.35 – -0.16)	<0.001	-0.09 (-0.19 – 0.01)	0.072
High physical activity	-0.42 (-0.59 – -0.26)	<0.001	-0.34 (-0.49 – -0.18)	<0.001	-0.11 (-0.27 – 0.05)	0.184

*Model 1: unadjusted, Model 2: adjusted for age and sex, Model 3: adjusted for age, sex, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, diabetes and current smoking.

9 Appendix

<table>
<tr><td colspan="3">Reference:
Boss HM, van der Graaf Y, Visseren FLJ, Van den Berg-Vos RM, Bots ML, de Borst GJ, et al. Physical Activity and Characteristics of the Carotid Artery Wall in High-Risk Patients-The SMART (Second Manifestations of Arterial Disease) Study. Journal of the American Heart Association. 2017;6(7).</td><td>Design: Cross-sectional study
Level of documentation: II
GRADE: ⊕○○○</td></tr>
<tr><th>Objective/Aim</th><th>Methods</th><th>Results</th><th>Discussion and commentaries</th></tr>
<tr><td>To examine the relationship of physical activity and characteristics of the carotid artery wall in patients with vascular disease or risk factors.

Conclusion
The results indicate that in patients with vascular disease or risk factors, increased physical activity was associated with smaller carotid diastolic diameter, decreased risk of carotid artery stenosis, and less carotid stiffness. It only showed benefits on carotid intima-media thickness in patients with vascular disease.

Country
The Netherlands.

Year of data collection
1996-2013</td><td>Data foundation: The SMART (Second Manifestations of Arterial Disease) Study.

Data material: 9578 patients were invited if they newly had been referred to the University Medical Center Utrecht.

Exclusions: 550 patients excluded due to missing data for physical activity, CIMT or CAS

Exposure outcome: IMT, carotid stiffness, carotid artery stenosis

Physical activity was assessed using questionnaires. Carotid intima-media thickness and carotid artery stenosis of both common carotid arteries was measured. In a subset of 3165 participants carotid diastolic diameter and distension were assessed. Carotid stiffness was expressed as the distensibility coefficient and Young's elastic modulus.

Statistical methods: Linear regression to investigate association between physical activity and characteristics of the carotid artery wall. Poisson regression model to assess association between physical activity at baseline and presence of CAS.</td><td>They found that physical activity was inversely associated with diastolic diameter, and decreased risk of carotid stenosis. A light level of physical activity was associated with less carotid stiffness, but there was no additional benefit with increasing levels of physical activity. In patients with vascular disease, physical activity was inversely associated with common carotid intima-media thickness, but not in patients with vascular risk factors.</td><td>Checklist:
-Did the study address a clearly focused issue? YES
-Was the study based on random selection of suitable participants? YES
-Were inclusion/exclusion criteria clearly defined? YES
-Were all participants representative of a specified population? YES
-Was the response rate high? YES

-Were objective criteria used to assess/validate outcome measures? YES
-Have authors identified all relevant confounders? YES
-Are prognostic/confounding factors considered in the study design and/or analysis? YES

-Do you trust the results? YES
-Can the results be applied to the local population? YES
-Do the results of this study support other available evidence? YES

Strengths: Large sample size.
Limitations: Cross-sectional design which did not allow causality from consequence. Also, possible that the observed associations are a consequence of the severity of vascular disease and vascular aging leading to decreased physical activity and not a consequence of physical activity itself. Additionally, physical activity was measured by a self-reported questionnaire, which can be a biased by patient recall and social desirability. This could have led to misclassification, especially in the most physically active group, and to an underestimation in the of the benefits of physical activity The influence of residual confounding cannot be excluded.</td></tr>
</table>

Reference: Stein RA, Rockman CB, Guo Y, Adelman MA, Riles T, Hiatt WR, et al. Association between physical activity and peripheral artery disease and carotid artery stenosis in a self-referred population of 3 million adults. Arteriosclerosis, thrombosis, and vascular biology. 2015;35(1):206-12.	**Design:** Cross-sectional study	
	Level of documentation	III
	GRADE	⊕⊕○○

Objective/Aim	Methods	Results	Discussion and commentaries
To investigate prevalence of peripheral artery disease (PAD) and carotid artery stenosis (CAS) in association with physical activity. **Conclusion** The results show that in a large population based study, higher levels of physical activity were independently associated with lower odds of vascular disease in the lower extremities and carotid arteries. **Country** USA **Year of data collection** 2003-2008	**Data material:** More than 3 million participants who completed a medical and lifestyle questionnaire in the Life Line screening program. Exclusions: Participants with non-compressible arterier or ABI values >1.40. **Exposure and outcome:** Physical activity was assessed with a questionnaire. Peripheral artery disease and carotid artery stenosis was determined with simple non-invasive procedures that have high sensitivity and specificity using the ankle brachial index (ABI) and carotid Duplex ultrasound. **Statistical methods:** Logistic regression models were used to estimate odds ratio of prevalent PAD and CAS, respectively. Estimated odds ratios were presented with 95% confidence intervals. Participants were divided into physical activity frequency groups and specific exercise activities presented in the questionnaire. Linear trend tests treated the categories of physical activity frequency as a single ordinal variable. Parallel analyses were performed for each type and intensity of physical activity, further adjusting for other components of physical activity to examine the independent association of each type of activity with odds of PAS and CAS, respectively.	The results show that subjects who reported any physical activity had a significantly lower odds of PAD (OR 0.64, 95% CI 0.63-0.65) and CAS (OR 0.80, 95% CI 0.79-0.81). The association between physical activity with PAD and AS was robust when stratified by sex, rae and age categories. Physical activity intensity frequency was associated with lower PAD and AS in a graded manner (p trend <0.0001 for both). Findings appeared unaffected by confounding by comorbidity or indication.	**Checklist:** -Did the study address a clearly focused issue? **YES** -Was the study based on random selection of suitable participants? **YES** -Were inclusion/exclusion criteria clearly defined? **YES** -Were all participants representative of a specified population? **YES** -Was the response rate high? **YES** -Were objective criteria used to assess/validate outcome measures? **YES** -Have authors identified all relevant confounders? **PARTLY** -Are prognostic/confounding factors considered in the study design and/or analysis? **YES** -Do you trust the results? **YES** -Can the results be applied to the local population? **YES** -Do the results of this study support other available evidence? **YES** **Strengths:** Large study population with more than 3 million. **Limitations:** Self-reported questions on physical activity. Cannot rule out other potential confounders.

Reference:
Walker TJ, Heredia NI, Lee M, Laing ST, Fisher-Hoch SP, McCormick JB, et al. The combined effect of physical activity and sedentary behavior on subclinical atherosclerosis: a cross-sectional study among Mexican Americans. BMC Public Health. 2019;19(1):161.

Design: Cross-sectional study	
Level of documentation	II
GRADE	⊕⊕◯◯

Objective/Aim

To examine the combined effects of sedentary behaviour and physical activity on carotid intima-media thickness (cIMT) and presence of carotid plaque in a Mexican American population on the Texas-Mexico border.

Conclusion

The results indicate that for Mexican Americans, there is a combined effect of sedentary behaviour and physical activity in presence of carotid plaque. Participating in moderate physical activity is optimal for having lower levels of carotid plaque in addition to avoiding excessive levels of TV/movie sitting (>3 h/day) and/or total sitting (>8.5 h/day).

Country

USA

Year of data collection

2010-2017

Methods

Data foundation: Cameron County Hispanic Cohort (CCHC)

Exclusions: 95 with missing data on sedentary behaviour, 22 due to reporting a non-normal week of sitting or extreme values.

Data material: 612 participants were recruited randomly to join the study.

Exposure and outcome: Carotid ultrasound was used to measure cIMT and presence of carotid plaque. Self-reported questionnaires were used to assess leisure time physical activity and sedentary behaviour (TV/movie sitting and total sitting).

Statistical methods: A multivariable linear regression model was used to determine whether physical activity and TV/movie sitting were significantly associated with mean cIMT. Two multivariable linear regression models were used to determine whether physical activity and TV/movie sitting were significantly associated with presence of plaque and with cIMT $\geq 75^{th}$ percentile. First set included physical activity and sedentary behaviour as independent variables. Second set included age and gender, and a third set included additional demographic and health variables.

Results

The study found no significant association between physical activity, sedentary behaviour and mean cIMT, or IMT thickness $\geq 75^{th}$ percentile for age and gender. However, there was a significant interaction between physical activity and TV/movie sitting with presence of carotid plaque. Participants who reported moderate levels of physical activity had significantly lower odds for presence of plaque compared to participants with no activity when TV/movie sitting time was <=3 hours per day. However, there was no significant difference in odds for presence of plaque between physical activity groups when TV/movie sitting exceeded 3 hours/day. These results were consistent with models examining total sitting time.

Discussion and commentaries

Checklist:
-Did the study address a clearly focused issue? **YES**
-Was the study based on random selection of suitable participants? **YES**
-Were inclusion/exclusion criteria clearly defined? **YES**
-Were all participants representative of a specified population? **YES**

-Was the response rate high? **UNCLEAR**
-Were objective criteria used to assess/validate outcome measures? **YES**
-Have authors identified all relevant confounders? **YES**
-Are prognostic/confounding factors considered in the study design and/or analysis? **YES TRADITIONAL, BUT NOT ALL**

-Do you trust the results? **YES**
-Can the results be applied to the local population? **YES**
-Do the results of this study support other available evidence? **YES**

Strengths: homogenous population of Hispanics. Analysed for interplay between physical activity and sedentary behaviour to gain better understanding of activity profile, which there lacks literature.
Limitations: cross sectional study, self-reported measures of physical activity and sedentary behaviour. Some confounding factors were not included.

Reference:
Lazaros G, Oikonomou E, Vogiatzi G, Christoforatou E, Tsalamandris S, Goliopoulou A, et al. The impact of sedentary behavior patterns on carotid atherosclerotic burden: Implications from the Corinthia epidemiological study. Atherosclerosis. 2019;282:154-61.

Design: Cross-sectional study	
Level of documentation	II
GRADE	⊕○○○

Objective/Aim

To examine the impact of different components of sedentary lifestyle on carotid atherosclerotic burden in an effort to generate new clues in the role of sedentary behaviour on cardiovascular health.

Conclusion

The results indicate that the findings have an important public health implication, providing a better understanding of the components of sedentary behaviour that are associated with atherosclerotic progression.

Country

Greece

Year of data collection

2015-2017

Methods

Data foundation: The Corinthia Study

Data material: 2043 inhabitants of the Corinthia region aged 40 years old or older.

Exposure and outcome: Evaluation of physical activity (exposure) was done using the self-reported International Physical Activity Questionnaire (IPAQ). Based on specific questions, the average hours per week spent on watching television, videos or DVD was calculated for each participant. IMT (outcome) was measured in both carotid arteries using ultrasonography. The average (mean IMT) and maximum thickness (max IMT) were determined as representative values of subclinical atherosclerosis.

Statistical methods: Linear regression analysis and logistic regression analysis were applied to examine – independently from confounders established in bibliography or from covariates proved significant by the univariate analysis – the association of increasing TV viewing time and total sitting time with the mean cIMT and to odds ratio of atheromatic carotid plaque, respectively. All reported p-values were based on two-sided tests. Exact values of $p<0.05$ were considered statistically significant.

Results

The results show that the prevalence of carotid atheromatic plaque was lower in the low TV viewing time group compared to the moderate and high TV viewing time groups ($p=0.02$). TV viewing time was associated with increased carotid IMT ($p=0.03$) and the prevalence of carotid atheromatic plaque ($p=0.02$), even after adjustment for age, body mass index, cardiovascular risk factors or history of cardiovascular disease. Subjects in the high TV viewing time group have 80% increase odds of carotid atheromatic plaque compared to patients categorized in the low viewing time group ($p=0.01$).

Discussion and commentaries

Checklist:
-Did the study address a clearly focused issue? **YES**
-Was the study based on random selection of suitable participants? **YES**
-Were inclusion/exclusion criteria clearly defined? **YES, INCLUSION**
-Were all participants representative of a specified population? **YES**
-Was the response rate high? **YES**

-Were objective criteria used to assess/validate outcome measures? **YES**
-Have authors identified all relevant confounders? **UNCLEAR**
-Are prognostic/confounding factors considered in the study design and/or analysis? **YES**

-Do you trust the results? **YES**
-Can the results be applied to the local population? **YES, FOR OTHER RURAL REGIONS**
-Do the results of this study support other available evidence? **YES**

Strengths: Random selection
Limitations: Physical activity and sedentary behaviour were self-reported. The results are based on subjects living in semi-rural regions and it may be difficult to extrapolate these findings in inhabitants of urban cities where patterns of activities and sedentary time differentiate. The possibility of residual confounding, by lifestyle or behavioural factors must be considered. Such a confounder could be the presence of osteomuscular disease, i.e. hip or knee osteoarthitis, arthritis or a past surgery, situations which consist a frequent cause of disability and immobility.

Reference:
Bergstrom G, Borjesson M, Schmidt C. Self-efficacy regarding physical activity is superior to self-assessed activity level, in long-term prediction of cardiovascular events in middle-aged men. BMC public health. 2015;15:820

Design: Cohort study	
Level of documentation	II
GRADE	⊕⊕○○

Objective/Aim

To evaluate the association between self-efficacy to perform physical activity, self-reported leisure-time physical activity and cardiovascular events in a population-based cohort of middle-aged Swedish men with no previous cardiovascular disease, or treatment with cardiovascular drugs.

Conclusion

The study found that self-efficacy to perform physical activity was strongly and independently associated with cardiovascular events and was superior to self-assessed physical activity in predicting cardiovascular events during 13-years of follow-up in a group of middle-aged men, without known CVD or treatment with cardiovascular drugs.

Country

Sweden

Year of data collection

1995-1997

Methods

Data foundation: Data gathered at the Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska University Hospital, Gothenberg, Sweden.

Exclusions: CVD, clinical diabetes mellitus (fasting blood glucose >= 6.1 mmol/L or medication), or other clinically overt disease, untreated diastolic blood pressure > 100 mmHg, treatment with cardiovascular drugs, or unwillingness to participate.

Data material: 377 men at age of 58 years. All participants were of Swedish ancestry and lived in the Gothenburg region.

Exposure and outcome: Exposure physical activity measured by the Saltin-Grimby Physical Activity Level Scale. Outcome being cardiovascular events.

Statistical methods: Descriptive statistics were used to summarize baseline characteristics in the total sample and by physical self-efficacy and leisure-time physical activity categories. Comparisons between groups were performed using the Mann-Whitney U or Chi-square-test. To analyse the relationship between two or more categorical variables or non-normal distributed variables, the Kruskal-Wallis test was used. Multi-variable logistic regression analysis was used to explore the association between risk factors and cardiovascular events, with cardiovascular events as the dependent variable and adjusting for a number of co-variates in different models. All data management and statistical analysis were conducted using PASW Statistics 18 (SPSS Inc.).

Results

The group with poor self-efficacy to perform physical activity had a significantly higher incidence of cardiovascular events compared with the group with good physical self-efficacy (32.1% vs 17.1%, p<0.01). Multivariate analysis showed that poor physical self-efficacy was associated with an increased relative risk of 2.0 (95% CI 1.2 to 3.0), of having a cardiovascular event during follow-up also after adjustments for co-variates such as waist to hip ratio, heart rate, fasting plasma glucose, serum triglycerides, systolic blood pressure, apoB/apoA-I ratio and leisure-time physical activity.

Discussion and commentaries

Checklist:
-Did the study address a clearly focused issue? **YES**
-Was the study based on random selection of suitable participants? **YES**
-Were inclusion/exclusion criteria clearly defined? **YES**
-Were all participants representative of a specified population? **YES**
-Was the response rate high? **UNCLEAR**
-Were objective criteria used to assess/validate outcome measures? **YES**
-Have authors identified all relevant confounders? **UNCLEAR**
-Are prognostic/confounding factors considered in the study design and/or analysis? **YES**
-Do you trust the results? **YES**
-Can the results be applied to the local population? **UNCLEAR, but probably for same age groups with same ethnicity**
-Do the results of this study support other available evidence? **YES**

Strengths:
Study group is homogenous which control for variation in age, sex and ethnicity. They were free of given diseases and therefore did not did not take any treatment which could have confounded the interpretation of the results.

Limitations:
VAS provide a subjective measure and may be subject to higher error rates. The MSE question used for assessing self-efficacy cannot distinguish if a subject has different self-efficacy for different activities. External validity that it only included men of Swedish ancestry within a limited age category and may not be extended to other groups.

<table>
<tr><td colspan="3">Reference:
Wang D, Jackson EA, Karvonen-Gutierrez CA, Elliott MR, Harlow SD, Hood MM, et al. Healthy Lifestyle During the Midlife Is Prospectively Associated With Less Subclinical Carotid Atherosclerosis: The Study of Women's Health Across the Nation. Journal of the American Heart Association. 2018;7(23):e010405.</td><td>Design: Cohort study
Level of documentation II
GRADE ⊕⊕○○</td></tr>
<tr><th>Objective/Aim</th><th>Methods</th><th>Results</th><th>Discussion and commentaries</th></tr>
<tr><td>To investigate prospective associations between a healthy lifestyle during the midlife and subclinical atherosclerosis.

Conclusion
The results show that healthy lifestyle during the menopausal transition is associated with less subclinical atherosclerosis, highlighting the growing recognition that the midlife is a critical window for cardiovascular prevention in women.

Country
USA

Year of data collection
1996-2015/2016</td>
<td>Data foundation: The Study of Women's Health Across the Nation (SWAN)

Exclusions: missing data on carotid measurements, self-reported heart disease or stroke, developed heart disease or stroke during follow up, reporting of too many or too few solid foods per day, skipped more than 10 food items on the food frequency questionnaire or reported a total energy intake that was too low or too high, incomplete data on the components of HLD for all visits, missing data for the major covariates.

Data material: 1143 women aged 42 to 52 years. Self-reported data on smoking, diet and physical activity in the SWAN study were used to construct a 10-year average HLS score during the midlife. Markers of subclinical atherosclerosis were measured 14 years after baseline and included common carotid artery intima-media thickness, adventitial diameter and carotid plaque.

Variables: smoking, physical activity and diet quality as exposure variables, atherosclerosis of carotid artery by ultrasound measurement as outcome variable.

Statistical methods: Linear models were used to assess the associations between average healthy lifestyle score (HLS) and carotid intima-media thickness and adventitial diameter. Cumulative logit models were used to assess association between average HLS and carotid plaque.</td>
<td>Average HLS was associated with smaller common carotid artery intima-media thickness and adventitial diameter in the fully adjusted models (p=0.0031 and <0.001, respectively).

Compared with participants in the lowest HLS level, those in the highest level had 0.024 mm smaller intima-media thickness (95% confidence interval: -0.048, 0.000), which equals 17% of the standard deviation of intima-media thickness, and 0.16 mm smaller adventitial diameter (95% confidence interval: -0.27, -0.04), which equals 24% standard deviation of adventitial diameter.

Among the 3 components of the HLS, abstinence from smoking had the strongest association with subclinical atherosclerosis.</td>
<td>Checklist:
-Did the study address a clearly focused issue? YES
-Was the study based on random selection of suitable participants? YES
-Were inclusion/exclusion criteria clearly defined? YES
-Were all participants representative of a specified population? YES
-Was the response rate high? YES, >70%

-Were objective criteria used to assess/validate outcome measures? YES
-Have authors identified all relevant confounders? YES
-Are prognostic/confounding factors considered in the study design and/or analysis? YES

-Do you trust the results? YES
-Can the results be applied to the local population? YES
-Do the results of this study support other available evidence? YES

Strengths: repeated measurements, broad diversity of racial/ethnic composition
Limitations: outcome variable (subclinical carotid atherosclerosis) was measured only once, thus no indication of change. Could not completely rule out the extent of reversed causation. Exposure data had some measurement errors, especially for the self-reported data on physical activity and dietary intake. Did not capture lifestyle exposure for the whole period.</td></tr>
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