

Arterial stiffness and risk factors for cardiovascular disease  
in young adults

*Education is the most powerful weapon we can use to change the world*  
*Nelson Mandela, 2003*

*To my beloved children,  
Ingrid and Karl-Johan*

*Örebro Studies in Medicine 201*



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**Arterial stiffness and risk factors for cardiovascular  
disease in young adults**

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## Abstract

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Atherosclerosis is a complex, chronic vessel wall disease that often leads to severe and acute cardiovascular diseases (CVD), such as myocardial infarction and stroke. CVD are the most common cause of death, both globally and in Sweden. Since most of the risk factors for atherosclerosis are preventable, it is of great importance to highlight the benefits of a healthy lifestyle to young adults who are about to create their own habits.

A general concern about physical inactivity, low cardiorespiratory fitness (CRF), and high body mass are supported by reports of an increased incidence and prevalence of obesity worldwide. In addition to this, the proportion of Swedish adults with low CRF almost doubled the last 20 years and the decline in CRF is more pronounced in the youngest age group.

The scientific work presented in this thesis was carried out to investigate the impact of different lifestyle related factors on vascular status, especially arterial stiffness, in young Swedish adults. In total 840 young adults in the age range 18-25 years were recruited to the cross-sectional Lifestyle, Biomarkers, and Atherosclerosis (LBA) study, to examine vascular status, biomarkers, and lifestyle related factors.

In the LBA study population of young adults in Sweden, 12% were classified as being at risk of future CVD. A high CVD risk was associated with low CRF and less physical activity. In the total study population 24% had unhealthy food habits, and 24% did not spend the recommended 30 minutes per day at moderate or vigorous intensities of physical activity. Low CRF, less physical activity, and overweight and obesity, were associated with stiffer arteries.

The results raises concerns about future CVD risk and highlights the health enhancing possibilities of high CRF and physical activity on vascular status in young Swedish adults.

*Keywords:* Cardiovascular disease, atherosclerosis, arterial stiffness, pulse wave analysis, intima media thickness, cardiorespiratory fitness, physical activity, body composition, young adults.

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## List of papers

This thesis is based on the following original papers, which will be referred to in the text by their roman numerals:

- I. Fernström M\*, Fernberg U\*, Eliason G, Hurtig-Wennlöf A. Aerobic fitness is associated with low cardiovascular disease risk: The impact of lifestyle on early risk factors for atherosclerosis in young healthy Swedish individuals – The Lifestyle, Biomarkers, and Atherosclerosis study. *Vasc Health Risk Manag* 2017; 13: 91-99.  
*\*Both authors contributed equally to this work*
- II. Fernberg U, Fernström M, Hurtig-Wennlöf A. Arterial stiffness is associated to cardiorespiratory fitness and body mass index in young Swedish adults: The Lifestyle, Biomarkers, and Atherosclerosis study. *Eur J Prev Cardiol* 2017; 24(17): 1809-1818.
- III. Fernberg U, op 't Roodt J, Fernström M, Hurtig-Wennlöf A. Body composition is a strong predictor of local carotid stiffness in Swedish, young adults – The cross sectional Lifestyle, Biomarkers, and Atherosclerosis study. *BMC Cardiovasc Disord.* 2019;19(1):205.
- IV. Fernberg U, Fernström M, Hurtig-Wennlöf A. Higher total physical activity is associated with lower arterial stiffness in Swedish, young adults – The Lifestyle, Biomarkers, and Atherosclerosis study. *Submitted manuscript.*

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## **Additional studies**

Studies not included in this thesis:

Fernström M, Fernberg U, Hurtig-Wennlöf A. Insulin resistance (HOMA-IR) and body fat (%) are associated to low intake of fruit and vegetables in Swedish, young adults: The cross sectional Lifestyle, Biomarkers, and Atherosclerosis study. *BMC Nutrition*. 2019; 5:15.

## List of abbreviations

AIx	Augmentation index
AIx_HR75	AIx adjusted to heart rate 75 beats /min
Art dist	Arterial distensibility
BMI	Body mass index
Body fat	Percentage of body fat
$\beta$ Stiffness	$\beta$ Stiffness index
cIMT	Carotid intima media thickness
CRF	Cardiorespiratory fitness
CVD	Cardiovascular disease
DBP <sub>brach</sub>	Brachial diastolic blood pressure
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
hs-CRP	high-sensitive C-reactive protein
LBA study	Lifestyle, Biomarkers, and Atherosclerosis study
LDL-C	Low-density lipoprotein cholesterol
LPA	Light physical activity
MAP <sub>brach</sub>	Brachial mean arterial pressure
MPA	Moderate physical activity
MVPA	Moderate and vigorous physical activity
PA	Physical activity
PP <sub>brach</sub>	Brachial pulse pressure
PWA	Pulse wave analysis
PWV	Pulse wave velocity
SBP <sub>brach</sub>	Brachial systolic blood pressure
SD	Standard deviation
Sed	Sedentary time
VO <sub>2</sub> max	Estimated maximal oxygen uptake
VPA	Vigorous physical activity
Waist	Waist circumference
WHO	World Health Organization
YEM	Young's elastic modulus

# Introduction

## **The Lifestyle, Biomarkers, and Atherosclerosis (LBA) study**

The Lifestyle, Biomarkers, and Atherosclerosis (LBA) study is an epidemiological, cross-sectional study conducted at Örebro University, Sweden. The main aim of the LBA study is to identify biomarkers and lifestyle factors that could be involved in the early development of atherosclerosis in young adults, to gain increased knowledge about the underlying mechanisms that cause cardiovascular diseases (CVD) in the long-term. This knowledge could contribute to preventive actions or therapeutic strategies against CVD.

## **Study design**

Data collection in the LBA study started in October 2014 and was closed in June 2016. In total, 840 individuals volunteered to participate in the study during the data collection period. The individuals were included if they were non-smokers, self-reported healthy, without any chronic disease, and in the age range 18.0-25.9 years. All study participants came for two visits with approximately one week in between visits.

At the first visit the study participants filled in a computerized, validated questionnaire [1] about their general mental and physical health. Questions about family background, exercise habits, and heredity of cardiovascular disease and diabetes were also answered. The study participants also filled in the questionnaire “Matvanekollen” [2], about food habits, from the Swedish National Food Agency. The examinations made at the first visit were blood pressure, flow-mediated dilation - an ultrasound examination of the brachial artery to assess endothelial function, blood sample collection for analyses of lipid- and glucose metabolism, and markers of inflammation and coagulation. Body composition measurements as height, length, waist circumference, and percentage of body fat was also taken, and at the end of the first visit all study participants were equipped with an accelerometer to assess physical activity (PA) during seven consecutive days.

At the second visit the study participants were examined with ultrasound at the carotid artery to measure intima media thickness (cIMT). Pulse wave analysis (PWA) was made at the radial artery for calculation of central blood pressure and augmentation index (AIx). Carotid-femoral pulse wave velocity (PWV) was performed to assess arterial stiffness. The second visit

ended with an ergometer bike test to estimate maximal oxygen uptake ( $\text{VO}_2\text{max}$ ) as a measurement of cardiorespiratory fitness (CRF).

The study participants was offered breakfast at the end of the first visit and at the second visit they were given some results from the blood analyses and the ergometer bike test. No other compensation was given to the individuals participating in the study.

### **My contribution in the LBA study**

During the summer of 2014, I was involved in planning the startup of the data collection period. I visited the Maastricht University Medical Center, Maastricht, Netherlands to learn and discuss some methods that was used in the Maastricht Study, that we also planned to use in the LBA study, and I trained at conducting the examinations over the summer. In September, I was responsible for beginning the recruitment of individuals who volunteered to participate in the study. The recruitment was performed in local schools, in classes at Örebro University, and in workplaces. Information about the study was given orally, through posters at announcement boards, through social media, by advertisement in the local newspaper, and on the University homepage during the whole data collection period. A contract was made with a booking site to make it possible for the study participants to book their own visits in our study schedule. In October 2014, the study team started to examine study participants from 8 a.m. until 4 p.m. One day every week we had examinations until 7 p.m. to meet study participants who wanted a later visiting hour. I performed examinations almost every day, and I also prepared and downloaded data from the accelerometers.

The recruitment of study participants and the data collection period continued until June 2016, with breaks for Christmas and summer vacation. Thereafter the process started of entering, cleaning, and analyzing data based on different research questions. The results are presented in this thesis. Periodically, during my research, I worked part time as a lecturer at Örebro University.

### **The burden of cardiovascular diseases (CVD)**

CVD are a group of disorders that affect the heart or the blood vessels in the body, for example coronary heart disease, cerebrovascular disease (stroke), peripheral artery disease, deep vein thrombosis, pulmonary embolism, rheumatic heart disease, and congenital heart disease. In 2016, approximately 17.9 million people died from CVD worldwide, making CVD the most common cause of death globally [3]. In Europe, CVD causes more

than half of all deaths [4]. CVD are also the most common cause of death in Sweden (37%). About 1.8 million people in Sweden are affected by CVD [5]. According to the global INTERHEART study, nine risk factors are associated with more than 90% of the risk of an acute myocardial infarction. These preventable risk factors are hypertension, hyperlipidemia, smoking, diabetes, abdominal obesity, psychosocial factors, a low consumption of fruit and vegetables, a high consumption of alcohol, and physical inactivity [6].

A review [7] addressing young adults' (18-34 years) knowledge of and attitudes to CVD and its risk factors demonstrates that the knowledge is limited regarding CVD and risk factors, and the attitude poor towards CVD prevention. The late manifestation of CVD may be one explanation for this lack of knowledge. Since most of the risk factors are preventable, except for increasing age, male sex, and hereditary factors, it is of great importance to highlight the benefits of a healthy lifestyle for young adults who are about to create their own habits [8].

### **Atherosclerosis**

Atherosclerosis is a complex, chronic vessel wall disease that often leads to severe and acute CVD, such as myocardial infarction and stroke. The atherosclerotic process is slow, starts already in childhood, and develops over decades [9, 10]. The formation of atherosclerotic plaques is initiated by endothelial damage and an inflammatory cell activation. After entrance of small low-density lipoproteins (LDL) into the vessel wall, the LDL is oxidized causing adhesion of monocytes and lymphocytes to the endothelium. Oxidized LDL (ox-LDL) stimulate smooth muscle cells (SMCs) and endothelial cells to secrete factors causing monocytes recruitment. The monocytes differentiate into macrophages and express several receptors. Interaction of ox-LDL and cluster of differentiation 36 receptors (CD36) activates the macrophages and initiates macrophage retention, while ox-LDL-scavenger receptor interaction leads to an uptake of ox-LDL in the macrophages and the formation of foam cells [11]. A large amount of foam cells form lesions ("fatty streaks"), and growth factors are released by the macrophages causing SMC proliferation and migration from the media into the intima, forming a fibrous plaque [8]. The SMC proliferation contributes to thickening of the atherosclerotic plaque that may calcify and obstruct the vessel lumen, causing ischemic damage and tissue death in vital organs, such as the brain and the heart [11].

The development of fatty streaks begins already in childhood. In adolescence, some fatty streaks develop into fibrous plaques by the accumulation of more lipids and the formation of a fibromuscular cap [10]. Taking this into account, you could expect some fatty streaks and infant fibrous plaques in the LBA population of young adults in the ages 18-25 years.

Possible risk factors for atherosclerosis are age, hypertension, hypercholesterolemia, adiposity, diabetes, cigarette smoking, physical inactivity, race, and male sex [8].

## **Lifestyle related factors**

According to The National Board of Health and Welfare in Sweden, lifestyle factors include tobacco use, alcohol consumption, physical activity, and food habits. Tobacco use, a high risk alcohol consumption, physical inactivity, and bad food habits can all lead to the development of different diseases, a low quality of life, and early mortality [12]. In the LBA study so far, we have focused on the lifestyle factors food habits, assessed by questionnaire, and physical activity, objectively measured by a motion sensor. We also broaden the perspective of lifestyle factors by using the concept of lifestyle related factors where we included body composition, CRF, and handgrip strength.

## **Body composition**

Obesity has nearly tripled worldwide during the last four decades and is of growing concern since it is a risk factor for CVD and several other non-communicable diseases [13, 14]. The adiposity levels rises fastest in children, adolescents, and young adults [15], and the prevalence of obesity is increasing in both developed and developing countries [14]. Using the WHO Body Mass Index (BMI) definitions [16], the Public Health Agency of Sweden reported in 2018 that 51% of the total Swedish population was overweight or obese. Overweight and obesity were more common in older than in younger people. In the age group 16-29 years, 31% was overweight or obese. However, during the last decade overweight and obesity increased most in the age group 16-29 years. From 2006-2018 it increased from 22%-31% [17].

Body composition can be measured with several methods. In addition to BMI ( $\text{kg/m}^2$ ), an estimation of percentage of body fat can be assessed using an impedance body composition analyzer [18], and waist circumference can be used for estimation of central fat distribution. Waist circumference is

included as a criteria in the definition of metabolic syndrome [19] as a marker of central obesity.

### **Fitness - Cardiorespiratory fitness and handgrip strength**

Cardiorespiratory fitness (CRF) reflects the ability of the circulatory and respiratory system to transport oxygen to the muscle cells to perform physical activity. CRF involves the function of numerous systems in the body and is considered to reflect total body health [20]. Approximately 50% of the variation in CRF is attributable to heritable factors [21].

An individual's CRF level can be measured directly in a laboratory setting, expressed as the individual's absolute (ml/min) or relative (ml/kg/min) maximal oxygen consumption ( $VO_2\text{max}$ ). The CRF level can also be assessed by estimating  $VO_2\text{max}$  from a submaximal exercise test, using the relation between the incremental heart rate response and the work rate [20, 22].

Low CRF is a strong independent predictor of CVD and all-cause mortality [23-26]. Furthermore, having moderate or high levels of CRF seems to be protective, even in the presence of other risk factors for CVD. In a large observational cohort study [23], "high fit persons", with multiple risk factors, had lower death rates than "low-fit persons" with no other risk factors for CVD. In a Swedish study, reporting associations between CRF, CVD morbidity, and all-cause mortality, in a large cohort including over 260 000 women and men in the ages 18-74 years, risk reduction per ml/kg/min for all-cause mortality was steeper in the groups with the lowest CRF. The authors conclude that preventive actions to increase CRF, especially in the vulnerable groups, is a clear public health priority [26].

Since 1995 there has been a decline in CRF in Swedish adults. The proportion of individuals with low CRF (<32 mL/kg/min) almost doubled, from 27% to 46% between 1995 and 2017. The decrease in  $VO_2\text{max}$  (both absolute and relative) was more pronounced in men than in women, and in the youngest age group (18-34 years) compared to the middle (35-49 years), and oldest age group (50-74 years) [27].

Grip strength measured with dynamometry is an indicator of an individual's overall strength and a predictor of cardiovascular and all-cause mortality [28]. Muscle strength was measured with a handgrip test in the LBA study.

## **Food habits**

In 2012, it was reported from a Swedish population-based 25 year follow-up study [29] that the intake of fat, especially saturated fat, had increased since 2004, in both women and men. The increasing intake of fat was followed by an increase in serum cholesterol and a continuously increasing BMI. The increased intake of fat came in parallel with the low-carbohydrate and high-fat diets that became popular for weight loss and control of blood glucose levels. In the LBA study, the food habits of the study participants were evaluated with a questionnaire from the Swedish National Food Agency. The questions were based on the dietary recommendations from the Swedish National Food Agency [30] and categorized into seven diet groups; bread (whole grain), fish and seafood, fruit and vegetables, fat, cheese, sweets (candy, buns, soft drink, and French fries), and fast food including sausages. The questions about fat, cheese, and fast food were related to the total intake of fat and type of fat, saturated or unsaturated. The results of the questionnaire mirrors the food habits.

## **Physical activity**

Physical activity (PA) is defined as “any bodily movement produced by skeletal muscles that result in energy expenditure beyond resting energy expenditure” [31]. It is well established that PA is protective in the development of atherosclerosis [32], that regular PA is associated with a decrease in cardiovascular and all-cause mortality [33], and that it reduces the risk of several health outcomes, such as hypertension, CVD, type 2 diabetes mellitus, thromboembolic stroke, obesity, osteoporosis, colon cancer, breast cancer, anxiety, and depression [34]. Several studies have also explored the relationship between objectively measured PA and different stiffness measurements [35-38], in young adults, and found that higher PA is associated with lower levels of arterial stiffness.

PA is divided into four main domains of activity; leisure-time PA, work- or school-related activity, household activities, and activity for transport. Exercise is a sub-category of leisure-time physical activity and is not synonymous with PA [39]. PA can also be classified according to the type of activity performed, e.g. walking, running, or bicycling; or according to when the activity is performed e.g. on weekdays or weekends; or according to whether the activity is e.g. intermittent or continuous, voluntary or compulsory [31].

Frequency, duration, and intensity are three basic characteristics when describing PA. The frequency describes how often the activity occurs over a

predetermined time period. The duration describes for how long time an activity is performed and the intensity refers to the level of effort that is needed to perform the activity [39]. The many ways to classify and describe PA mirrors the fact that PA is a complex behavior.

PA can be measured with subjective and objective methods. An objective way to measure PA over time is to use an accelerometer, a monitor that measure body movements in terms of mainly vertical acceleration [40].

### Physical activity recommendations

PA recommendations for healthy adults, 18-65 years, state that it is important to have a physically active lifestyle to promote and maintain good health. According to recommendations you should perform aerobic physical activity at a moderate intensity level of at least 150 minutes per week, or do aerobic vigorous-intensity physical activity for at least 75 minutes per week. Combinations of moderate and vigorous aerobic activities can be performed to meet the recommendations. The recommended amount of PA can be divided into bouts lasting at least 10 minutes. Muscle strengthening activities should also be performed at least two times a week involving large muscle groups [41]. In the updated Physical Activity Guidelines for Americans from 2018, the recommendation of performing PA in at least 10 minutes bouts has been removed. The message to the Americans is, sit less and move more. There is no lower threshold for benefits of PA, all activity is better than nothing [42].

### **Biomarkers**

There are several biomarkers of lipoprotein metabolism, glucose metabolism, and inflammation that are considered to be risk factors for CVD [10, 43]. An elevated total cholesterol level is a traditional risk factor associated with atherosclerosis in both women and men. The most common risk factors for elevated cholesterol levels are a diet with a high intake of saturated fat, particularly elevating the low-density lipoprotein cholesterol (LDL-C) level, physical inactivity, smoking, overweight and obesity, and heredity [44].

A low level of high-density lipoprotein (HDL-C), which is protective against the development of CVD, is also a risk factor for atherosclerosis. On the other hand, aerobic fitness has effects on the blood lipid profile, giving a reduction in total cholesterol, LDL-C, and triglycerides, independent of weight reduction [45].

C-reactive protein (CRP) is a sensitive marker of systemic inflammation and has been shown to be a predictor of future cardiovascular outcomes [46]. Increased PA leads to lower circulating levels of CRP [47], and PA and exercise training are inversely associated with CRP [48].

A frequently used marker of insulin resistance is the Homeostasis Model Assessment Insulin Resistance (HOMA-IR). Glucose and insulin concentrations are incorporated in the calculation of HOMA-IR, which is considered to be a stronger marker of CVD than glucose and insulin by themselves [49]. PA and aerobic fitness are also known to prevent insulin resistance due to their effects on insulin sensitivity [50].

### **Wildman risk score**

The definition by Wildman [51, 52] was used to classify individuals who are at risk for atherosclerosis and CVD. Individuals having two or more of the following characteristics were classified as being at risk according to Wildman's cut off values; elevated blood pressure (130/85 mmHg), elevated triglycerides ( $\geq 1.70$  mmol/L), decreased HDL-C (women  $< 1.30$  mmol/L, men  $< 1.04$  mmol/L), elevated glucose ( $\geq 5.6$  mmol/L), Insulin resistance (HOMA-IR  $> 2.52$ ), and elevated hs-CRP ( $> 5.07$  mg/L).

### **Blood pressure**

Brachial blood pressure is a well-established risk marker and predictive of cardiovascular outcome. Hypertension, a sustained elevated brachial blood pressure, is a major risk factor for CVD [53, 54]. According to the WHO, 1.3 billion people worldwide suffer from hypertension, and less than one of five have their hypertension under control. Risk factors for hypertension are physical inactivity, tobacco use, overweight and obesity, and an unhealthy diet containing a low intake of fruit and vegetables and a high intake of saturated fats and salt [54].

Peripheral and central blood pressure have been measured in the LBA study. The diastolic and mean arterial blood pressure are relatively similar in the arterial tree while the systolic blood pressure is higher in the peripheral than in the central arteries. The phenomenon is called pulse amplification and is more pronounced in younger individuals than in older [8]. Central blood pressure is considered to be more relevant from a pathophysiological perspective for the pathogenesis of CVD than peripheral pressure [55]. The longitudinal Strong Heart Study demonstrated that central aortic pressure was more strongly associated with atherosclerosis than brachial

blood pressure, and that central pulse pressure more strongly predicted cardiovascular outcome than did brachial blood pressure [56].

Brachial blood pressure can be measured non-invasively in several ways. By decreasing the air pressure in an inflated sphygmomanometer cuff placed around the upper arm, the arterial blood pressure can be determined by palpation or by audible detection with a stethoscope over a. brachialis. More automated techniques have been developed in the last decades, and the oscillometric method is one of them. The oscillations in the arterial wall, produced by the pulsatile flow, are transmitted to a cuff around the upper arm. After inflation of the cuff, over the expected systolic pressure, the air pressure in the cuff is decreased (deflation). During the deflation, the amplitude of the pressure oscillations increases, reaches a maximum, and decreases again. The cuff pressure at the initial increase in pressure oscillations corresponds to the systolic pressure, the maximum amplitude of pressure oscillations to the mean arterial pressure, and the cuff pressure just before the oscillations stop decreasing in amplitude, the diastolic pressure [8].

The central pressure can be determined by applanation tonometry. By putting a light pressure with the tonometer over the arterial pulse, the underlying vessel wall is flattened, and pressure wave forms can be registered [57]. The central aortic pressure can be approximated through different methods:

Firstly, by applying a general transfer function to the recorded radial pressure wave (or the carotid pressure wave), the ascending aortic pressure can be generated [58]. This method, when applanating the radial artery, is approved in expert consensus documents [55, 59].

Secondly, by use of the second systolic peak of the radial waveform. It has been shown that the second systolic peak of the radial waveform approximates with the maximal systolic pressure in the aortic waveform [60]. This method works only when the second systolic peak is present, which is not the case in most young adults [8].

Thirdly, by assuming that the diastolic and mean arterial pressure are similar in a peripheral artery and the carotid artery [61] it is possible to determine carotid systolic pressure, by using the peripheral diastolic and mean arterial pressure for calibration and extrapolating to the systolic carotid pressure. The carotid pressure wave can then be used as a surrogate of the proximal and the ascending aortic pressure, and is used as a measure of central pressure [8]. The systolic blood pressure is approximately 2 mmHg higher in the carotid artery than in the aorta [62].

## Arterial markers

Carotid intima media thickness (cIMT) and arterial stiffness are considered to be two arterial markers with the ability to predict the risk of future cardiovascular events and cardiovascular mortality [8].

### Carotid intima-media thickness

The distance between the lumen-intima and the media-adventitia interfaces reflects the intima media in the arterial wall and makes the carotid intima-media thickness (cIMT) useful as a marker of preclinical atherosclerosis [63, 64]. A linear relationship between age and cIMT has been demonstrated in several studies [65-67], and an increased cIMT is associated with vascular risk factors and the presence of more advanced atherosclerosis. cIMT is also a strong predictor of future cardiovascular events. However, in young populations with lower cIMT, the relationship between cIMT and vascular risk factors needs further study [63]. The cIMT is measured with high-resolution ultrasound according to international guidelines [68]. Because of the non-invasive examination technique, the measurement is easy to use in large population studies [63]. In the age group 24-39 years, the average increase in cIMT is close to 0.6  $\mu\text{m}/\text{year}$  [69].

### Arterial stiffness

Arterial stiffness describes the rigidity of the arterial walls [70] and is determined by the vascular smooth muscle tone, the arterial pressure, and the elastin and collagen content of the vessel wall [8]. Along with healthy ageing, there is a progressive stiffening of the elastic arteries, this has been described in several longitudinal cohort studies [71-73]. The repetitive pulsations in the arteries causes the elastin lamellae in the media to become frayed and fractured by mechanical stress and the collagen fibers to increase. The arteries respond with stiffening and dilation [74]. Arterial stiffness is associated with the presence of cardiovascular risk factors and atherosclerotic disease in adults [75], and in children and adolescents obesity is associated with arterial stiffness [76].

A recently published study [77] investigated the relationship between adiposity in children and adolescents and arterial stiffness (measured as carotid-femoral pulse wave velocity) at the age of 17. The results showed that a high fat mass during childhood, measured with dual-energy x-ray absorptiometry (DEXA), was associated with greater arterial stiffness at age 17. This adverse effect on arterial stiffness was noticeable regardless the meta-

bolic profile of the study participants. However a vulnerable metabolic profile (i.e. three or more of the following: high levels of systolic blood pressure, triglycerides, glucose (all >75th percentile), and low HDL-C (<25th percentile)) further aggravated the arterial stiffness. The study also showed that the children who normalized their fat mass during adolescence had a normal PWV at the age of 17 years, comparable to those who had normal fat mass throughout.

Arterial stiffness can be assessed over an arterial segment in the arterial tree, a regional stiffness measurement, or at a specific location, a local stiffness measurement [78].

### Regional stiffness measurements

Carotid-femoral pulse wave velocity (PWV) is a measure of regional arterial stiffness of the segment between the two measurement sites. PWV is considered as the gold standard method for assessing arterial stiffness, mainly in the aortic tract [59, 79], and it is a non-invasive, robust, and reproducible technique [80]. Several longitudinal studies have reported the predictive value of using PWV as a measurement of arterial stiffness to estimate cardiovascular mortality [59]. PWV is calculated by the formula:

$$\text{PWV (m/s)} = \text{dist. between measurement locations (m)} / \text{transit time (s)}$$

Another indirect index of regional arterial stiffness is the augmentation index (AIx). The arterial pressure wave, Figure 1, is a combination of the forward pressure wave, generated by the left ventricle, and the reflected wave originating from peripheral points in the arterial tree, for example at bifurcations [70]. In elastic arteries with low PWV, the reflected wave will arrive during diastole or late systole. It then contributes to improved diastolic perfusion of the coronary arteries. In stiffer arteries, with higher PWV, the reflected wave will return faster to the aortic root. It then causes augmentation of the systolic pressure, an increased load for the left ventricle, and less diastolic coronary perfusion [81, 82].

The arterial pressure wave is detected by applanation tonometry, usually at the radial artery, and analyzed with pulse wave analysis (PWA) [59]. By applying a generalized transfer function [58], an aortic pressure waveform can be calculated from the radial pressure wave form. The AIx can be derived from the aortic pressure waveform and is defined as the difference

between the second and the first systolic peaks (augmentation pressure) expressed as a percentage of the pulse pressure [8, 70], Figure 1. For interpretation, higher values of PWV and AIx indicate stiffer vessels [8].

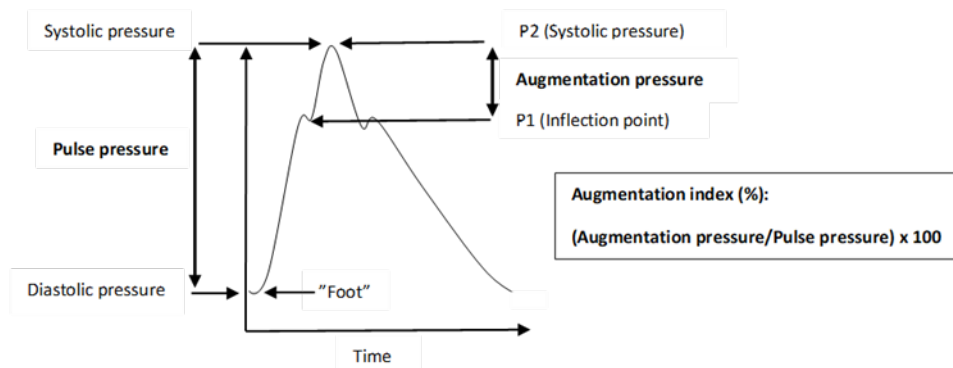


Figure 1. The augmentation is defined as the difference between the height of the late systolic peak (P2) and the inflection point (P1), which is the beginning upstroke of the reflected wave. The ratio of augmentation pressure to pulse pressure defines the augmentation index (AIx) in percent. AIx is adjusted to heart rate 75 bpm (AIx<sub>HR75</sub>).

### Local stiffness measurements

Since atherosclerosis is common in the carotid artery, the specific local carotid stiffness can be of particular interest [59]. Increased carotid stiffness is associated with atherosclerotic plaque presence and stroke risk [83].

There are several descriptors of local carotid stiffness. The change in vessel diameter between systole and diastole is the absolute distention ( $\mu\text{m}$ ). The distention is included together with local pulse pressure in the calculation of arterial distensibility ( $\text{kPa}^{-1}$ ) [83]. The distensibility measures the ability of the arteries to expand in response to changes in blood pressure caused by cardiac relaxation and contraction. A formula that, in addition to blood pressure, also takes into account the arterial wall thickness is Young's elastic modulus ( $\text{kPa}$ ) [59]. Finally,  $\beta$  Stiffness index (unit-less), an index that accounts for the effect of blood pressure by including the logarithm of the systolic to diastolic ratio in the equation, can be used to assess local arterial stiffness.

The formulas for the local stiffness measurements are as follows [8]:

Absolute distention:

Systolic diameter (Ds) – Diastolic diameter (Dd)

Arterial distensibility:

$(D_s - D_d) / ((\text{Systolic pressure (Ps)} - \text{Diastolic pressure (Pd)}) \times D_d)$

Young's elastic modulus (YEM):

$((P_s - P_d) \times D_d) / ((D_s - D_d) \times h)$  where h is the arterial wall thickness.

$\beta$  Stiffness index:

$(D_d \ln(P_s/P_d)) / (D_s - D_d)$

The intima media thickness is used as a surrogate for total arterial wall thickness in the YEM formula [59]. For interpretation, lower values of arterial distensibility and higher values of YEM and  $\beta$  stiffness index indicate stiffer vessels [83]. The gold standard is to use the local blood pressure in the calculations of the different descriptors of local carotid elasticity. Because of pulse pressure amplification in young subjects with a higher blood pressure in the peripheral arteries, it is important to use the local blood pressure from the same site as where the relative diameter change is measured [59]. The use of brachial pulse pressure may overestimate pulse pressure in central arteries, which results in false lower values of arterial distensibility and false higher values of YEM and  $\beta$  stiffness index [84].

## Rationale

A general concern about physical inactivity, low CRF, and high body mass are supported by reports of an increased incidence and prevalence of obesity worldwide. The increase in overweight and obesity occurs mostly in children, adolescents, and young adults.

In addition to this, the proportion of Swedish adults with low CRF almost doubled the last 20 years and the decline in CRF is more pronounced in the youngest age group. Since most of the risk factors for atherosclerosis are preventable, it is of great importance to highlight the benefits of a healthy lifestyle to young adults who are about to create their own habits, and to find easy tools to detect young adults who need cardiovascular risk follow-up and lifestyle counselling.

Healthy young adults are underrepresented in the CVD literature compared to different patient groups, and based on this fact, this thesis was carried out to investigate the impact of different lifestyle related factors on arterial stiffness in young Swedish adults. This age group could contribute to the detection of early changes affecting the development of CVD.

# Aims

The overall aim of this thesis is to explore the impact of different lifestyle related factors on arterial stiffness in young adults, which could be involved in the early development of atherosclerosis. The knowledge could contribute to preventive actions against cardiovascular disease.

The specific aims for each included paper in the thesis were to:

- Paper I: Assess cardiometabolic biomarkers, cIMT as a marker of subclinical atherosclerosis, and lifestyle factors (food habits, handgrip strength, and maximal oxygen uptake,  $VO_{2max}$ ). Analyze the associations between cIMT and lifestyle factors, and identify subjects at risk of CVD and compare the characteristics of subjects with and without risk of CVD.
- Paper II: Examine the associations between arterial stiffness measurements, PWV and  $AIx_{HR75}$ , and lifestyle related factors, such as body composition and cardiorespiratory fitness.
- Paper III: Explore the hypothesis that local measurements of the common carotid artery are associated with body composition. The measurements used were thickness (cIMT) and stiffness (i.e. arterial distensibility, Young's elastic modulus, and  $\beta$  stiffness index). The study was carried out in a subsample from the Lifestyle, Biomarkers, and Atherosclerosis study.
- Paper IV: Present data of the PA pattern and time spent sedentary, including analyses of gender differences, and to explore the association between PA and arterial stiffness, in this age group.



# Methods

## Study participants

In the LBA study, young individuals volunteered to participate during the data collection period. To be included in the LBA study, the individuals fulfilled the inclusion criteria of being 18.0-25.9 years, non-smoking, self-reported healthy, and without any chronic disease. In total 840 young adults were recruited to the study, and the individuals who met the inclusion criteria made two visits approximately one week apart, to examine vessel status, biomarkers, and lifestyle related factors.

## LBA subsample

A highly specific vessel analysis of the common carotid artery [85] was performed on 220 study participants, randomly selected from the cross sectional LBA study (n=840). The selected study participants were included in the subsample (here after called the LBA subsample) based on the Sphygmocor quality criteria in the Sphygmocor equipment and the ultrasound image. The quality criteria (pulse length variation, pulse height variation, shape deviation, and diastolic variation) in the Sphygmocor equipment needed to be fulfilled with as high quality index as possible (maximum 100%, no one had an index below 80%), and the near and far wall boundaries of the carotid artery needed to be clear and visible in the ultrasound image. The LBA subsample is representative of the LBA study with respect to gender distribution and also evenly distributed across the data collection period. The edge wall tracking of ultrasound B-mode recordings was not performed in all study participants in the LBA study due to technical and time limitations.

## Body composition

Height, weight, percentage of body fat, and waist circumference were measured in the study participant in a fasting state. Height was measured to the nearest 0.5 cm with a fixed stadiometer. The study participants were standing without shoes, with heels together, and with the arms extended alongside the body.

Waist circumference was measured with a flexible, non-stretchable measuring tape to the nearest 0.5 cm. The measurement was performed around the abdomen between the iliac crest and the lowest rib on exhalation [86]. Waist circumference was categorized according to gender-specific cut-off

values where <80 cm for women and <94 cm for men were desirable. A waist circumference >88 cm in women and >102 were classified as central obesity [19].

Weight was measured and percentage of body fat was calculated using an impedance body composition analyzer (Tanita Europe B.V. Tanita BC-418 MA, Amsterdam, Netherlands). The study participants were standing barefoot on the metal surface conductive equipment, holding metal handles, according to the manufacturer's guidelines [18]. Adjustments were made with 1 kg for clothes and the standard setting was used. The study participants gender, age, and length were registered in the body composition analyzer, and the output were, except for BMI and percentage of body fat, an estimation of the basal metabolic rate, fat free mass, and total body water [18]. BMI (kg/m<sup>2</sup>) was calculated and categorized into underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9), and obese (≥30) according to the WHO classification [16].

## **Food habits**

The study participants filled in the computerized food frequency questionnaire "Matvanekollen" from the Swedish National Food Agency [2]. The questions were based on the dietary recommendations from the Swedish National Food Agency [30], and the results were based on the total response to the questionnaire and presented to the study participants directly after the test as a score from 1 to 12 points. Individuals with 1 to 4 points were considered to be unhealthy and were recommended to improve their food habits. Having 5 to 8 points was considered as healthy food habits with some potential for improvement, and the ones categorized with 9-12 points had food habits according to the recommendations from the Swedish National Food Agency. A short feedback from the software was given to the study participants, based on their results.

## **Handgrip strength**

Muscle strength was assessed by Dynamometer (Fabrication Enterprises inc, Baseline® HiRes™ hydraulic hand dynamometer, Irvington, NY, US). Initially, the hand size was measured with a measuring tape, and the dynamometer was adjusted to fit the hand size [87]. Handgrip strength was measured in the dominant hand and the study participants sat with the arm in an angle of 90°. All study participants performed four measurements, one

practice test and three measurements, with one minutes rest in between. The result was calculated as an average of the three measurements.

The study participants where categorized as having low, normal or high muscular strength according to reference values [88]. Limits for handgrip strength categories for females were: low  $\leq 22$  kg, normal 22.1- 34.9 kg, and high  $\geq 35$  kg. For males the corresponding levels were: low  $\leq 37$  kg, normal 37.1 – 56.9 kg, and high  $\geq 57$  kg.

### **Cardiorespiratory fitness (CRF)**

To assess CRF, a submaximal exercise test was performed to calculate maximal oxygen uptake ( $VO_2\text{max}$ ) [89]. The exercise test was performed on a Monark 939E (Monark Sports & Medical, Monark 939E, Vansbro, Sweden) with simultaneously ECG registration to monitor heartrate (Cardiolex, EC Sense, Solna, Sweden). The exercise test started on an individually adjusted level between 50 and 100 W depending on the study participants' earlier exercise habits. The cycling continued until a steady-state level was reached and then the workload was increased by 25-50 W to reach the next steady-state level. The exercise test was ended when the study participant reached a steady-state at two workload levels, with a heart rate above 130 and 150 respectively. The estimated  $VO_2$  max was calculated using the straight line equation from the heart rates at the two steady-state levels and the expected oxygen consumption per work rate. Maximal heart rate as estimated through the formula  $220 - \text{age in years}$ . The study participants were categorized as having low, normal or high  $VO_2\text{max}$  according to European reference values [90]. Categories for  $VO_2\text{max}$  for women were: low ( $\leq 30$  ml/kg/min), normal (30.1 - 39.9 ml/kg/min), and high ( $\geq 40$  ml/kg/min)  $VO_2\text{max}$ . Categories for men were: low ( $\leq 40$  ml/kg/min), normal (40.1 – 49.9 ml/kg/min), and high ( $\geq 50$  ml/kg/min)  $VO_2\text{max}$ .

The method was validated with a comparison against the Åstrand test, and a maximal test in 32 individuals (not included in the LBA study) in the age range 18-25 years [91].

### **Physical activity (PA)**

The study participant's daily PA pattern was objectively measured with an accelerometer (ActiGraph, model GT3X+, Pensacola, FL, USA). The frequency, intensity, and duration of PA, as well as sedentary time [39], were recorded over one week. The study participants were instructed to wear the

accelerometer on an elastic belt around their waist at their lower back during all waking time, except during water activities [92]. The accelerometer data was processed and analyzed with the Actilife software (ActiLife, version 6.13.3, ActiGraph, Pensacola, FL, USA). The accelerometer was initialized with a raw data sampling frequency of 30 Hz, and uniaxial (vertical) analyses with 60-s epoch was used. Non-wear time was defined by an interval of at least 60 consecutive minutes of 0 counts per minute with an allowance for maximum 2 minutes of counts between 0-100. Wear-time was defined by subtracting non-wear time from 24 hours [93] and the study participants included in the analyses needed at least 10 hours or more of monitor wear per day, and at least four valid days [92, 94].

The cut-off points used to define different PA intensity levels were for light intensity PA (LPA) <2020 counts, for moderate intensity PA (MPA) 2020-5999 counts, and for vigorous intensity PA (VPA) >5999 counts per minute. Moderate- and vigorous physical activity (MVPA) were defined as  $\geq 2020$  counts without any distinction between MPA and VPA [93]. Sedentary time was defined as registration time with < 100 counts per minute [95]. Number of steps was also registered by the accelerometer. The time spent in different PA intensity levels were presented as minutes/day by dividing total minutes in each PA intensity level with the number of registered days.

## **Biomarkers**

Blood samples were collected from the study participants after approximately 20 minutes rest, following an 8-12h fasting period. The area for venipuncture was warmed up with a heating pad and cleaned with antiseptics before the tourniquet was placed approximately 10 cm above the venipuncture site. The venipuncture was performed with a 21 gauge butterfly needle (Greiner Bio-One International GmbH, Vacuette®, Rainbach im Mühlkreis, Austria) and the tourniquet was immediately released when blood flow was established. After blood collection, all tubes (BD Vacutainer; BD AB, Stockholm, Sweden) were gently inverted several times. All blood samples were analyzed at the accredited clinical chemistry laboratory at Örebro university hospital.

For analyzing HDL-C, triglycerides, and hs-CRP, blood collection was made into lithium-heparin tubes. Plasma was obtained by centrifugation for 8 min at 2000xg in room temperature, and afterwards placed in +4°C until transportation to the laboratory. A citric acid-citrate-NaF tube was used to collect blood to analyze glucose. The tube was placed in room temperature or +4°C until transportation. HDL-C, triglycerides, and glucose (mmol/L)

were analyzed on an Ortho Clinical Diagnostics™ (Clinical Chemistry instruments, Vitros 5,1™ FS, Raritan, New Jersey, U.S.A.). The method was dry chemistry (colorimetric method) according to the manufacturer's (Ortho) instructions. hs-CRP was analyzed with the method from Siemens [96] (Siemens, ADVIA 1800 chemistry system, Upplands Väsby, Sweden).

Serum for insulin analysis was obtained by collecting blood in a standard serum tube with clot activating substances. The blood was allowed to clot for at least 30 min before centrifugation was performed at 2000xg for 8 min at room temperature. Insulin (mU/L) was analyzed on an Architect i2000SR instrument from Abbott (Illinois, U.S.A.), with their reagent according to their instructions on antibody-based technologies.

Insulin resistance HOMA-IR was calculated by using the mathematical equation by Matthews, (insulin (mU/ml)\*glucose (mmol/L)/22.5). In the present study the ratio HOMA-IR was used as a measure of insulin resistance [97].

## **Blood pressure**

### **Peripheral blood pressure**

Brachial blood pressure was measured during both visits after approximately 10-15 minutes rest in a seated respectively a supine position using a digital automated device (GE Healthcare, Dinamap V100, Buckinghamshire, UK) with Dura-Cuf (GE Medical Systems, GE Criticon Dura-cuf, Milwaukee, WI, US). At both visits, the brachial blood pressure was measured in the left arm with the study participant in a supine position. At least three measurements were conducted with two minutes intervals. When the difference between the two latest systolic pressures were less than 5 mmHg the measurement was ended. The results for blood pressure and heart rate were reported as an average of the two latest results. Depending on the other variables included in different analyses, the blood pressure measurement from the first or the second visit was used.

The oscillometric blood pressure device measured systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) from the oscillation pattern that occurred in the cuff during deflation. The cuff pressure and the pressure oscillations were measured and analyzed by an intra-arterial reference algorithm [98]. The algorithm was developed based on blood pressure values received from an intra-arterial catheter in the aorta. The algorithm stores the pattern of the individual's oscillation size as a function of the pressure steps during the cuff deflation. When two

subsequent pulsations have a relatively equal amplitude, the cuff deflates another step. The algorithm measures the agreement of pulse size to assess if more steps are needed. Pulse pressure (PP) was calculated manually by subtracting DBP from SBP.

Blood pressure measurements were also performed between the pulse wave analysis (PWA) and the pulse wave velocity (PWV) measurements to ensure a stable and representative blood pressure for the full examination period. If the DBP was stable within  $\pm 5$  mm Hg after the PWA examination, the PWV measurements started. Otherwise a new series of blood pressure measurements were performed as earlier described, to get a representative resting blood pressure before the PWV registrations.

### **Central blood pressure**

The right common carotid artery was examined with applanation tonometry, SphygmoCor (AtCor Medical Pty Ltd, SphygmoCor, Sydney, Australia) during the pulse wave velocity examination, see below. The common carotid pulse waves were recorded with the subject in a supine position, in a temperature-controlled room (22-24°C). At least three measurements were made on each test subject. The carotid blood pressure was obtained by a calibration method [57] using the brachial artery pressure and wave, which is based on the observation, assuming that mean and diastolic blood pressure are constant throughout the large artery tree. The measurement with the highest quality index on the SphygmoCor equipment [99] was reported for each subject.

### **Applanation tonometry**

Applanation tonometry was measured with SphygmoCor (AtCor Medical Pty Ltd, SphygmoCor, Sydney, Australia) to perform PWA and PWV measurements. The arteries that were examined were the radial artery, the carotid artery, and the femoral artery. Each artery was palpated before the applanation tonometry to find the most prominent part. The arteries were flattened by a slight downward pressure and the pulse waveforms were recorded from each site [8].

### **Pulse wave analysis (PWA)**

The study participants rested in a supine position for approximately 10 minutes before a resting blood pressure was measured, according to the routines earlier described. Radial artery tonometry was performed at the study participant's right wrist, in a temperature-controlled room (22-24°C), with

the study participant in a supine position. The aortic pressure waveform was derived from the radial waveform by a validated transfer function [58]. At least three measurements were made on each study participant and the measurements were ended when all quality parameters (pulse height variation, pulse length variation, diastolic variation, and shape deviation) were fulfilled in the SphygmoCor equipment [99]. An average of AIx adjusted to heart rate 75 (AIx\_HR75) from three measurements were reported for each study participant. After acquisition of PWA, blood pressure measurements were repeated to ensure a stable and representative blood pressure for the full examination period.

### **Pulse wave velocity (PWV)**

PWV was measured, after approximately 20-30 minutes rest for the study participant, in a supine position. Carotid and femoral pulse waves were recorded with applanation tonometry with simultaneously ECG recording. The time between the R-wave of the electrocardiogram and the “foot” (the deviation from baseline, see Figure 1) of the carotid and femoral waves respectively, were measured to get the transit time [78, 79]. At least three measurements were performed, and PWV was calculated. Distance was measured as a straight line from the sternal notch to the carotid site, and from the sternal notch to the femoral site via the umbilicus. The length between the sternal notch and the carotid site was subtracted from the length between the sternal notch and the femoral site to get the distance [100]. When the PWV was stable within 0.5 m/s over three measurements, the examination was ended and an average of the three velocities was calculated.

### **Ultrasonography of the carotid artery**

Ultrasound measurements were performed using a high-resolution B-mode system (Vivid E9; GE Healthcare, Chicago, IL, USA) with a 12 MHz linear array transducer. The study participants were examined in a supine position with their heads slightly extended and turned approximately 45° to the left, according to guidelines [68, 101]. The right carotid artery was scanned with transverse and longitudinal views and a simultaneous ECG-recording was made during the ultrasound examination. A Doppler flow measurement was made to verify a correct location in the artery. The quality of the images were optimized by adjustments of, depth, focus and gain. The ultrasound images were saved in the format of DICOM (Digital Imaging and Communications in Medicine) clips. The DICOM clips were 5-8 seconds long.

### **Carotid intima media thickness (cIMT)**

The cIMT was measured 10 mm proximal to the carotid bulb with a lateral probe position, over a 10 mm segment in the longitudinal view of the far wall, in the common carotid artery [68, 101]. The intima media thickness was identified by using the Vivid E9 semi-automated edge detection program. Measurements were made on at least three images, for reproducibility, and an average of three measurements with a difference less than 0.05 mm was reported for each study participant, as was an average for the maximum values. The borders that defined the cIMT could be slightly adjusted manually if not satisfactory, but at first hand, new images were collected to improve the image quality for easier detection of the cIMT.

### **Carotid diameter**

The end-diastolic carotid diameter was measured in the longitudinal view with manually placed calipers. The diameter was measured over a 10 mm segment, 10 mm proximal to the carotid bulb, between the media-adventitia boundaries on the near and far wall. Three diameter measurements were made on at least three images, for reproducibility, and an average of all nine measurements was reported for each study participant. To assess the reproducibility of the diameter measurements, the coefficient of variation (CV) was calculated as  $((\text{standard deviation}/\text{mean}) \times 100)$ . The CV for mean end-diastolic carotid diameter was 1.6%.

### **Edge wall tracking**

In a subsample of 220 study participants from the LBA study, analyses of the carotid artery distention and cIMT was performed with edge wall tracking of ultrasound B-mode recordings by an ultrasound specialist, blinded for the study, using custom built Matlab software developed at Maastricht University Medical Centre (MUMC, Maastricht, The Netherlands). The software is based on previously published algorithms [85, 102]. Initially, before the B-mode edge tracking started, a region of interest was selected in the DICOM clip, and four media-adventitia transitions were manually plotted on the near and far wall. Subsequently, the vessel wall in the region of interest was divided into thirteen segments, and the media-adventitia transitions of the anterior and posterior walls were automatically determined for each segment, Figure 2. If necessary, it was possible to make manually adjustments of the media-adventitia transitions. Analyses of the distention and the cIMT were made in each segment.

To assess the reproducibility of ultrasound edge wall tracking analyses, the coefficient of variation (CV) was calculated as ((standard deviation/mean) x 100). The CV for mean distention and mean cIMT were 6.0% and 6.9% respectively.

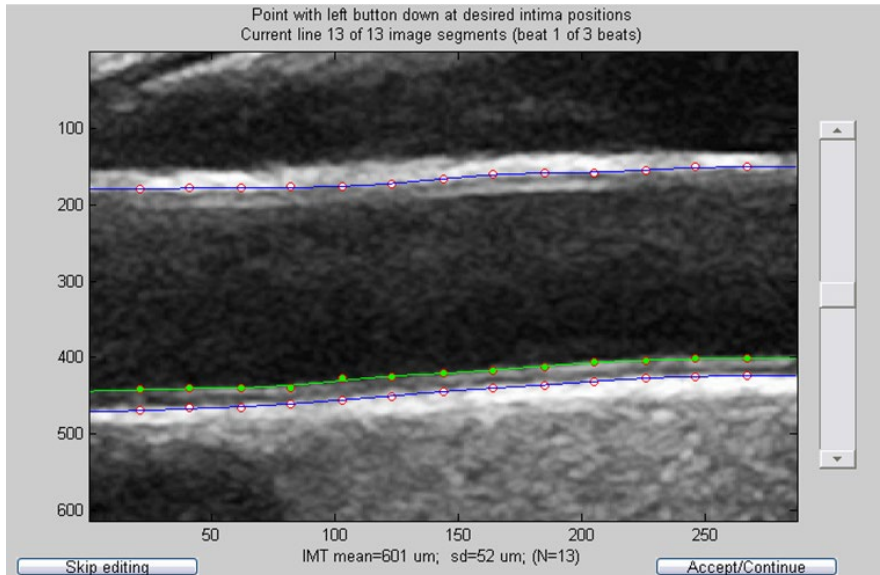


Figure 2. Automatic analysis of the common carotid artery distention and cIMT made with edge wall tracking of ultrasound B-mode recordings. The media-adventitia boundaries are defined by the blue lines, and the distance between them is the carotid diameter. The lumen-intima boundary is shown as the green line and defines the cIMT on the far wall together with the blue line.

### Local stiffness measurements

Calculations of the local stiffness measurements, arterial distensibility, Young's elastic modulus, and  $\beta$  stiffness index were made according to the formulas presented in the background (page 27).

### Statistical analysis

Statistical calculations were performed using IBM SPSS Statistics, version 23, 24, and 25 for Windows (IBM Corp, Armonk, NY, USA). The Kolmogorov Smirnov and the Shapiro Wilk test were used to check all variables for normal distribution. Descriptive data in normal distributed variables

were presented as mean and standard deviation, and skewed variables were presented as median and interquartile range (Q1-Q3).

In general, unpaired Student's *t*-test was used when comparing means in independent groups. The natural logarithms of the skewed variables were used in the unpaired Student's *t*-test. Comparisons between genders in qualitative variables were compared using the non-parametric tests. For comparison of means across more than two categories, one-way analysis of variance (ANOVA) was used in combination with an adequate post-hoc test. Pearson's correlation coefficient (*r*) was used to study associations between quantitative normal distributed variables. Spearman's correlation coefficient (*r*<sub>ho</sub>) was used to study the associations between skewed variables.

Simple linear regression analyses were used to study relationships between dependent and independent variables. Correction for multiple analyses were made according to Bonferroni.

Multiple regression analyses were performed to assess the independent variables' individual effect on a dependent variable. Covariates included in multiple regression models were earlier described in the literature as determining factors of the dependent variable. Model validations were performed to check that the error terms were normally distributed, that the residuals had constant variance, that the regression function was linear, and to avoid multicollinearity and outliers that could disturb the regression model. Across the analyses, the level of significance was set at  $P < 0.05$ , with exception for simple linear regression analyses with a high number of studied relationships, where a Bonferroni correction was applied.

In the results that follow, Unpaired Student's *t*-test was used when comparing means between the genders (Table 1, 2, and 7), between subjects *at risk* and subjects *not at risk* according to Wildman (Table 3A, 3B, and 4), between the study participants with valid PA data (i.e. at least 4 valid days with at least 10 hours per day), and the excluded study participants without valid PA data, between study participants *reaching 30 minutes MVPA per day* vs study participants *not reaching 30 minutes MVPA per day*, and between the total LBA study population and the LBA subsample. If skewed, the variables were normalized by ln-transformation. The natural logarithms of the skewed variables were used in the unpaired Student's *t*-test. Comparison of mean between genders in the qualitative variable (food habits score) was analyzed with the Mann-Whitney U test and *Chi-2* test.

## One-way analysis of variance

For comparisons of central and peripheral mean SBP across BMI categories, one-way ANOVA with Hochberg's post-hoc test was applied (Figure 5). For comparisons of mean PWV (Figure 6 and 7) and AIx\_HR75 (Figure 9 and 10) across categories (BMI and VO<sub>2</sub>max respectively), one-way ANOVA with Dunett post-hoc test was applied. "Low VO<sub>2</sub>max" and "obese" were used as reference categories in the post-hoc test.

When combining BMI categories and VO<sub>2</sub>max categories, overweight and obese were merged together because of the relatively low number of obese subjects. Nine BMI/VO<sub>2</sub>max categories in the total population were created. The category "Overweight+Obese/Low VO<sub>2</sub>max" was used as a reference category in the one-way ANOVA analysis, with Dunett post hoc test, of mean values across the nine BMI/VO<sub>2</sub>max categories, based on both women and men (Figure 8).

## Bivariate correlations

Pearson's correlation coefficient ( $r$ ) was used to study associations between cIMT and quantitative variables. Spearman's correlation coefficient ( $\rho$ ) was used to study the associations between cIMT and qualitative or skewed variables.

## Simple linear regression analyses

Simple linear regression analyses were used to study associations between the end-diastolic carotid diameter, height, cIMT, and VO<sub>2</sub>max in the total population, and between the end-diastolic carotid diameter and height in the LBA subsample.

Furthermore, simple linear regression analyses were used to study the associations between time spent in different intensity levels and arterial stiffness measurements.

Simple linear regression analyses were also used in the LBA subsample to study the associations between cIMT and local stiffness measurements as dependent variables, and body composition measurements and blood pressure as independent variables, respectively.

Correction for multiple comparisons were made according to Bonferroni. Based on the number of tests (here using the example of 48 tests) the Bonferroni correction resulted in the following: Significant level  $P < 0.05$  requires  $P < 0.00104$  ( $P < 0.05/48$ ), significant level  $P < 0.01$  requires  $P < 0.00021$  ( $P < 0.01/48$ ), and significant level  $P < 0.001$  requires  $P < 0.000021$  ( $P < 0.001/48$ ).

### **Multiple linear regression analyses**

To study the impact of  $VO_{2max}$  on the end-diastolic carotid diameter, a multiple regression analysis was performed.

Multiple regression analyses with PWV (Table 5) and AIX\_HR75 (Table 6), respectively, as dependent variables were performed. The unstandardized  $\beta$  coefficients and the standardized  $\beta$  coefficients were used to report the individual effect of  $VO_{2max}$  and BMI on the dependent variables. The other included covariates had earlier been described in the literature as determining factors for PWV (age and MAP), and AIX\_HR75 (age, MAP, height, and PWV), respectively [59, 82].

Furthermore, multiple regression analyses were performed with arterial stiffness measurements as dependent variables to explore the effect of PA per day. In addition to PA, other risk factors for CVD and variables earlier described in the literature as determining factors of arterial stiffness were included in the multiple regression model [59].

Finally, in the LBA subsample, multiple regression analyses, with the variables known to affect arterial stiffness (age and MAP) [1], were performed with arterial distensibility as dependent variable and body composition measurements as independent variables in separate models (Table 8). In addition, total cholesterol, glucose, and insulin were included in the models. The standardized  $\beta$  coefficients were used to report the individual effect of the covariates.

## Ethical considerations

The LBA study has been conducted in accordance to the Helsinki Declaration. All study participants were given a study code, the LBA code, which was used in all documentation of study results. The project manager and the project coordinator are the only ones with access to the key code.

In the case of pathological findings, the study participants were informed. In the LBA study it was in particular elevated cholesterol values, elevated insulin levels, and elevated brachial blood pressure. The study participants with pathological values were recommended to contact the local health care center for examination and counselling. No other ethical issues or risks have been identified. The study participants were covered by an insurance (*Särskilt personskadeskydd*) through The Legal Financial Administrative Services Agency (Kammarkollegiet).

All individuals had oral and written information and gave their written consent to participate. They were informed that they had the right to end their participation in the study at any time, without any negative effects for them personally. They had the possibility to ask questions during oral information sessions or by e-mail. The study design was approved by the Regional Ethics Committee in Uppsala, Sweden (Dnr: 2014/224).



# Results

## The LBA study population

Of the 840 study participants that were recruited to the LBA study during the data collection period from October 2014 until June 2016, six individuals were excluded because of chronic diseases (e.g. type I diabetes mellitus and Chron's disease). A total of 834 study participants (577 women and 257 men) fulfilled the inclusion criteria of being 18.0-25.9 years old, non-smoking, and self-reported healthy. In the LBA study, 10% of the study participants reported having been born outside Sweden, and 24% of the participants reported to having at least one parent born outside Sweden. Due to drop out before visit number two or technical difficulties there are some missing values. Results are missing as follows: 1 subject: waist circumference, 13 subjects: glucose, 19 subjects: insulin, 25 subjects: HOMA-IR, 5 subjects: HDL-C, 6 subjects: LDL-C, 5 subjects: total cholesterol, 4 subjects: triglycerides, 12 subjects: hs-CRP, 20 subjects: heart rate, 13 subjects: VO<sub>2</sub>max, 10 subjects: handgrip, 11 subjects: cIMT, 14 subjects: PWV, and 15 subjects: AI<sub>X</sub>\_HR75. The basic characteristics of the study participants split by gender are presented in Table 1.

## LBA subsample

The LBA subsample (n=220) (paper III) did not differ in basic characteristics from the full LBA study population (n= 834).

## Lifestyle related factors

In addition to the lifestyle factors, food habits and physical activity, descriptive data of body composition, cardiorespiratory fitness, and handgrip strength will be presented below, to describe lifestyle related factors in the LBA study population.

## Body composition

Mean values and standard deviations of the body composition measurements are presented in Table 1. The BMI in the study population varied from 15.1 kg/m<sup>2</sup> to 49.8 kg/m<sup>2</sup> in women, and from 16.3 kg/m<sup>2</sup> to 35.0 kg/m<sup>2</sup> in men. When dividing the study population in BMI categories according to WHO [16], the population was distributed as follows: In women, 7% were underweight, 77% were normal weight, 13% were overweight,

and 4% were obese. In men, 3% were underweight, 70% were normal weight, 25% were overweight, and 2% were obese.

In the study population, waist circumference varied from 57.0 cm to 123.0 cm in women, and from 60.0 cm to 107.5 cm in men. When dividing the study population into categories according to the cut-off values [19], the population was distributed as follows: In women, 82.5% had a normal waist circumference <80 cm, 13% had a waist circumference between 80-88 cm, and 4.5% had central obesity with a waist circumference >88 cm. In men, 93% had a normal waist circumference <94 cm, 5% had a waist circumference between 94-102 cm, and 2% had central obesity with a waist circumference >102 cm.

Percentage of body fat varied from 10.3% to 55.9% in women, and from 1.8% to 35.5% in men.

**Table 1.** Basic characteristics of the study participants in the LBA study, split by gender.

Variables	Women (n=577)		Men (n=257)		P-value
	Mean	SD	Mean	SD	
Age (years)	21.8	1.9	22.0	2.0	0.232
Height (cm)	168.6	6.3	181.8	6.7	<0.001
Weight (kg)	63.7	11.4	77.4	11.4	<0.001
BMI (kg/m <sup>2</sup> )	22.4	3.6	23.4	3.1	<0.001
Body fat (%)	28.0	6.6	14.8	5.6	<0.001
Waist (cm)	73.9	7.9	81.9	7.3	<0.001
Glucose (mmol/L)	4.9	0.3	5.2	0.3	<0.001
Insulin (mU/L)	8.0	4.5	7.5	3.7	0.136
HOMA-IR	1.8	1.1	1.8	0.9	0.814
HDL-C (mmol/L)	1.5	0.4	1.2	0.3	<0.001
LDL-C (mmol/L)	2.3	0.7	2.3	0.7	0.798
Cholesterol (mmol/L)	4.3	0.8	4.1	0.8	<0.001
Triglycerides (mmol/L)	0.8	0.4	0.8	0.4	0.389
hs-CRP (mg/L) <sup>a</sup>	0.7	0.4-1.9	0.6	0.3-1.3	<0.001
SBP <sub>brach</sub> (mmHg)	111	9	125	12	<0.001
DBP <sub>brach</sub> (mmHg)	65	7	67	7	<0.001
MAP <sub>brach</sub> (mmHg)	81	7	87	9	<0.001
PP <sub>brach</sub> (mmHg)	46	7	58	10	<0.001
Heart rate (beats/min)	66	10	63	10	<0.001
VO <sub>2</sub> max (ml/kg/min)	37.8	8.5	42.9	9.9	<0.001
Handgrip strength (kg)	34.4	6.5	53.1	10.1	<0.001
Food habits (points) <sup>a, b</sup>	6	5-7	6	4-7	0.053
cIMT (mm)	0.49	0.06	0.50	0.06	<0.05
PWV (m/s)	5.2	0.7	5.6	0.9	<0.001
Alx_HR75	-5.0	9.9	-8.4	9.5	<0.001

**Notes:** Data are presented in women and men as mean and SD. Mean differences between the women and men were analyzed by unpaired Student's *t*-test. Natural logarithms were made of the skewed variables before the analyses. <sup>a</sup>Data are presented as median and interquartile range (Q1-Q3). <sup>b</sup>Mann-Whitney U test was performed to compare mean values between women and men in the qualitative variable "Food habits". Level of significance was set at *P*<0.05 in all tests.

**Abbreviations:** BMI, body mass index, Body fat, percentage of body fat, Waist, waist circumference, HOMA-IR, Homeostasis model assessment of insulin resistance, HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol, hs-CRP, high-sensitive C-reactive protein, SBP<sub>brach</sub>, Brachial systolic blood pressure, DBP<sub>brach</sub>, Brachial diastolic blood pressure, MAP<sub>brach</sub>, Brachial mean arterial pressure, and PP<sub>brach</sub>, Brachial pulse pressure, VO<sub>2</sub>max, estimated maximal oxygen uptake, cIMT, carotid intima media thickness, PWV (m/s), pulse wave velocity, Alx\_HR75, augmentation index adjusted to heart rate 75 beats per minute.

### **Cardiorespiratory fitness and handgrip strength**

Mean values and standard deviations of VO<sub>2</sub>max and handgrip strength are presented in Table 1. The CRF level in the study population varied from 15.7 ml/kg/min to 87.4 ml/kg/min in women, and from 21.6 ml/kg/min to a maximum value of 77.9 ml/kg/min in men. When dividing the study population in VO<sub>2</sub>max categories according to the European reference values [90] the population was distributed as follows: In women, 17% had low VO<sub>2</sub>max, 48% had normal VO<sub>2</sub>max, and 35% had high VO<sub>2</sub>max. In men, 41% had low VO<sub>2</sub>max, 37% had normal VO<sub>2</sub>max, and 22% had high VO<sub>2</sub>max.

The handgrip strength varied in the study population from 16 kg to 73 kg in women, and from 22 kg to 90 kg in men. When categorizing the study population as having low, normal, or high handgrip strength, according to reference values [88], the population was distributed as follows: In women, 3% had low handgrip strength, 49% had normal, and 47% had high handgrip strength. In men, 4% had low handgrip strength, 66% had normal, and 30% had high handgrip strength.

### **Food habits**

In the study population 23% of the women and 26% of the men scored 1-4 points at the food frequency questionnaire, indicating unhealthy food habits, 68% of the women and 66% of the men scored 5-8 points, indicating normal food habits with some potential for improvement, and 10% of the women and 8% of the men scored 9-12 points, indicating healthy food habits. Compared with the general Swedish population, no statistical difference was found,  $p=0.65$ . Nor any difference was found between women and men in the food questionnaire score,  $p=0.053$ .

### **Physical activity**

Of the total population (834 individuals), 828 individuals used and returned their accelerometer. A total of 658 individuals (73% women) who fulfilled the PA wear-time criteria were included in the analyses, Figure 3. In comparisons between study participants with valid PA data and the study participants excluded because of no valid PA data, the analyses showed that the women with valid PA data had significantly lower BMI ( $P<0.05$ ) and percentage of body fat ( $P<0.01$ ) than the excluded women. The men with valid PA data had significantly lower glucose ( $P<0.05$ ), HOMA-IR ( $P<0.05$ ), and AIx\_HR75 ( $P<0.05$ ) than the excluded men.

Daily median accelerometer wear time was 13.2 h per day in the total population with no significant differences between women and men. Women spent significantly more time per day in LPA ( $P<0.01$ ), were less sedentary ( $P<0.001$ ), and took significantly more steps per day ( $P<0.05$ ), than men. There were no significant differences between women and men in how much time they spent in MPA, VPA, and MVPA per day, Table 2.

Women and men accumulated on average, median (Q1-Q3), 45 (31-58) and 44 (29-56) minutes per day, respectively, in MVPA with large individual variations. Total daily time spent in MVPA varied from 7 to 203 minutes in women and from 4 to 294 minutes in men. In total 76% of the study participants spent on average at least 30 minutes per day in MVPA. There were no significant difference between women and men, 77% of women and 74% of men spent on average at least 30 minutes per day in MVPA.

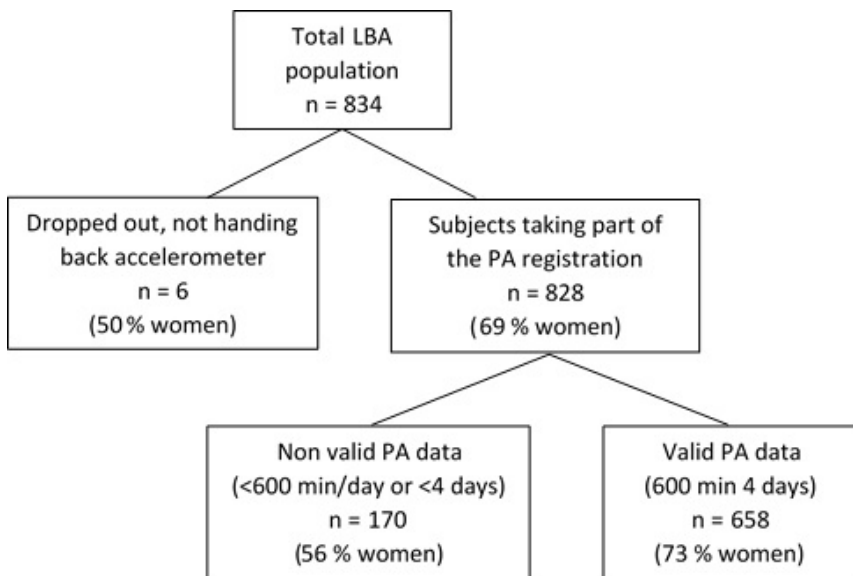


Figure 3. Sampling procedure of the physical activity data in the Lifestyle, Biomarkers, and Atherosclerosis study.

**Table 2.** Distribution in time spent in different physical activity intensity levels (min/day) and steps/day, in women and men. Mean differences between the women and men were analyzed by unpaired Student's *t*-test.

Variables	Women (n=478)		Men (n=180)		<i>P</i> -value
	Median	Q1-Q3	Median	Q1-Q3	
Sed (min/day)	523	481-565	547	506-593	<0.001
LPA (min/day)	214	187-244	202	176-231	<0.01
MPA (min/day)	38	28-51	38	27-47	0.339
VPA (min/day)	3	0-9	3	0-8	0.152
MVPA (min/day)	45	31-58	44	29-56	0.527
Steps (steps/day)	7796	6123-9402	7336	5773-8749	<0.05

**Notes:** Data are presented in women and men as median and interquartile range (Q1-Q3). Mean differences between the women and men were analyzed by unpaired Student's *t*-test. Natural logarithms were made of the skewed variables before the analyses. Level of significance was set at  $P<0.05$ .

**Abbreviations:** Sed, Sedentary time per day, LPA, Light intensity physical activity, MPA, Moderate intensity physical activity, VPA, Vigorous intensity physical activity, MVPA, Moderate and Vigorous intensity physical activity.

## Wildman's risk score

In the LBA study population of self-reported healthy young adults, 12% (98 study participants) had two or more risk factors according to Wildman's definition. They were classified as having a metabolic phenotype vulnerable to CVD and are in the following text called "subjects *at risk*". 15% of the LBA study population were defined as insulin resistant with HOMA-IR > 2.52, and 35% of the women and 25% of the men had HDL-C lower than recommended according to Wildman's cut-off values [51, 52].

When comparing subjects *at risk* and subjects *not at risk*, according to Wildman's risk score, significant differences were seen in women and men *at risk* compared to women and men *not at risk* in body composition ( $P<0.001$  and  $P<0.05$  respectively), in the risk factors included in the Wildman risk score ( $P<0.01$  and  $P<0.05$  respectively), except for the lack of differences in blood pressure in men. Significant differences were also seen in the lifestyle related factors, VO<sub>2</sub>max ( $P<0.001$  in women and  $P<0.05$  in men), and in handgrip strength in women ( $P<0.05$ ), Table 3A and 3B.

There were also significant differences between subjects *at risk* compared to subjects *not at risk* in time spent in LPA ( $P<0.01$ ), and total PA ( $P<0.01$ ) per day, Table 4.

When dividing the study population into the VO<sub>2</sub>max categories, low, medium, and high with gender specific cut off values [90], 43% of the subjects

at risk had a low CRF level, compared to 22% of the subjects not at risk. Only 15% of the subjects at risk had a high CRF level, compared to 33% of the subjects not at risk according to Wildman's risk score, Figure 4.

### **Peripheral and central blood pressure**

Peripheral blood pressure was measured in the study participants both at the first and the second visit. Mean values and standard deviations of brachial SBP, DBP, MAP, and PP are presented in Table 1. When measuring the central blood pressure from the carotid artery, 224 study subjects (49% women) were excluded due to technical issues. Their central blood pressure was similar or higher in comparison with their peripheral blood pressure. The central SBP were in women and men, mean and (SD), 103 (9) mmHg and 115 (11) mmHg respectively, the central DBP were 64 (6) mmHg and 65 (7) mmHg respectively, MAP were 80 (7) mmHg and 84 (7) mmHg respectively, and the central pulse pressure were 38 (8) mmHg and 50 (10) mmHg in women and men respectively.

When dividing the study population into BMI categories, underweight, normal weight, overweight, and obese, the overweight and obese women had significantly higher central and peripheral SBP than the under- and normal weight women, ( $P<0.001$ ). The overweight men had significantly higher central and peripheral SBP than the normal weight men, ( $P<0.01$ ), and the overweight and obese men also had significantly higher peripheral SBP than the underweight men, ( $P<0.01$ ), Figure 5.

**Table 3A.** The differences in body composition, Wildman's risk factors, and lifestyle related factors compared between *subjects at risk* and the *subjects not at risk* according to Wildman's risk score.

Variables	Women (n=577)		P value
	<i>At risk</i> (n=68)	<i>Not at risk</i> (n=509)	
<b>Body composition</b>			
BMI (kg/m <sup>2</sup> )	25.6 (6.5)	22.0 (2.8)	<0.001
Body fat (%)	33.7 (8.5)	27.3 (6.0)	<0.001
Waist (cm)	80.8 (13.4)	73.0 (6.4)	<0.001
<b>Wildman's risk factors</b>			
SBP <sub>brach</sub> (mmHg)	116 (13)	110 (8)	<0.001
DBP <sub>brach</sub> (mmHg)	67 (7)	64 (6)	<0.001
Glucose (mmol/L)	5.1 (0.5)	4.9 (0.3)	<0.01
HOMA-IR	3.1 (1.9)	1.6 (0.7)	<0.001
HDL-C (mmol/L)	1.1 (0.3)	1.5 (0.4)	<0.001
Triglycerides (mmol/L)	1.3 (0.5)	0.7 (0.3)	<0.001
hs-CRP (mg/L) <sup>a</sup>	2.9 (1.3-6.8)	0.7 (0.3-1.4)	<0.001
<b>Lifestyle related factors</b>			
VO <sub>2</sub> max	32.4 (7.7)	38.6 (8.4)	<0.001
Handgrip strength (kg)	32.7 (5.8)	34.7 (6.6)	<0.05
Food habits (points) <sup>a, b</sup>	6 (4-7)	6 (5-7)	0.156

**Notes:** Data are presented in women *at risk* and women *not at risk* according to Wildman's risk score, as mean and (SD). Mean differences between the women *at risk* vs *not at risk* were analyzed by unpaired Student's *t*-test. Natural logarithms were made of the skewed variables before the analyses. <sup>a</sup>Data are presented as median and interquartile range (Q1-Q3). <sup>b</sup>Mann-Whitney U test was performed to compare median values between women *at risk* vs *not at risk* in the qualitative variable "Food habits". Level of significance was set at *P*<0.05 in all tests.

**Abbreviations:** BMI, body mass index, Body fat, percentage of body fat, Waist, waist circumference, SBP<sub>brach</sub>, Brachial systolic blood pressure, DBP<sub>brach</sub>, Brachial diastolic blood pressure, HOMA-IR, Homeostasis model assessment of insulin resistance, HDL-C, high-density lipoprotein cholesterol, hs-CRP, high-sensitive C-reactive protein, VO<sub>2</sub>max, estimated maximal oxygen uptake.

**Table 3B.** The differences in body composition, Wildman's risk factors, and lifestyle related factors compared between the *subjects at risk* and the *subjects not at risk* according to Wildman's risk score.

Variables	Men (n=257)		P-value
	<i>At risk</i> (n=30)	<i>Not at risk</i> (n=227)	
<b>Body composition</b>			
BMI (kg/m <sup>2</sup> )	25.1 (4.1)	23.2 (2.8)	<0.05
Body fat (%)	18.0 (7.4)	14.4 (5.2)	<0.05
Waist (cm)	85.5 (8.2)	81.4 (7.1)	<0.01
<b>Wildman's risk factors</b>			
SBP <sub>brach</sub> (mmHg)	127 (15)	124 (11)	0.400
DBP <sub>brach</sub> (mmHg)	68 (9)	66 (7)	0.155
Glucose (mmol/L)	5.5 (0.4)	5.1 (0.3)	<0.001
HOMA-IR	2.9 (1.3)	1.6 (0.7)	<0.001
HDL-C (mmol/L)	1.0 (0.2)	1.3 (0.3)	<0.001
Triglycerides (mmol/L)	1.0 (0.7)	0.8 (0.3)	<0.05
hs-CRP (mg/L) <sup>a</sup>	1.1 (0.4-3.9)	0.5 (0.2-1.1)	<0.001
<b>Lifestyle related factors</b>			
VO <sub>2</sub> max	38.8 (10.6)	43.5 (9.7)	<0.05
Handgrip strength (kg)	53.2 (12.1)	53.1 (9.8)	0.987
Food habits (points) <sup>a, b</sup>	5.5 (4-7)	6 (5-7)	0.176

**Notes:** Data are presented in men *at risk* and *not at risk* according to Wildman's risk score, as mean and (SD). Mean differences between the men *at risk* vs *not at risk* were analyzed by unpaired Student's *t*-test. Natural logarithms were made of the skewed variables before the analyses. <sup>a</sup>Data are presented as median and interquartile range (Q1-Q3). <sup>b</sup>Mann-Whitney U test was performed to compare median values between men *at risk* vs *not at risk* in the qualitative variable "Food habits". Level of significance was set at *P*<0.05 in all tests.

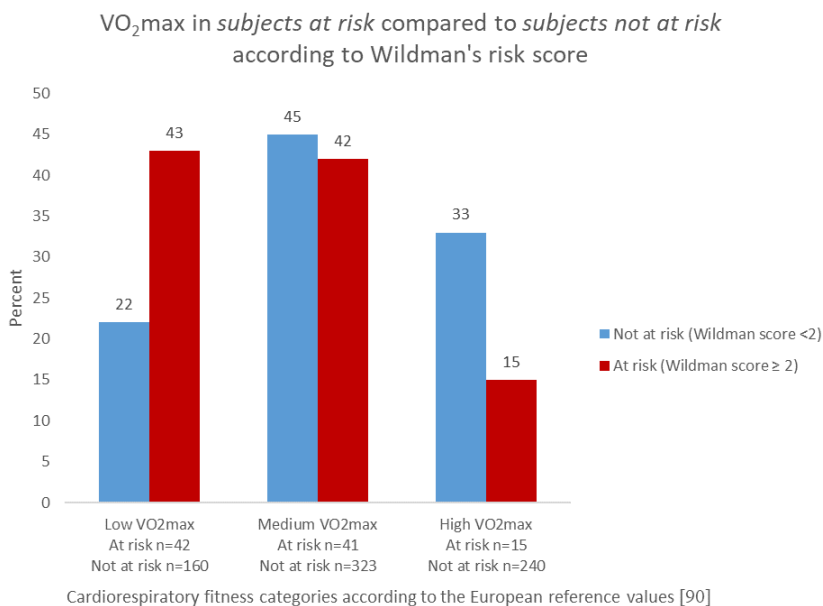
**Abbreviations:** BMI, body mass index, Body fat, percentage of body fat, Waist, waist circumference, SBP<sub>brach</sub>, Brachial systolic blood pressure, DBP<sub>brach</sub>, Brachial diastolic blood pressure, HOMA-IR, Homeostasis model assessment of insulin resistance, HDL-C, high-density lipoprotein cholesterol, hs-CRP, high-sensitive C-reactive protein, VO<sub>2</sub>max, estimated maximal oxygen uptake.

**Table 4.** Time spent in different physical activity levels, compared between the *subjects at risk* and the *subjects not at risk* according to Wildman's risk score.

	Total population with valid PA data (n=658)		P-value
	At risk (n=68)	Not at risk (n=590)	
Sed (min/day)	538 (481-588)	522 (480-565)	0.646
LPA (min/day)	203 (169-233)	216 (188-245)	<0.01
MVPA (min/day)	41(27-57)	46 (32-58)	0.178
Total PA (min/day)	238 (213-238)	262 (226-296)	<0.01
Steps (steps/day)	7254 (5650-8699)	7625 (6077-9334)	0.062

**Notes:** Data are presented in the categories *at risk* and *not at risk* according to Wildman's risk score, as median and interquartile range (Q1-Q3). Mean differences between the categories were analyzed by unpaired Student's *t*-test. Natural logarithms were made of the skewed variables before the analyses. Level of significance was set at  $P<0.05$ .

**Abbreviations:** Sed, Sedentary time per day, LPA, Light intensity physical activity, MPA, Moderate intensity physical activity, VPA, Vigorous intensity physical activity, MVPA, Moderate and Vigorous intensity physical activity.



*Figure 4. VO<sub>2</sub>max in subjects at risk compared to subjects not at risk, according to Wildman's risk score. The European reference values [90] for the VO<sub>2</sub>max (ml/kg/min) categories were gender specific, and the limits for women were: low ( $\leq 30$  ml/kg/min), normal (30.1-39.9 ml/kg/min), and high ( $\geq 40$  ml/kg/min). For men, the limits were: low ( $\leq 40$  ml/kg/min), normal (40.1-49.9 ml/kg/min), and high ( $\geq 50$  ml/kg/min).*

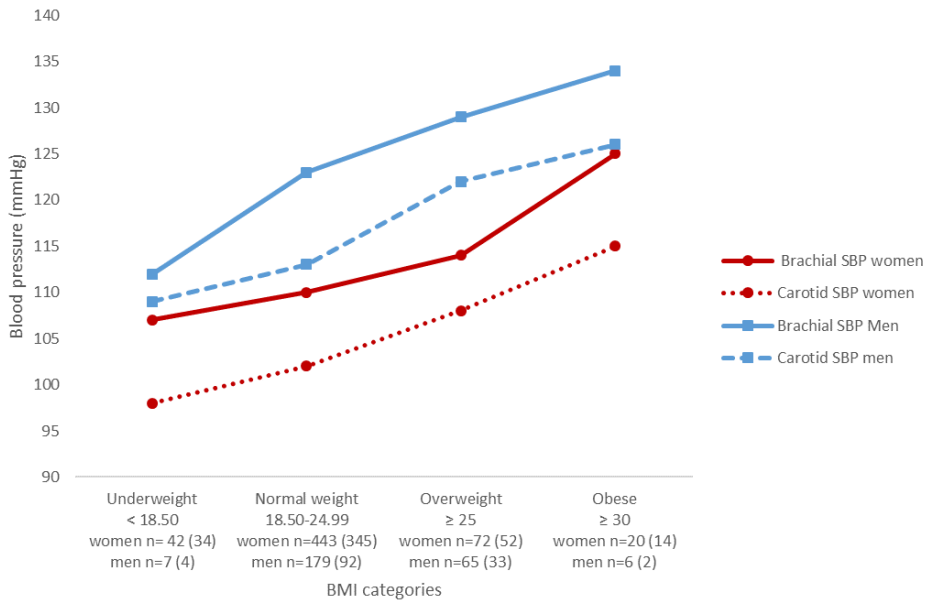


Figure 5. Number of women and men in each BMI category are presented for peripheral and (central) blood pressure. One-way ANOVA was used for comparison of mean systolic blood pressure across BMI categories. The women with overweight and obesity had significantly higher central and peripheral SBP than the under- and normal weight women,  $P < 0.001$ . The overweight men had significantly higher central and peripheral SBP than the normal weight men,  $P < 0.01$ . The men with overweight and obesity had also significantly higher peripheral SBP than the under-weight men,  $P < 0.01$ .

## **Carotid intima media thickness and carotid diameter**

Carotid intima media thickness (cIMT) was measured in the total study population with the semiautomated edge-detection program in the ultrasound machine. Due to drop out from visit number two, cIMT from nine women and two men was missing. cIMT was also analyzed in the LBA subsample with edge wall tracking in 156 women and 64 men.

### **Semiautomated edge-detection**

The mean cIMT in women and men were 0.49 mm with a standard deviation (SD) of 0.06 mm, and 0.50 mm with a SD of 0.06 mm, respectively, Table 1. The mean of the maximum cIMT in women and men were 0.60 mm with a SD of 0.07 mm, and 0.60 mm with a SD of 0.07 mm, respectively. The mean cIMT varied from 0.33 mm to 0.69 mm in women and from 0.34 mm to 0.82 mm in men. There was a significant difference in mean cIMT between women and men ( $P<0.05$ ), Table 1. No correlation was found between cIMT and time spent in MVPA or cIMT and  $VO_2\max$  in women or in men. In women, a positive correlation was found between cIMT and food habits,  $r_s=0.089$  ( $P<0.05$ ). In men, a positive correlation was found between cIMT and handgrip strength,  $r=0.137$  ( $P<0.05$ ).

The mean carotid end-diastolic diameter was 5.83 mm with a SD of 0.36 mm, and 6.22 mm with a SD of 0.43 mm, in women and men respectively. There was a significant difference in mean carotid end-diastolic diameter between women and men ( $P<0.001$ ). The mean carotid end-diastolic diameter was positively associated with height (Standardized  $\beta$  0.415,  $P<0.001$ ), cIMT (0.119,  $P<0.001$ ), and  $VO_2\max$  (0.141,  $P<0.001$ ) in the total population. When adjusting for height, cIMT, and MAP, a positive association between carotid end-diastolic diameter and  $VO_2\max$  was still present (0.079,  $P<0.05$ ).

### **Edge wall tracking**

The mean cIMT in women and men were 0.61 mm with a standard deviation (SD) of 0.08 mm, and 0.65 mm with a SD of 0.11 mm, respectively. The mean of the maximum cIMT in women and men were 0.73 mm with a SD of 0.11 mm, and 0.78 mm with a SD of 0.12 mm, respectively. The mean cIMT varied from 0.40 mm to 0.82 mm in women and from 0.45 mm to 0.90 mm in men. There was a significant difference in mean cIMT between women and men ( $P<0.05$ ). No significant associations were found between cIMT and body composition measurements (i.e. BMI, percentage of body

fat, and waist circumference) or brachial blood pressure, in the LBA subsample, data not shown.

The mean carotid end-diastolic diameter in women and men was 6.03 mm with a SD of 0.35 mm, and 6.41 mm with a SD of 0.42 mm, respectively. There was a significant difference in mean carotid end-diastolic diameter between women and men ( $P<0.001$ ). The mean carotid end-diastolic diameter was positively associated with height (Standardized  $\beta$  0.386,  $P<0.001$ ) in the LBA subsample.

### Pulse wave velocity

The mean PWV in women was 5.2 m/s with a SD of 0.7 m/s (Table 1) and a maximum mean PWV of 8.7 m/s. In men, mean PWV was 5.6 m/s with a SD of 0.9 m/s (Table 1) and a maximum mean of 9.9 m/s. In ANOVA analyses of mean PWV across the VO<sub>2</sub>max and BMI categories, women with low VO<sub>2</sub>max had significantly higher PWV than women with medium and high VO<sub>2</sub>max,  $P<0.001$ . No significant difference in mean PWV was found between the CRF categories in men, Figure 6.

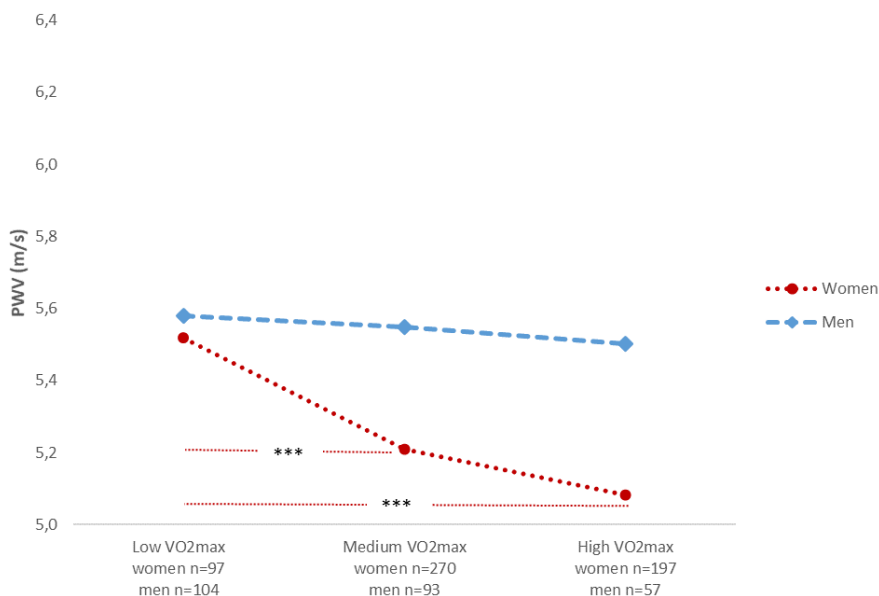


Figure 6. PWV in VO<sub>2</sub> categories (gender specific) for women and men. One-way ANOVA was used for comparison of mean PWV between different VO<sub>2</sub> categories in women and men respectively. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .

The obese women had significantly higher PWV than the women in the other BMI categories  $P<0.001$ . The obese men had significantly higher mean PWV than the underweight men,  $P<0.05$ , Figure 7.

When combining BMI and  $VO_2\text{max}$  in nine categories, the study participants in the category Overweight+Obese/Low  $VO_2\text{max}$  had the highest PWV. The lowest PWV was found in the category Normal weight/High  $VO_2\text{max}$ , Figure 8.

In multiple regression analyses with PWV as the dependent variable, the separate effect of  $VO_2\text{max}$  and BMI were explored. After including the earlier described covariates, age and MAP, the analyses showed that  $VO_2\text{max}$  had a stronger effect on PWV than BMI in women, but not in men, Table 5.

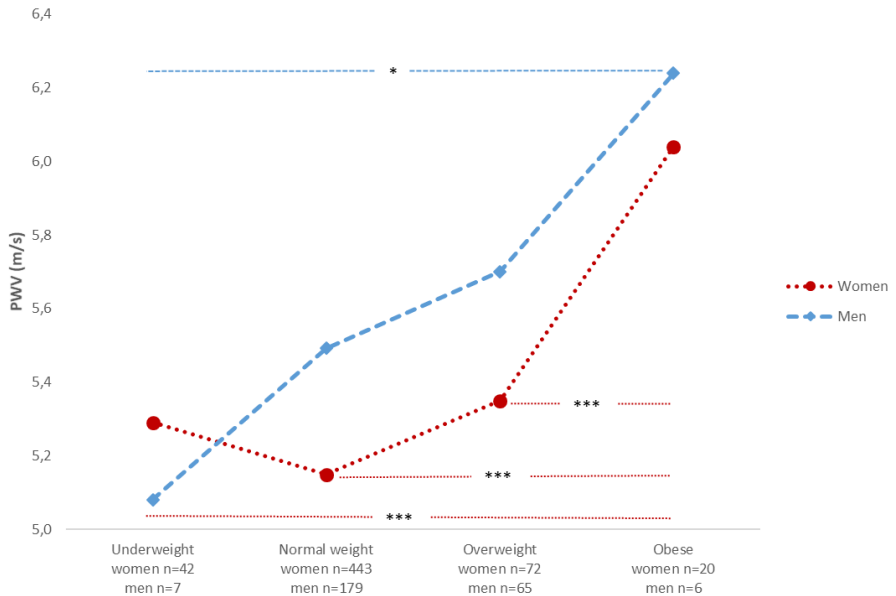


Figure 7. PWV in BMI categories, for women and men: underweight ( $<18.5$ ); normal weight ( $18.5-24.9$ ); overweight ( $25.0-29.9$ ); and obese ( $\geq 30$ ). One-way ANOVA was used for comparison of mean PWV between different BMI categories in women and men respectively,  $*P<0.05$ ,  $**P<0.01$ ,  $***P<0.001$ .

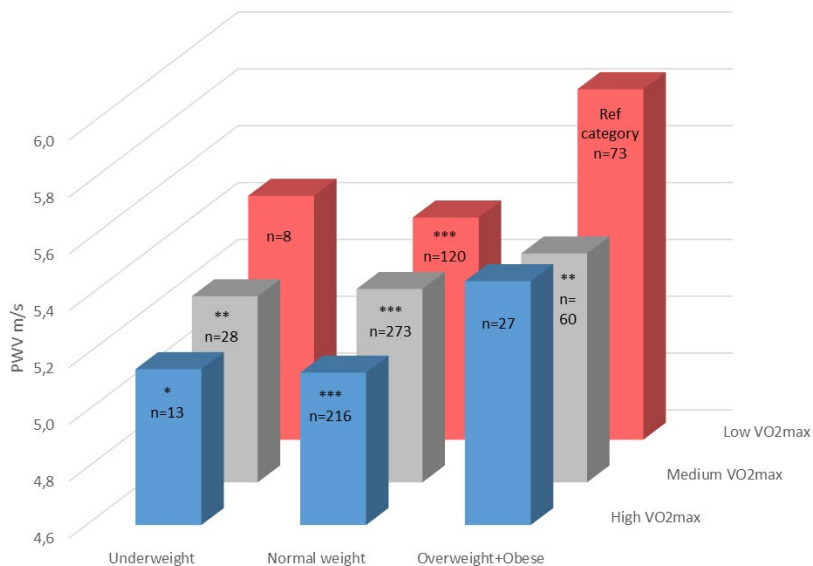


Figure 8. Nine combined BMI/VO<sub>2</sub>max categories in the total population. The highest mean PWV was found in the combined BMI/VO<sub>2</sub>max category Overweight+Obese/Low VO<sub>2</sub>max. The lowest mean PWV was found in the category Normal weight/High VO<sub>2</sub>max. Overweight+Obese/Low VO<sub>2</sub>max was used as a reference category when comparison of mean PWV was performed with one-way ANOVA. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001

Table 5. Multiple regression model to explore the separate effect of the lifestyle related factors VO<sub>2</sub>max and BMI, on PWV (m/s).

Covariates	PWV (m/s)					
	Women (n=562)			Men (n=253)		
	Unstand. β	Stand. β	P-value	Unstand. β	Stand. β	P-value
VO <sub>2</sub> max	-0.013	-0.158	<0.001	-0.007	-0.081	0.179
BMI	0.016	0.080	0.065	0.031	0.112	0.083
Age	0.031	0.084	<0.05	0.073	0.170	<0.01
MAP	0.028	0.264	<0.001	0.026	0.228	<0.001

**Notes:** Unstandardized β, Standardized β, and P-values are presented for the covariates in the model, in women and men separately.

**Abbreviations:** PWV (m/s), pulse wave velocity, VO<sub>2</sub>max (ml/kg/min), estimated maximal oxygen uptake, BMI (kg/m<sup>2</sup>), body mass index, Age (years), and MAP (mmHg), mean arterial pressure.

In simple linear regression analyses, significant inverse associations were seen between PWV and MVPA (standardized  $\beta$  -0.104;  $P < 0.05$ ), and PWV and steps (-0.128;  $P < 0.01$ ) per day in women (i.e. lower stiffness when more PA).

## **Augmentation index**

The mean AIx\_HR75 in women was -5.0% with a SD of 9.9% (Table 1) and a maximum value of 24.0%. In men, mean AIx\_HR75 was -8.4% with a SD of 9.5% (Table 1) and a maximum value of 29.7%.

In ANOVA analyses of mean AIx\_HR75 across the VO<sub>2</sub>max and BMI categories, women with low VO<sub>2</sub>max had significantly higher (i.e less negative) AIx\_HR75 than women with high VO<sub>2</sub>max,  $P < 0.001$ . Men with low VO<sub>2</sub>max had significantly higher AIx\_HR75 than men with medium and high VO<sub>2</sub>max,  $P < 0.05$ , Figure 9. The obese women had significantly higher AIx\_HR75 than the overweight and normal weight women,  $P < 0.05$  and  $P < 0.01$  respectively. No significant difference in mean AIx\_HR75 were found across the BMI categories in men, Figure 10.

In multiple regression analyses with AIx\_HR75 as dependent variable, the separate effect of VO<sub>2</sub>max and BMI were explored. After including the earlier described covariates, age, MAP, height, and PWV, the analyses showed that VO<sub>2</sub>max had a stronger effect on PWV than BMI in both women and men, Table 6.

In simple linear regression analyses, significant inverse associations were seen in women between AIx\_HR75 and MVPA (standardized  $\beta$  -0.166;  $P < 0.001$ ), total PA (-0.116  $P < 0.05$ ), and steps (-0.150;  $P < 0.001$ ) per day. In men, a significant inverse association was seen between AIx\_HR75 and total PA (-0.153;  $P < 0.05$ ).

Multiple regression analyses were performed to explore the effect of total PA per day on AIx\_HR75, after adjustment for risk factors (BMI, HDL-C, and hs-CRP) and other previously described determining factors (MAP, Height, Age, PWV). After including all covariates an inverse association was seen between AIx\_HR75 and time spent in total PA per day (standardized  $\beta$  -0.100;  $P < 0.05$ ), in women. No significant association was seen between total PA and AIx\_HR75 in men (-0.115;  $p = 0.118$ ).

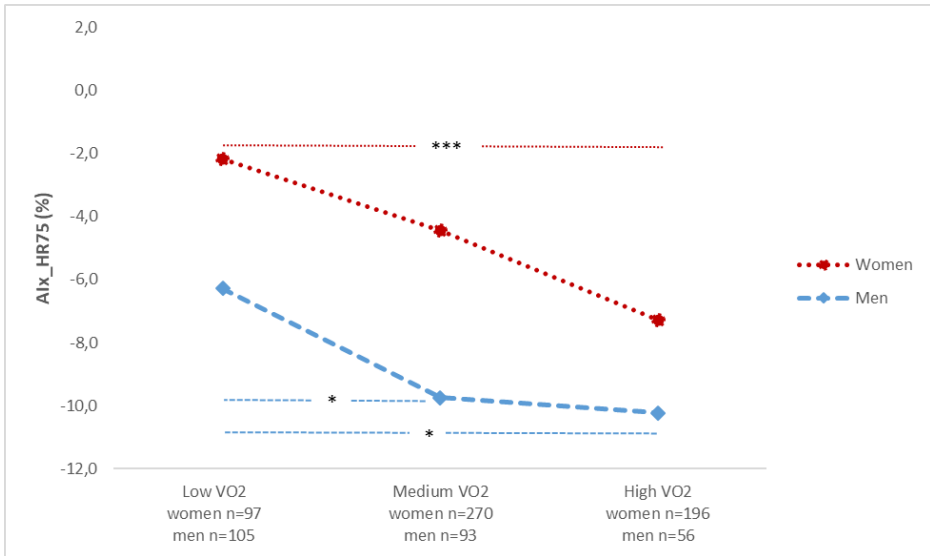


Figure 9. Aix\_HR75 in VO<sub>2</sub> categories (gender specific) for women and men. One-way ANOVA was used for comparison of mean PWV between different VO<sub>2</sub> categories in women and men respectively. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

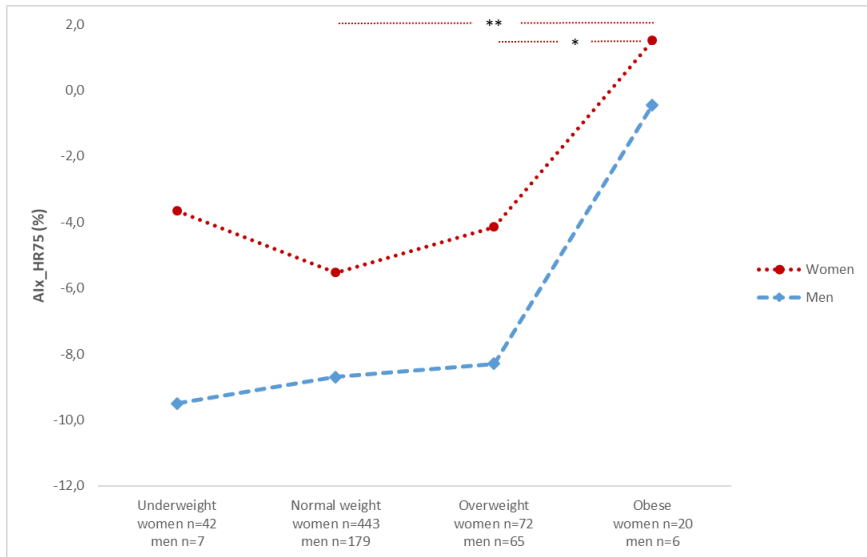


Figure 10. Alx\_HR75 in BMI categories, for women and men: underweight (<18.5); normal weight (18.5-24.9); overweight (25.0-29.9); and obese (≥30). One-way ANOVA was used for comparison of mean Alx\_HR75 between different BMI categories in women and men respectively. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001

**Table 6.** Multiple linear regression models to explore the separate effect of the life-style related factors VO<sub>2</sub>max and BMI, on Alx\_HR75 (%).

Covariates	Alx_HR75 (%)					
	Women (n=561)			Men (n=252)		
	Unstand. β	Stand. β	P-value	Unstand. β	Stand. β	P-value
VO <sub>2</sub> max	-0.231	-0.200	<0.001	-0.161	-0.167	<0.01
BMI	-0.254	-0.094	<0.05	0.050	0.016	0.809
Age	0.912	0.178	<0.001	0.048	0.010	0.872
MAP <sub>brach</sub>	0.173	0.118	<0.01	0.209	0.164	<0.05
Height	-0.482	-0.310	<0.001	-0.213	-0.152	<0.05
PWV	2.317	0.169	<0.001	0.658	0.060	0.364

**Notes:** Unstandardized β, Standardized β, and P-values are presented for the covariates in the model, in women and men separately.

**Abbreviations:** Alx\_HR75 (%), augmentation index adjusted to heart rate 75 beats per minute, VO<sub>2</sub>max (ml/kg/min), estimated maximal oxygen uptake, BMI (kg/m<sup>2</sup>), body mass index, Age (years), MAP<sub>brach</sub> (mmHg), brachial mean arterial pressure, Height (cm), and PWV (m/s), pulse wave velocity.

## Local stiffness measurements

There were significant differences between women and men ( $P<0.001$ ) in the calculated local stiffness measurements. Women had higher arterial distensibility and lower YEM, and  $\beta$  stiffness index than men, all  $P<0.001$ , Table 7.

**Table 7.** Results of local stiffness measurements (i.e. arterial distensibility, Young's elastic modulus, and  $\beta$  stiffness index) in the common carotid artery in the LBA sub-sample (n=220).

Variables	Women (n=164)		Men (n=56)		P-value
	Mean	SD	Mean	SD	
Art dist (kPa <sup>-1</sup> )	0.020	0.006	0.017	0.004	<0.001
YEM (kPa)	0.077	0.027	0.104	0.037	<0.001
$\beta$ Stiffness	4.22	1.26	5.38	1.68	<0.001

**Notes:** Data are presented in women and men as mean and standard deviation (SD). Mean differences between the women and men were analyzed by unpaired Student's *t*-test. Level of significance was set at  $P<0.05$ .

**Abbreviations:** Art dist (kPa<sup>-1</sup>), Arterial distensibility, YEM (kPa), Young's elastic modulus,  $\beta$  Stiffness,  $\beta$  Stiffness index.

Inverse associations were found between arterial distensibility and BMI, and between arterial distensibility and waist circumference (standardized  $\beta$  for BMI -0.310, and for waist circumference -0.268), in women. Positive associations were also found between YEM and BMI, and between  $\beta$  stiffness index and BMI (standardized  $\beta$  for BMI 0.267 and 0.294 respectively) in women. In men, inverse associations were found between arterial distensibility and BMI, and between arterial distensibility and percentage of body fat (standardized  $\beta$  for BMI -0.437, and for percentage of body fat -0.474). No significant associations were found between YEM and the body composition measurements, or between  $\beta$  stiffness index and body composition measurements in men.

Based on the observation that arterial distensibility was the local stiffness measurement that was significantly associated to most body composition measurements, in both women and men, arterial distensibility was chosen as the dependent variable in the multiple regression analyses to explore which of the body composition measurements contributed mostly to arterial distensibility.

Multiple regression analyses showed that BMI contributed slightly more to the variation in arterial distensibility than percentage of body fat and waist circumference in women, and that BMI and percentage of body fat contributed equally to the variation in arterial distensibility, in men. The

other covariates in the multiple regression models, age, MAP, and the biomarkers (i.e. total cholesterol, glucose, and insulin), contributed less than body composition measurements to the variation in arterial distensibility in this age group, Table 8.

Table 8. Multiple linear regression models with arterial distensibility as dependent variable and BMI, percentage of body fat, and waist circumference as independent variables. The models are adjusted for age, MAP, total cholesterol, glucose, and insulin. The analyses were performed in the LBA subsample (n=220).

Covariates	Arterial distensibility (kPa <sup>-1</sup> )			
	Women (n=164)		Men (n=56)	
	Stand. $\beta$	P-value	Stand. $\beta$	P-value
<b>BMI model</b>				
BMI (kg/m <sup>2</sup> )	-0.277	<0.01	-0.440	<0.01
Age (years)	0.013	0.874	-0.053	0.700
MAP <sub>brach</sub> (mmHg)	-0.101	0.220	-0.177	0.183
Total cholesterol (mmol/L)	0.002	0.980	0.195	0.152
Glucose (mmol/L)	-0.064	0.449	-0.088	0.544
Insulin (mU/L)	0.048	0.617	0.028	0.843
<b>Body fat model</b>				
Body fat (%)	-0.213	<0.05	-0.450	<0.01
Age (years)	-0.006	0.938	-0.159	0.208
MAP <sub>brach</sub> (mmHg)	-0.113	0.174	-0.170	0.192
Total cholesterol (mmol/L)	0.015	0.856	0.199	0.133
Glucose (mmol/L)	-0.092	0.293	-0.109	0.440
Insulin (mU/L)	0.022	0.824	0.044	0.755
<b>Waist model</b>				
Waist circumference (cm)	-0.218	<0.05	-0.375	<0.05
Age (years)	-0.013	0.872	-0.096	0.488
MAP <sub>brach</sub> (mmHg)	-0.115	0.165	-0.188	0.167
Total cholesterol (mmol/L)	-0.003	0.971	0.186	0.181
Glucose (mmol/L)	-0.068	0.432	-0.156	0.284
Insulin (mU/L)	0.019	0.845	0.082	0.600

**Notes:** Standardized  $\beta$ , and P-values are presented for the covariates in the model, in women and men separately.

**Abbreviations:** BMI (kg/m<sup>2</sup>), body mass index, Body fat (%), percentage of body fat, and MAP<sub>brach</sub> (mmHg), mean arterial pressure.



# Discussion

## Study population

Of the 840 self-reported healthy individuals that volunteered to participate in the LBA study, six individuals were excluded because of chronic diseases. The individuals with chronic diseases were on medical therapy, but they explained that they felt healthy and wanted to participate in the study. They did not consider themselves to be ill. The fact that the individuals included in the study were self-reported healthy could be considered as a limitation. We didn't include individuals with chronic disease, but we accepted for example individuals with obesity, which is a reversible condition and an important risk factor for CVD. In addition to chronic disease, smoking was one exclusion criteria in the study. We included only non-smokers in the LBA study because we wanted to focus on other lifestyle related risk factors and biomarkers in the population of young adults. Smoking is a well-known risk factor for CVD [6] and we wanted to exclude the impact of smoking at the young adult's vascular status.

Of the 834 study participants who came for visit number one, 11 individuals did not show up for visit number two. They were contacted with a reminder about visit number two and the ones that answered refused to continue as study participants. The drop out is only 1.3% which could be considered as low.

For some reason there were more women who volunteered to participate in the study: about one third (31%) of the study population was men. Why it was harder to get men to volunteer in the study is unknown. We observed that many of the women were interested in health and fitness and appreciated to have feedback from some of the examinations. Perhaps that interest is lower in young men.

From a family background perspective, the LBA study population represented the source population well. In the study population, 24% had at least one parent born outside Sweden. The corresponding age-group specific number in Sweden was 25%.

## Lifestyle related factors

### Body composition

Compared to the 31% of overweight and obese young adults in Sweden, reported in 2018 by the Public Health Agency of Sweden [17], the women

and men in the LBA study were less fat. In women and men, 17% and 27% respectively, were overweight or obese. The discrepancy between the general Swedish population and the LBA study population can be explained by the fact that some of the individuals that volunteered in the LBA study, especially the women, had an interest in health and fitness. They were curious to know about their health status and had an interest in participating to get feedback about their blood pressure, CRF, and blood variables.

Only a few percent (4.5% in women and 2% in men) of the study participants had a waist circumference over the recommended cut-off, indicating central obesity. In the definition of the metabolic syndrome [19], central obesity, as assessed by waist circumference, is essential to diagnosis. Prevalence of metabolic syndrome was not assessed in the LBA population. Instead we used the Wildman risk score [51] to classify individuals at risk of future CVD. Neither waist circumference, nor any other body composition measurement was included in the Wildman risk score. Central obesity can also be assessed with the waist to hip ratio, but a disadvantage can be that you overestimate the waist to hip ratio if you have individuals with a weak pelvic-gluteal musculature. That can result in a high ratio without necessarily indicating abdominal/central obesity [44].

### **Cardiorespiratory fitness and handgrip strength**

In the LBA study a greater proportion of men had low CRF compared to women, 41% vs. 17%. That is a large difference that partially can be explained for the same reason as discussed above. The women that participated in the LBA study were more aware of health and fitness compared to the men in the study. The result is also in line with the CRF pattern in the general Swedish population. The decline in CRF from 1995 to 2017 in the Swedish working force was more pronounced in men than in women. Relative  $\text{VO}_2\text{max}$  decreased by 9.4% (3.6 ml/kg/min) in women and by 12.4% (4.8 ml/kg/min) in men. The decrease in  $\text{VO}_2\text{max}$  (both absolute and relative) was also more pronounced in the youngest age group (18-34 years) compared to the middle (35-49 years) and oldest age group (50-74 years) [27]. This fact, together with the large proportion of men with low CRF in the LBA study of young adults, raises concerns about future risk for CVD.

Compared to age- and sex-specific reference values [88], less than 5% were categorized as having low muscle strength and 47% of the women and 30% of the men had high handgrip strength. This was in line with data from the questionnaire that the study participants filled in, which indicated that muscle strength training was popular in the study population.

## **Food habits**

Regarding the food habits in the LBA study population, approximately one quarter of the study participants had unhealthy food habits and scored 1-4 points in the Swedish National Food Agency test. No significant difference was found compared with the general Swedish population. Nor was any difference found between women and men in the food questionnaire score. The low score may have several explanations, a low intake of fruit and vegetables, lack of fiber, and a high intake of saturated lipids. In the LBA study population, the dietary habits are further explored showing significant associations between having more beneficial levels of HOMA-IR when following a recommended intake of fish, fruit and vegetables, and sweets [103].

## **Physical activity**

Women spent less time sedentary ( $P < 0.001$ ) and more time in LPA ( $P < 0.01$ ) in comparison with men. These findings are in line with the findings in another Swedish study, the SCAPIS Pilot Study, which observed that men spent more time sedentary and less time in LPA compared to women [104]. However, no significant differences between the genders were seen in MPA, VPA, or MVPA in the present study. This finding differs from several other studies [104-106] where most commonly men are reported to be more physical active. This finding mirrors the high percent women in the LBA study with normal or high CRF, and the understanding that a lot of women were physically active and interested in being well-trained. This could possibly explain why we don't observe the previously reported differences between the genders in time spent in MVPA.

In total, 76% of the study participants spent on average at least 30 minutes per day in MVPA. In an earlier publication from the Swedish SCAPIS pilot Study in 2015 [104], 75.7% in the age group 50-64 years fulfilled the recommendations of 150 minutes of MVPA per week. These results are in line with the findings in the present study where 77% of women and 74% of men spent on average at least 30 minutes per day in MVPA. Data from a national survey in Sweden, the Physical Activity Fact Sheet from 2018 [107], presented similar results, 67% in the age group 18-64 years met the PA recommendations of at least 150 minutes of MVPA per week [41]. However, the PA level was assessed subjectively with questionnaire unlike the PA data in the LBA study, and the age group was considerably wider in its range than in the LBA study, so comparisons can be less relevant.

When describing what percentage of the study population spent on average at least 30 minutes per day in MVPA, no account was taken of whether the MVPA was performed in bouts of at least 10 minutes, or spread out during most days of the week (or on most days of the registration period, if not a week). The requirement of performing the activity in bouts is present in the WHO Physical Activity guidelines from 2010 [41], and in the national Swedish guidelines from 2011 [108]. However, in the recently published Physical Activity Guidelines for Americans, 2<sup>nd</sup> edition [42], the recommendation of 10 minute bouts is removed. The updated guidelines shift direction towards the message that some PA is better than nothing, and that there is no lower threshold to receive benefits from PA. The main recommendation for substantial health benefits in adults is still though to perform at least 150-300 minutes of MPA, or 75-150 minutes of VPA, or an equivalent of MVPA, preferably on most days of the week.

The encouraging message in the updated guidelines from 2018 [42], that there is no lower level to reach benefits from PA, are supported by the recently published systematic review and harmonized meta-analysis [109], with individual data from a large sample (n=36 383). The meta-analysis showed that all intensities of PA were associated with a reduced risk of mortality, highlighting the fact that any PA is better than nothing. It is not only the high intensities of PA that have beneficial effects for health.

### **Wildman's risk score**

In the LBA study population, 12% had two or more risk factors according to Wildman's definition, 15% of the LBA study population were defined as insulin resistant with HOMA-IR > 2.52, and 35% of the women and 25% of the man had HDL-C lower than recommended according to Wildman's cut-off values [51, 52].

In a study carried out in university students in Brazil [110] (median age 23 and 22 years respectively in women and men), the prevalence of metabolic syndrome and insulin resistance were estimated. They found a lower prevalence of insulin resistance, 7.3%, than in the LBA study, but they used a slightly higher cut-off value, HOMA-IR > 2.7 [111]. If we had used HOMA-IR > 2.7 as a cut off, the prevalence of insulin resistance in the LBA study would have been 13.5%, still higher than in the Brazil study. On the other hand, 61.2% in the Brazil study had low HDL-C levels, with no difference between the sexes. Those findings differ from the LBA study where a lower prevalence of low HDL-C levels with significant differences between women and men were found. The divergent results in the studies may be

explained by different lifestyle factors such as food and PA habits. PA was assessed with a short version of the International physical activity questionnaire (IPAQ) in the Brazil study, and almost 70% were assessed as sedentary according to the IPAQ guidelines [112].

The present study showed that the study participants *at risk* according to Wildman were less physically active than the study participants *not at risk* according to Wildman. The findings are supported by the recently mentioned systematic review [109]. In accordance with the LBA study, more women than men (72.8%) were included in the sample, but the mean age was considerably older, mean age 62.6 years. The meta-analysis showed that higher levels of total PA and less time spent sedentary were associated with a reduced risk of premature mortality. This is in line with the findings in the LBA study where we demonstrate that young adults without risk factors for CVD have a higher level of total PA compared to those at risk for future CVD who are less physically active. The meta-analysis showed, as earlier mentioned, that all intensities of PA were associated with a reduced risk of mortality: not only high intensities of PA have beneficial effects for health. The greatest risk reduction for mortality were seen at about 375 min/day in LPA or 24 min/day of MVPA. In addition, a higher risk of death was seen if spending 9.5 hours or more per day sedentary [109].

## Arterial markers

### Regional stiffness measurements

The mean PWV in women and men in the LBA study are in line with previously published reference values in healthy children and teenagers [113]. Compared to reference values from a large European population of healthy adults [114], the mean PWV in women and men in the LBA population is somewhat lower. The reference values in the age group <30 years, was 6.2 m/s while in the LBA study the mean PWV were 5.2 m/s in women and 5.6 m/s in men. Compared to published reference values from a large population study [115], the mean AIx\_HR75 in women and men in the LBA study were low. The reference values for AIx\_HR75 in the low-risk cohort with similar age as in the LBA study, were approximately between 0-10 % compared to -5.0 % in women and -8.4 % in men in the LBA study.

Women had a higher mean AIx\_HR75 than men and that is in line with the reference values. The gender difference in AIx\_HR75 could partly be explained by the differences in height, since AIx has been described earlier

to be inversely associated with height [116]. Several studies have earlier described a positive association between AIx and age. The relationship has been described as non-linear with a greater change in AIx in younger individuals below 50 years. These findings suggest that AIx might be a more sensitive marker than PWV in younger individuals [116].

Significant inverse associations were found between PWV and VO<sub>2</sub>max in women, and between AIx\_HR75 and VO<sub>2</sub>max in both women and men indicating that higher VO<sub>2</sub>max is associated with more elastic arteries. These findings are in line with the results from other studies [117-119] demonstrating that VO<sub>2</sub>max is of high importance for the prevention of CVD and that improvements in CRF are desirable to get benefits in arterial stiffness by exercise. In the comparison of mean PWV between the underweight, normal weight, overweight, and obese women and men, the obese women had significantly higher PWV than the women in the other BMI categories. The obese men had significantly higher mean PWV than the underweight men. These findings indicate that the more fat, the stiffer the arteries. The findings are in line with results from a study of adolescents and young adults with obesity and obesity-related type 2 diabetes mellitus [120]. The obese individuals had a mean PWV similar to the obese individuals in the LBA study. A two-year follow-up study [121] demonstrated that weight loss was associated with a decrease in arterial stiffness. Furthermore, the longitudinal Cardiovascular Risk in Young Finns study reported that the risk of cardio-metabolic outcomes was normalized in those who went from being an overweight/obese child to becoming a non-obese adult [122]. The finding is encouraging, since weight is a factor that could possibly be influenced by changes in lifestyle.

When comparing the combined BMI/VO<sub>2</sub>max categories, the graphic showed that “*Overweight+Obese/Low VO<sub>2</sub>max*” was the most unfavorable combination with the highest mean PWV and AIx\_HR75. The finding was perhaps not so surprising; more interesting was the fact that the study participants in the category “*Overweight+Obese/High VO<sub>2</sub>max*” had a mean PWV similar to the normal- and underweight study participants with low VO<sub>2</sub>max. This indicates that even if you are overweight and obese you can benefit from keeping a high CRF level.

Several studies have investigated the relationship between arterial stiffness [35, 37, 38] and objectively assessed PA in young adults and adolescents. It has been described, in both healthy individuals and in individuals with type 2 diabetes [35], that higher PA is associated with lower levels of arterial stiffness. These findings are consistent with the results in the LBA

study where inverse associations were seen between AIx\_HR75 and total PA, in both women and men. The relationship demonstrates that the higher the level of total PA, the less stiff the arteries. In a multiple regression analysis, after adjusting for several covariates, the inverse association between AIx\_HR75 and total PA was significant in women, but not in men. However, the standardized  $\beta$  coefficient in men was similar in magnitude to the standardized  $\beta$  coefficient in women. Failure to observe a significant standardized  $\beta$  coefficient for total PA may be due to low statistical power resulting from the smaller number of men.

Thus, in this age group of young adults it is important to maintain or strive for more time as physically active, all intensities of PA seems to be beneficial for the vascular health.

### **Local stiffness measurements**

There were significant differences ( $P<0.001$ ) between women and men in the LBA subsample in the calculated local stiffness measurements in the common carotid artery. Women had higher arterial distensibility, and lower YEM and  $\beta$  Stiffness index, than men, indicating that men had stiffer carotid arteries. The findings in the present study are in line with results from several other studies [123-126] showing similar gender differences where women have more beneficial values than men. Comparing the local stiffness measurements with reference values and results from other studies was difficult since there is a lack of standards [127] with many different measures of local stiffness being used.

Of the three local measurements calculated in the LBA Study, arterial distensibility was the measurement with the strongest associations with BMI, body fat, and waist circumference. To explore which of the body composition measurements contributed most to the variability in arterial distensibility, multiple regression analyses were performed. The analyses showed that BMI had the highest impact on arterial distensibility in women and that BMI and percentage of body fat had equal impact on arterial distensibility in men. The gender difference could be explained by the different fat distribution in women and men. A man with a high percent of body fat is more likely to have a more central fat distribution in the abdominal area, while in women it is more common with fat distributed to the hips and thighs [128]. Central fat in the abdominal area is associated with diabetes, increased blood pressure, and CVD [13, 129, 130], and it may be that the difference in fat distribution can explain why percentage of body fat has a greater impact on arterial distensibility in men than in women.

### **Carotid intima-media thickness and carotid diameter**

There was a significant difference in mean cIMT between women and men in the total population when assessed with the semi-automated edge-detection ( $P<0.05$ ) and when assessed with edge wall tracking ( $P<0.05$ ) in the LBA subsample. Women had a thinner cIMT than men. The findings are in line with results from the Young Finns Study, where the cIMT were compared between women and men in the same age group as in the LBA study [131]. The same findings are demonstrated from a multi-center study in a healthy sub-population that measured cIMT with echo-tracking [65]. No correlation was found in the total population between cIMT and time spent in MVPA or between cIMT and  $VO_2$ max in women or men. The variation in cIMT in the LBA population of young adults was relatively small, and a majority of the study participants, especially the women, were quite well-trained and had a normal BMI. These can be two possible explanations for the lack of association between cIMT and PA level in the study participants.

cIMT is a well-known marker of preclinical atherosclerosis [63]. The longitudinal Cardiovascular Risk in Young Finns Study has found that children with risk factors, e.g. obesity, physical inactivity, and high LDL-C, have an increased progression in cIMT in adulthood [131]. However, there is no consensus about the effect of PA and exercise on cIMT between the different studies. In a study where the arterial wall properties in females, in the ages 17-25, were compared between athletes, sedentary controls, and normally active controls, cIMT was similar between the groups [132]. The findings were supported by another study where no difference in cIMT was observed between endurance-trained and sedentary controls [133]. On the other hand, a study that compared elite squash players with less active controls found that the elite trained group had significantly lower cIMT [134]. A review that describes the effect of exercise training on arterial wall thickness summarizes that exercise seems to have a modest effect on cIMT in young subjects [135]. Regular exercise training results in repeated increases in blood flow, shear stress, and blood pressure. A decreased arterial wall thickness is most likely depending on systemic effects. The transmural pressure, which is modulated as a result of increased blood pressure during exercise, is one factor that probably affects the arterial wall thickness [136]. However, changes in carotid arterial wall thickness may require intense exercise, as in athletes, over a long period of time [135].

In the LBA study there was also a significant difference in mean carotid end-diastolic diameter between women and men in the total population when assessed manually with calipers ( $P<0.001$ ), and when assessed with

edge wall tracking ( $P < 0.001$ ) in the LBA subsample. Men had a larger mean diameter than women and the diameter difference is most likely explained by the difference in body height. The mean carotid end-diastolic diameter was positively associated with height, both when assessed manually in the total population and when assessed with edge wall tracking in the LBA subsample, indicating that the taller person, the larger diameter.

It has been shown that athletes have an increased arterial lumen diameter. The increased arterial lumen dimension seems to be modified by local mechanisms, such as increased shear stress [136]. The increased shear stress, which causes release of nitric oxide (NO) from the endothelium in the vessel wall, seems to be a key player in the vascular adaptation and remodeling of the arteries due to exercise training [137].

In the LBA study we found a positive association between the end-diastolic carotid diameter and  $VO_2\max$  in the total population, supporting the hypothesis that a high CRF gives an increased arterial lumen diameter. The association between the lumen diameter and  $VO_2\max$  was quite weak which probably can be explained by the fact that very few of the study participants with a high CRF exercised at the level of an athlete.

## **Methodological considerations**

### **Detection of cIMT and carotid diameter with different methods**

We used two different ways to detect the cIMT and carotid end-diastolic diameter. The semi-automated detection in the GE Vivid ultrasound machine showed lower values of cIMT both in women and men (0.49 mm and 0.50 mm respectively), compared to the edge wall tracking analyses of the DICOM clips (0.61 mm in women and 0.65 mm in men). Similar results were found when comparing the two methods for measuring the carotid end-diastolic diameter. The mean diameter was larger in both women and men (6.03 mm and 6.41 mm respectively) when measured with the edge wall tracking system compared to the manually caliper measurements (5.83 mm and 6.22 mm in women and men respectively).

The differences were probably due to differences in the software analyses of cIMT, and in the automatic analyses of the diameter compared to the manually performed diameter measurements. The edge wall tracking system works with an edge threshold of 65% of the maximal grey value of the local adventitia, at the near- and far wall respectively, to detect the boundary between the media and adventitia. When measuring the end-diastolic diameter manually with calipers, it is only a visual assessment of the boundary

between the media and adventitia, at the near- and far wall, that determines the diameter. Generally, it seems likely that the semi-automated detection of cIMT and the manually measurement of the carotid end-diastolic diameter underestimated the values compared to the edge wall tracking system. However, since we primarily investigated associations with other variables, the small absolute differences between the methods were of little relevance. The accuracy and precision of the edge wall tracking system has been assessed compared to radiofrequency phase tracking and the methods are equal regarding the accuracy and the precision of the distention in an elderly patient population [85]. The authors assume that since the distention can be assessed properly in the elderly, with expected curved arteries with irregularities in the vessel wall, it is likely that the edge wall tracking method works properly in younger adults without overt atherosclerotic disease.

### **Pulse amplification in young adults**

Because of the pulse amplification between central and peripheral arteries in young adults, guidelines recommend the use of local blood pressure instead of arm blood pressure when calculating the carotid local stiffness measurements [59]. In the LBA study, the local blood pressure in the common carotid artery was used in the calculations of the local stiffness measurements. However, several other studies used the brachial blood pressure [123, 125, 126, 138]. One study reported a strong correlation between brachial and local pulse pressure, but the correlation was strongest in the oldest tertile ( $r=0.82$ ) and weakest in the youngest tertile ( $r=0.57$ ) of the study population [125]. In the LBA subsample, the local stiffness measurements were calculated both with carotid pressure and brachial pressure (data not shown). The mean values of the local stiffness measurements differed significantly both in women and men depending on which blood pressure measurement (carotid or brachial) was used in the calculations. However, the size and direction of the associations with the independent variables did not differ much. So despite the small significant differences in mean values, the associations were similar and not affected by the choice of blood pressure.

In the LBA study, pulse amplification between the brachial artery and the carotid artery was slightly lower than expected. In a comparison with data from the Anglo-Cardiff Collaborative Trial II, young adults in the same age as the study participants in the LBA study, had higher pulse amplification with absolute differences between peripheral and central systolic blood pressure of approximately 15 mmHg [62, 139]. They generated their central

pressure data from applanation tonometry of the radial artery, and estimated the ascending aortic pressure by using the general transfer function, which makes the data not fully comparable with the data from the LBA study. When the radial pressure wave is non-invasively applanated and calibrated with intra-arterial pressures, the calculation of aortic pressure is accurate [58, 140]. The accuracy decreases when the radial pressure wave is calibrated by the cuff pressure from the brachial artery [141]. The same applies when applanating the carotid artery, using cuff pressure from a peripheral artery for calibration [55].

In the LBA study we generated calculated central aortic pressures from applanating the radial artery and using the general transfer function (data not shown), but we choose also to perform PWA of the applanation registrations from the carotid artery because we wanted to include the local pressure from the carotid artery in the calculations of local stiffness measurements. The unexpectedly low pulse amplification between the carotid and brachial artery was probably due to technical issues and the accuracy of using cuff pressure when calibrating the carotid arterial pressure waves. Except for the use of cuff calibration, several methodological issues must be considered when examining the carotid pressure waves. It can be difficult to applanate the carotid artery because of its anatomical location, the mobility of the artery and the depth in the neck. Reproducible pulse wave recordings are more difficult to obtain at the carotid artery compared with the radial artery due to the anatomical preconditions [62]. The carotid baroreceptors can be stimulated mechanically, resulting in changes in heart rate. Respiratory fluctuations can also affect the possibility of obtaining a stable applanation of the carotid artery [8]. In addition, one cannot rule out the possibility that some of the study participants experienced the examination as somewhat stressful and as a response to that had an increased systolic blood pressure during the assessment of the central blood pressure.

### **The submaximal ergometer bike test**

The study participant's CRF was assessed with a submaximal ergometer bike test. To perform a maximal exercise test is strenuous, and because of the large study population in the LBA study, a submaximal test was chosen. A method validation study was made in 32 individuals, 18-25 years (not LBA study participants), to compare the submaximal test used in the LBA study with a maximal exercise test, and an Åstrand test. A repeated measures ANOVA showed no significant difference between the estimated  $\text{VO}_2\text{max}$  from the submaximal test, based on two steady state levels, the

Åstrand test, or the maximal exercise test [91]. The advantages of making a “pre-test” before a submaximal test, to make the test subject familiar with the test equipment and the testing procedure, have been discussed. Conducting only one test may result in an underestimation of  $\text{VO}_2\text{max}$  because of anxiety in the patient, resulting in a higher pulse rate during the exercise test [142].

In the LBA study, we did not perform a “pre-test” before the submaximal exercise test in all study participants. Firstly, and most important, because of the choice of method. Since we wanted the study participants to have a heart rate above 130 beats/min at the first steady-state level, we reduced the risk of catching a higher pulse rate because of a stress reaction. The effort it takes to perform a physical activity at approximately 65% of maximum heart rate, overrides the increase in heart rate due to stress [143]. Secondly, it would have been an extra test for the study participants, which might have discouraged some of them.

### **The objectively measured physical activity**

In March of 2016, ActiGraph identified an issue that caused raw data attenuation on wGT3X-BT activity monitors that were shipped between November 2013 and March 2014. Unfortunately, several accelerometers used in the LBA study were purchased during this period, and hence they were affected by the raw data attenuation. Of the 827 study participants that had PA registrations, 313 were affected (38%), and 514 were unaffected (62%). ActiGraph provided file repair options to affected customers and we choose to ship our affected accelerometers to Actigraph for repair. Of course, we were deeply disappointed about this issue because of the time delay it caused and because of uncertainty in the effectiveness and reliability of the repair method.

In paired comparisons between the original affected files and the “fixed” affected files, there were significant differences at group level in all PA intensities and in steps ( $P < 0.01$ ). The counts had increased in all PA intensity levels in the “fixed” affected files compared to the original affected files. However, in a paired comparison between the “fixed” affected files and the unaffected files there were no significant differences at group level in any PA intensity or in steps. After discussions with national experts in the field (Nätverk för objektiv rörelsemätning – NORM), the consensus was to “accept” the adjusted files from the Actigraph company and to include them as valid results [144].

We had valid PA data from 79.5% of the study population who completed their accelerometer registration. Analyses between the study participants with valid PA data and the study participants excluded because of no valid PA data, showed that the women with valid data had lower BMI and percentage of body fat while the men with valid PA data had more beneficial levels of glucose, HOMA-IR, and AIx\_HR75, compared to the women and men respectively, who did not reach the required of 600 minutes per day on at least 4 days. Thus, an overrepresentation of healthier individuals, according to the tests in the LBA study, had full PA registrations. This decreases the variability in the data set and decreases the probability in detecting differences. Despite a healthy active cohort we saw associations between higher levels of PA and lower stiffness, and it could be speculated that less healthy are at risk of higher arterial stiffness.

Finally, PA is a complex behavior and the accelerometer does not capture all types of activity, e.g. swimming. Some activities can also be underestimated by the accelerometer, such as riding a bike, walking on stairs, or carrying a heavy load. Those types of activities can result in an underestimation of total energy expenditure when registered with an accelerometer [106]. However, the variability in the validity and reliability is higher with objectively measured PA compared to self-reported PA [145].

## Future perspectives

A longitudinal follow-up of the LBA population is something that I really would like to carry out in the future. The cross-sectional design of the LBA study prevents us from drawing conclusions in terms of causality, we can only describe relationships between the study participant's vascular status and lifestyle related factors. A longitudinal follow-up could address other research questions.

Pending a longitudinal follow-up, there are more research questions to answer in the available data. The questionnaire that the study participants filled in about their general mental and physical health [1] have not been processed yet. For example, there are data about the study participants sleep behavior and experienced stress in everyday life that can be important to investigate and to relate to their habitual physical activity pattern.

Shorter sleep duration has been associated with the development of obesity [146], and a review published two years ago concluded that insufficient sleep in traditional-age students had been associated with weight-gain, hypertension, insulin resistance, diabetes, and stress [147]. A study from Canada investigated the associations between PA and sleep in young adults [148]. They assessed PA subjectively with questionnaire and found that more time spent sedentary was associated with poorer sleep quality. I would like to address these questions in the LBA population with objectively measured PA.

The current PA recommendations are similar for adults from 18-65 years [41, 42], which is a quite wide age range. I would like to further explore PA habits in young adults to investigate whether individuals in this age group could receive additional health benefits from more targeted PA recommendations. Longitudinal studies of PA can contribute to strengthening the evidence of received health benefits.

Finally, there is a large challenge in finding convenient tools to identify CVD risk in young adults. Different risk scores can be used [51, 149, 150], but often several variables are included which can result in a lot of measurements, causing high costs and inconvenience for the individual. In the LBA population, higher body mass and lower CRF and PA were associated with higher arterial stiffness. Therefore, it would be interesting to further explore whether BMI, as a simple indicator of body composition, and resting heart rate, as a simple approximation of the individual's cardiovascular fitness, can be two simple tools for identifying CVD risk in young adults.

## Conclusions

In the LBA study population of young adults in Sweden, it is clear that 12% were classified as being at risk of future CVD. A high CVD risk was associated with low cardiorespiratory fitness and less physical activity. In the total study population 24% had unhealthy food habits, and 24% did not spend the recommended 30 minutes per day at moderate or vigorous intensities of physical activity. Low cardiorespiratory fitness, less physical activity, and overweight and obesity, were associated with stiffer arteries.

The results raises concerns about future CVD risk and highlights the health enhancing possibilities of high cardiorespiratory fitness and physical activity on vascular status in young Swedish adults.

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