Adiposity and carotid atherosclerosis in two populations at very different risk of cardiovascular mortality: Norway and Russia

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Declaration

I, Yume Imahori, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed

Date

8th Sep 2019

Abstract

Obesity is a key risk factor for cardiovascular disease (CVD). The aim of this PhD study was to investigate the associations of obesity and adiposity with carotid atherosclerosis, and, in particular, to examine the effect of general vs abdominal adiposity on the burden of carotid plaque. These associations were investigated in the Tromsø study in Norway and in the Know Your Heart study in Russia. These populations have very different cardiovascular mortality rates, with Russia having one of the highest rates in the world. In this context, the thesis looked at whether differences in carotid plaque burden between these populations can be explained by adiposity.

Four anthropometric measures of adiposity were included, which involved body mass index, waist circumference, waist-to-hip ratio, and waist-to-height ratio. Carotid plaque burden, i.e. the presence of plaques, number of plaques, and total plaque area (TPA), was assessed using carotid ultrasound. In the Tromsø Study 5th survey (2001), there was strong evidence of an association between TPA and adiposity, especially abdominal adiposity, after adjustment for potential confounders (sex, smoking status, education). This association was largely mediated by cardio-metabolic CVD risk factors (blood pressure, cholesterol, glycosylated haemoglobin). These findings were confirmed in a prospective analysis assessing changes in plaque burden between the Tromsø Study 4th (1994-95) and 5th surveys.

Comparison of the cross-sectional results from the Know Your Heart study (KYH) and Tromsø Study 7th wave (Tromsø 7) showed that women in KYH had higher levels of adiposity than in Tromsø 7, while levels were similar for men. Despite evidence that adiposity was associated with carotid plaque (presence of plaques and number of plaques), adiposity failed to explain the high burden of carotid plaque in KYH compared to Tromsø 7 in women as well as men. Explanations for the greater carotid plaque burden in Russia must be sought elsewhere, although reducing levels of obesity in Russia and Norway must be a priority for other reasons.

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Abbreviations

ASDRs	Age-standardised death rates
BIA	Bioelectric impedance analysis
BMI	Body mass index
CAC	Coronary artery calcium
CCA	Common carotid artery
CEE/FSU	Central and Eastern Europe and the former Soviet Union
CHD	Coronary heart disease
CI	Confidence interval
CT	Computed tomography
CVD	Cardiovascular disease
DALY	Disability-adjusted life year
DXA	Dual-energy X-ray absorptiometry
ESSE-RF	Epidemiological Survey of cardiovascular diseases in different regions of the Russian Federation
HbA1c	Glycosylated haemoglobin
HDL	High-density lipoprotein
ICA	Internal carotid artery
ICC	Intraclass correlation coefficient
IPCDR	The International Project of Cardiovascular Disease in Russia
IHD	Ischemic heart disease
IMT	Intima-media thickness
KYH	Know Your Heart Study
LDL	Low-density lipoprotein
MRI	Magnetic resonance imaging
OR	Odds ratio
PA	Physical activity
SBP	Systolic blood pressure

SCORE	Systematic Coronary Risk Evaluation
SNPs	Single nucleotide polymorphism
TPA	Total plaque area
Tromsø 4-7	Tromsø Study the 4-7th survey
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio

1 Introduction

1.1 Background

Although the burden of cardiovascular disease (CVD) in Europe, as well as other high-income countries, has been decreasing for decades, a huge inequality exists in the CVD burden across the European region. While high-income Western European countries enjoy unprecedentedly low CVD mortality rates, the rates in Russia are among the highest in Europe. The European Society of Cardiology categorises Russia as a very-high-risk country with CVD risk levels more than double of those in low-risk countries (1). The gap between the CVD mortality rate among people of working-age in Russia and elsewhere is even wider, resulting in low life expectancy for this industrialised country (2). Furthermore, the estimated Disability-Adjusted Life Years (DALY) for CVD in Russia is among the highest in Europe (1, 3). The reasons for this high CVD burden and associated risks of carotid atherosclerosis are not well understood.

Previous studies have suggested that differences in the prevalence of conventional CVD risk factors such as hypertension, hyperlipidemia, and smoking status do not fully explain this gap in CVD burden between Russia and high-income Western Europe (4, 5). However, these studies are relatively old. In spite of its high CVD burden, CVD risk factors have not been systematically monitored in the general population in Russia, which makes understanding of the distribution and trends of risk factors challenging (6). Further, little research has investigated the association between CVD risk factors and CVD burden over the past 10-15 years. Up-to-date information on the distribution of CVD risk factors and estimation of its impact on CVD burden will be an important step towards implementing a tailored strategy to improving cardiac health in Russia and to reducing excess CVD mortality.

Among risk factors for CVD, obesity is one of few that is increasing not only Russia but also in high-income Western European countries (7). Obesity and its consequent type 2 diabetes may reverse the downward trend in CVD burden in Western Europe and further add to the considerable burden in Russia. Obesity can, thus, be considered as a critical CVD risk factor in the European region. So far, no attempt has been made to investigate to what extent the differences in CVD burden between Russia and high-income Western European countries can be explained by differences in their obesity levels.

Atherosclerosis is a major cause of CVD both through ischemic heart disease and ischaemic stroke, being responsible for over 80% of CVD deaths (8). Atherosclerotic changes occur far in advance of CVD events, having been found even in young adults (9-11). Therefore, the identification of sub-clinical atherosclerosis holds great promise as an effective biomarker of CVD. Several non-invasive imaging modalities have the potential to predict CVD risk beyond conventional CVD risk factors by capturing regional atherosclerotic changes (12, 13). Among them, carotid atherosclerosis can be easily and safely assessed at relatively low-cost using ultrasound, making it suitable for large studies using healthy populations. In particular, detection of carotid plaque is promising with its good predictive ability for future CVD events (14). While increased intima-media thickness (IMT), a widely-used measure of carotid atherosclerosis, does not necessarily indicate atherosclerotic changes, carotid plaque is characteristic of a later stage of atherosclerosis, outperforming IMT regarding predictive value for CVD (15). Not only can carotid plaque be used as an imaging biomarker of CVD burden, but can also potentially be used to compare CVD burden directly across different populations with different CVD risks.

The Know Your Heart (KYH) Study is a population-based cross-sectional study conducted in two Russian cities during the years 2015-18 (2). In this study, data were collected on carotid plaque variables and several anthropometric adiposity measures, along with data on traditional CVD risk factors, from a random population-based sample aged 35-69 years. Efforts were made to align the main protocols of KYH with those of a concurrent population-based study in Norway: the Tromsø Study seventh survey (Tromsø 7) conducted in 2015-16, with the aim of increasing the validity of planned comparisons between the two study populations. In the Tromsø 7 study, data were collected on similar variables to KYH, including carotid plaque variables and adiposity measures from a random population sample. Norway is a typical Western European country with moderately low CVD mortality. However, the CVD mortality rate at working age is eight times higher in Russia than in Norway (2). Thus, KYH and Tromsø 7 provide a unique opportunity to examine differences in carotid plaque burden and the associations with adiposity in untreated older people across two populations with a contrasting CVD risk. Furthermore, analysis of the two studies enables us to examine whether excess carotid plaque burden in Russia, if any, can be explained by adiposity level.

1.2 Aims and Objectives

The aim of this thesis is to examine the contribution of adiposity to atherosclerotic changes in the carotid artery in a general population in Russia compared to that in Norway, a low CVD risk European country. A further aim is to determine whether adiposity explains the difference in carotid plaque burden between the two populations.

The thesis has two main objectives:

1) To investigate the role of general and abdominal adiposity on measures of carotid plaque assessed by ultrasound in population-based studies from Norway (the Tromsø study seventh survey: Tromsø 7) and Russia (Know Your Heart Study: KYH);
2) To determine whether differences in carotid plaque burden (the presence of plaques, number of plaques) between the two populations, if any, can be statistically accounted for by various measures of adiposity

1.3 Structure of the thesis

This thesis has been organised as follows. After this introduction, I review the literature on CVD/atherosclerosis, adiposity, and the association between them to provide the overall context of this thesis (Chapter 2: literature review). This is followed by a systematic review of the published evidence on the association between ultrasound-assessed carotid atherosclerosis and adiposity, which identifies knowledge gaps in this area (Chapter 3: systematic literature review). In the next two chapters, I describe the study setting and design of the two population studies I use (KYH and the Tromsø Study) (Chapter 4: Study setting and design) and their main exposures and outcomes (Chapter 5: Main exposure, outcome, and other variables).

I report the results of my analyses in four chapters (6 to 9). In Chapter 6, I investigate the cross-sectional association between carotid plaque burden and four adiposity measures to confirm the pathway between adiposity and carotid atherosclerosis in the fifth survey of the Tromsø study. In Chapter 7, I investigate the same issue using longitudinal rather than cross-sectional data from the Tromsø study to investigate the prospective association between the progression of carotid atherosclerosis and baseline adiposity. In Chapter 8, I examine and compare the general/abdominal adiposity level and the prevalence of obesity in KYH and the Tromsø 7 study. Finally, I compare the association of carotid atherosclerosis with general/abdominal adiposity in KYH and Tromsø 7, leading onto an analysis of whether

adiposity explains the difference in carotid atherosclerosis between these two population-based studies (Chapter 9).

2 Literature review (CVD, adiposity, the association between adiposity and CVD)

This chapter aims to provide the context of the thesis with a literature review on cardiovascular disease (CVD)/ atherosclerosis, adiposity, and the association between adiposity and CVD. In the first section, current knowledge about the burden of CVD worldwide, in Western Europe and in Russia is summarised. This is followed by an overview of the pathology of atherosclerosis, a major cause of CVD, and imaging techniques to assess atherosclerosis. In the second section, the burden of obesity in Western Europe and Russia is described, followed by a discussion of the complexity of obesity, its assessment, and risk factors for obesity. In the final section, the current evidence on the association between adiposity and CVD is reviewed.

2.1 Cardiovascular disease (CVD)

2.1.1 Cardiovascular disease (CVD) and atherosclerosis

CVD is a leading cause of death worldwide. In 2015, it was estimated that nearly a third of all global deaths (17.7 million) were attributable to CVD (16). CVD not only has a negative impact on the lives of individuals due to premature mortality or disability but also imposes a huge economic burden on society. Further effort is needed to reduce the CVD burden.

Atherosclerosis is a major cause of CVD. Atherosclerotic CVD mainly comprises two major conditions: ischemic heart disease (IHD) and cerebrovascular disease, particularly ischemic stroke. IHD and stroke accounted for 84.5% of CVD deaths in 2013 (8).

Atherosclerosis is a chronic inflammatory disease of the arteries, which narrows or blocks important vessels such as coronary and cerebral arteries with serious consequences. The major risk factors for atherosclerosis are well established and preventable. Thus, major guidelines aiming to reduce CVD focus on the prevention of atherosclerotic CVD (12, 17, 18).

Broadly, risk factors for atherosclerosis are divided into two groups: behavioural risk factors and cardio-metabolic risk factors. The American Heart Association has specified four health behavioural factors (smoking, obesity, physical activity, diet) and three cardio-metabolic factors (cholesterol, blood pressure, blood glucose) which have significant impacts on

cardiovascular health (19). Similarly, the European Society of Cardiology specifies eight major CVD risk factors: four behavioural risk factors (diet, low physical activity, smoking, alcohol use) and four medical risk factors (high systolic blood pressure, high total cholesterol, high fasting plasma glucose, high body mass index) (20). Among them, blood pressure, cholesterol, and smoking are particularly important. These three, along with age, are included in main CVD score systems to predict future CVD risks (12). The control of these cardio-metabolic and behavioural risk factors is a vital step to achieving a reduction in CVD.

Fortunately, CVD mortality has experienced a dramatic decrease since 1980, particularly in high-income countries (21-24). This reduction has largely been due to two factors: reduction in major risk factors and evidence-based medical and surgical treatment. The improvement in hypertension treatment, widespread statin use and a decrease in the prevalence of smoking have resulted in the reduced distribution of CVD risk factors (22). The analysis of the prospective population-based cohort Tromsø Study revealed that change in CVD risk factors contributed to 66% of the reduction in CVD deaths between 1995 and 2010 (25). At the same time, appropriate delivery of evidence-based treatment played a significant role in reducing CVD mortality rates, including secondary preventive therapies after myocardial infarction, revascularisation, initial treatments for coronary artery disease, and treatment for heart failure (22).

In spite of this success story in CVD control in high-income countries, there remain two major concerns which must be addressed. First, CVD mortality rates show a huge regional difference with some countries still suffering from a heavy burden of CVD compared to their neighbours. Second, although some risk factors such as blood pressure and hyperlipidemia are well-controlled with a sustained reduction in the prevalence in high-income countries, the prevalence of obesity has increased, accompanied by an increase in type 2 diabetes in almost all countries. This increase in obesity and diabetes might offset or reverse the downward trend of the CVD burden. Indeed, signs of deceleration have already been observed (26). For example, analysis of the US national data showed that the decline in CVD in the US has substantially decelerated since 2011, which may be attributable to a rapid increase in obesity and diabetes (27). Therefore, the prevention and treatment of obesity might be crucial to further success in the control of CVD.

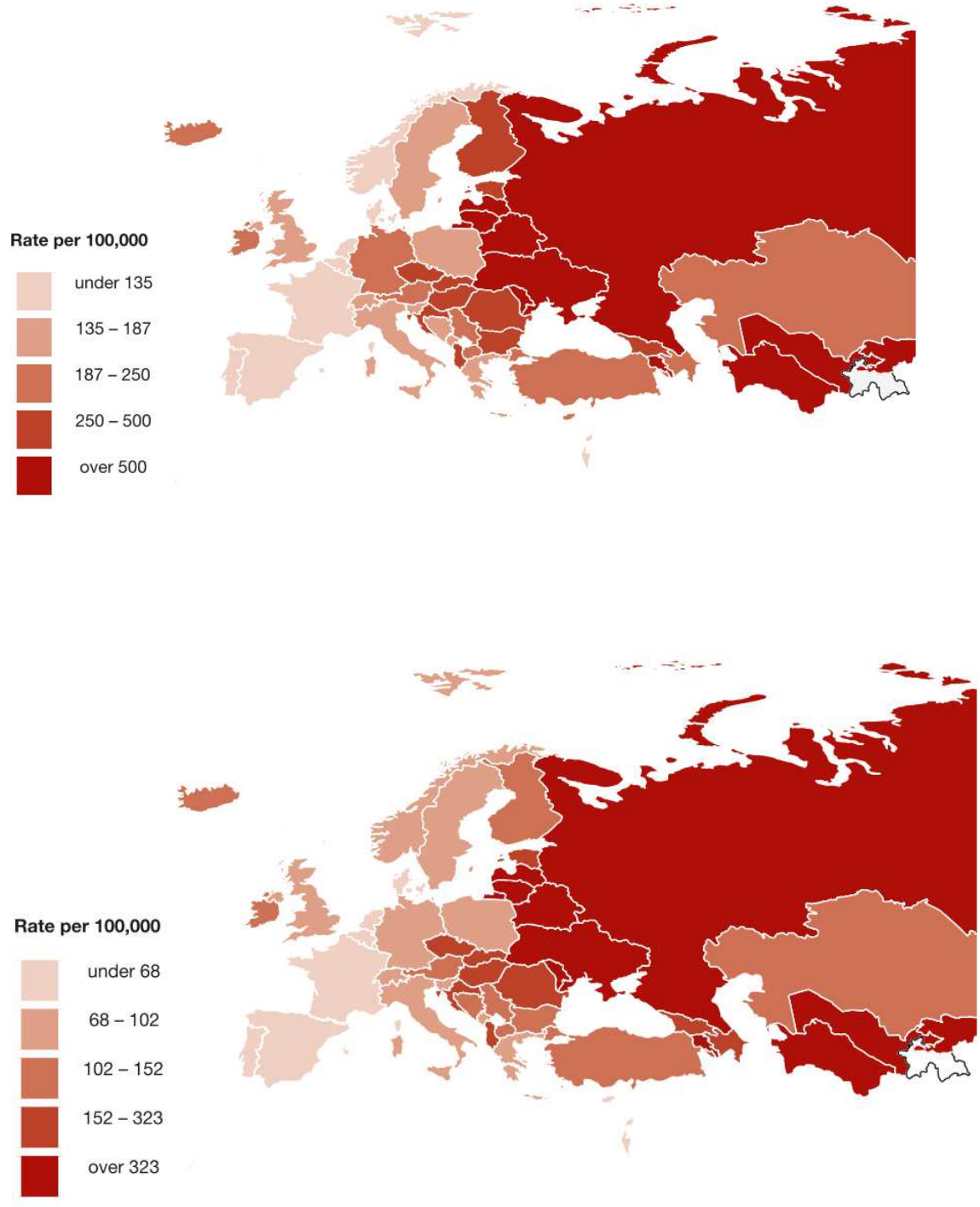
The European region faces both problems: a huge gap in CVD burden between regions and an increasing obesity burden. Although the CVD mortality rate has decreased dramatically in

high-income Western European countries, Russia still suffers from a high but declining CVD burden which results in low life expectancy for such an industrialised country (65.2 years for men and 76.5 years for women) (28). Detailed information on CVD burden and the distribution of risk factors in the European region was provided in the next section.

2.1.2 Russia and Western Europe: Comparison of CVD burden and CVD risk factors

A huge difference in CVD burden exists between Western Europe and non-EU countries, although the CVD mortality rate has decreased overall in the European region (Figure 2-1). According to a recent report, there are 4 million CVD deaths per year in Europe as a whole, accounting for 45% of total deaths (1). Furthermore, 1.4 million of these deaths occur in people under 75 and 700,000 in people under 65. However, as Table 2-1 shows, the CVD burden varies widely according to the European region. The CVD burden in EU-28 countries and non-EU members is clearly higher than that in high-income Western Europe. In Western European countries, deaths from CVD make up 33% of total deaths, which is a lower percentage than in other European regions with lower age-standardised death rates (ASDRs). On the other hand, in non-EU countries, 54% of deaths are attributable to CVD with higher ASDRs. In Russia, ASDRs in men and women are 1423.1/100,000 and 914.0/100,000, respectively. This exemplifies the enormous inequality of the CVD burden among European countries.

Regarding trends in CVD mortality, there has been a substantial decrease in Western Europe, ranging from 25.2% in Austrian men to 49.7% in Luxembourger men, and from 25.3% in Italian women to 42.9% in Portuguese women. Non-EU countries also show downward trends generally, but the decrease is not as sharp as in Western European countries, and there is wide variation. Since 2005 there has been a 10-year decrease in the Russian Federation of 19.0% in men and 23.9% in women. There is also a large difference in Disability-Adjusted Life Years (DALY) attributable to CVD between European countries with Russia having the second-highest (181 per 1000 population) (1).



Data from European Cardiovascular Disease Statistics 2017 edition (29)

Figure 2-1 Age-standardised death rates from ischemic heart disease under 65 years, 1980 to 2015 (upper panel: males, lower panel: females)

Table 2-1 Differences in the burden of CVD: Range of ASDRs according to EU regions (over ten years)

	Percentage of CVD/total deaths	ASDRs per 100,000 for CVD in men		ASDRs per 100,000 for CVD in women	
		Minimum	Maximum	Minimum	Maximum
EU-15 countries	33% (1.3 million)	France 275.2	Finland 480.7	France 174.1	Greece 391.3
EU-28 countries	38% (1.9 million)	Malta 407.7	Bulgaria 1299.5	Malta 317.0	Bulgaria 959.6
Non-EU member	54% (2.1 million)	Israel 255.0	Ukraine 1544.9	Israel 194.9	Kyrgyzstan 1087.4

Data taken from Townsend et al. (1)

The distribution of traditional CVD risk factors is an important determinant of CVD burden, which may explain the gap in CVD burden between Western Europe and Russia. Recently, the European Society of Cardiology reported detailed data on the distribution and trend of CVD risk factors in Europe (20). They concluded that the prevalence of some risk factors has shown a downward trend in Europe. For example, the prevalence of raised systolic blood pressure has decreased over the last three decades, although the extent of this decline varies widely by country and national income with high-income countries showing lower prevalence. In high-income Western European countries, the mean prevalence of raised blood pressure (systolic blood pressure >=140 mmHg or diastolic blood pressure>=90 mmHg) was 27.3% in men and 18.3% in women according to data from the WHO Global Health Observatory in 2014. On the other hand, there is a higher prevalence of raised blood pressure in Russia for both men and women at 33.5% and 24.5%, respectively. Similarly, blood cholesterol concentration levels in Europe have decreased over the last 30 years. The age-standardised prevalence of raised blood cholesterol (>6.2 mmol/l) in Western Europe was 19.0% in men and 18.9% in women. Interestingly, Russian men had a much lower prevalence at 12.3% than their Western European counterparts, while Russian women also showed a slightly lower prevalence (17.1%) (20).

In contrast to hypertension and hyperlipidemia, there has been a marked rise in the prevalence of obesity in both high- and middle-income European countries in the past three decades (30). This may sound paradoxical, considering obesity is a risk factor for hypertension and hyperlipidemia. However, the control of obesity seems more difficult than

that of hypertension and hyperlipidemia: a meta-analysis shows that the long-term maintenance of weight-loss by lifestyle change or medication is challenging (31), which makes prevention and treatment of obesity difficult compared to other CVD risk factors. The increase in obesity could potentially hinder the decline in CVD mortality and could, in the future, result in a reversal of the progress made. The prevalence of general obesity assessed by BMI was 23.1% in men and 22.9% in women in Western Europe. Russian men had lower general obesity prevalence at 20.3%, whereas Russian women showed a higher prevalence at 27.4% (20).

Another increasing risk factor for CVD is type 2 diabetes, which can be a consequence of increasing obesity. Diabetes is characterised by hyperglycemia and the development of microvascular pathology; this results in detrimental health conditions, including blindness and renal dysfunction. In addition, diabetes contributes to the acceleration of atherosclerotic disease, leading to CVD events (32). A recent meta-analysis from 68 prospective studies showed that men with diabetes have almost two times higher risk of deaths from occlusive vascular diseases such as ischemic heart disease and stroke while the risk among women tripled after adjustment for traditional CVD risk factors (33). Although currently available data appear to show a higher prevalence of diabetes in high-income than middle-income European countries, the prevalence of raised blood glucose is higher in middle-income than high-income European countries, suggesting either underdiagnosis or poor control of existing diabetes in middle-income European countries (20). The prevalence of raised blood glucose in men and women was 7.2% and 5.2% respectively in Western Europe while it was 7.4% and 8.0% respectively in Russia, suggesting the burden of diabetes may be higher in Russia than Western Europe, especially for women (20).

With respect to health behaviours, smoking prevalence shows the most dramatic difference between Western European men (26.3%) and Russian men (55.0%). Smoking prevalence in women is similar at 17.0% in Western Europe and 16.3% in Russia (29, 34). However, increasing smoking prevalence among Russian women is a concerning trend (34). Information on other behaviours such as physical activity and diet may be less reliable because of self-reporting nature and measurement error. Caution is needed when interpreting these data. Regarding alcohol consumption patterns, there are some differences between Western Europe and Eastern Europe including Russia. Although recorded per-capita consumption of alcohol is similar between Western Europe and Eastern Europe, Eastern

Europe is characterised by high consumption of unrecorded intake, including home-made alcohol, resulting in higher per-capita consumption of alcohol in Eastern Europe (26.4 l/drinker) than Western Europe (15.0 l/drinker). Furthermore, heavy drinking is more common in Eastern Europe (17.7%) (35).

To summarise, available data suggest a higher prevalence of smoking, hypertension, and heavy drinking among Russian men and higher prevalence of hypertension, raised glucose, and obesity among Russian women than their Western European counterparts when the distributions of CVD risk factors are compared (20). However, it should be noted that evidence from Russia is limited compared to Western Europe. In spite of its high CVD burden, there is no systematic monitoring system for CVD risk factors in the general population in Russia. Updated epidemiological data on CVD risk factors would be of the utmost importance in any investigation of the reasons behind the high CVD burden in Russia.

2.1.3 Pathology of atherosclerosis and its non-invasive assessment using imaging modality

In the following section, I briefly summarise the pathology of atherosclerosis and its assessment using imaging techniques. I also describe current evidence on how imaging modality predicts future CVD risks.

Pathology of atherosclerosis

Atherosclerosis is a chronic condition which occurs in the large and medium-sized arteries. Normal large and medium-sized arteries have three layers: the innermost layer is the intima, which is a monolayer of endothelial cells and is in contact with blood. The second layer, the tunica media, consists of smooth muscle cells which control vessel tone. The intima and media layers are separated by the internal elastic lamina. The role of the outer layer, the adventitia, is not clear, although it contains the vasa vasorum and nerve endings in addition to collagen fibrils (36). The main location of atherosclerosis is the intima. The following is a brief description of the development of atherosclerosis, divided into two steps: atherosclerosis initiation and evolution of atheroma.

First step: atherosclerosis initiation: The process of atherosclerosis begins with the accumulation of lipoprotein particles in the intima (36). They bind to proteoglycans and become more susceptible to various modifications, especially oxidative modification. A further important step is the recruitment and retention of leukocytes. They enter the intima,

accumulate lipids, and create foam cells. While monocyte and T lymphocytes are both involved in this process, the main player is the monocyte. Monocytes receive the signal to penetrate the endothelial monolayer from the chemoattractant cytokines and enter the arterial wall, where they differentiate into inflammatory macrophages and become lipid-rich foam cells by digesting oxidized lipoproteins, starting the formation of the fatty streak (37, 38). At this stage, the structure of the fatty streak is not complicated and may be reversible to some extent (36).

Second step: evolution of atheroma: It is widely accepted that inflammation plays a critical role in the evolution of early atheroma to the more complex plaque (39). Macrophage-derived foam cells synthesise inflammation mediators, which promote inflammation. Another important player is the smooth muscle cell; these cells migrate from the media into the intima, gathering, and producing extracellular matrix molecules. These processes make simple fatty streaks into complicated fibrofatty lesions (39). These atherosclerotic changes progress slowly, often for decades during which patients usually have no symptoms (36).

Eventually, narrowing (stenosis) of the affected artery prevents blood flow when the demand for oxygen increases. This can cause, for example, stable angina if it occurs in the coronary artery. However, even if the stenosis is not severe, the rupture of smaller non-occlusive plaques may cause thrombosis and occlusion of the artery, resulting in acute coronary syndrome. Indeed, it is well-known that plaque stability and vulnerability play an important role in CVD events (40).

Imaging techniques to identify atherosclerosis

As mentioned above, atherosclerosis occurs far in advance of CVD events. Thus, it is expected that detection of an atherosclerotic change in arteries can predict future CVD events in an apparently healthy population. Various imaging techniques can detect sub-clinical atherosclerosis non-invasively, and some can predict future CVD events beyond conventional CVD risk factors. As a result, they have been receiving considerable attention recently as a way to identify individuals at high CVD risk.

Among a variety of non-invasive imaging techniques, MRI and CT can provide accurate information on atherosclerosis but are costly. On the other hand, B-mode ultrasound is widely available, relatively cheap and less time-consuming, and may be more suitable for large-scale epidemiological studies. The following section describes the main imaging techniques used to

evaluate atherosclerosis in the carotid and coronary arteries, which are the main arteries affected by atherosclerosis.

Non-invasive assessment of Carotid artery atherosclerosis:

Intima-media thickness (IMT): Ultrasound has been used to assess carotid atherosclerosis for more than three decades. Assessment of the carotid artery using ultrasound is easy due to the superficial location of the artery. Most traditional ultrasound measurement is the IMT; this technique has been used since the mid-1980s in numerous studies. IMT is a double-line pattern visualised by ultrasound, with the lines representing the lumen-intima and the media-adventitia interfaces (41). Due to the low frequency of the ultrasound, it is not possible to resolve the media and intima separately. The Mannheim consensus recommends that IMT is measured on the far wall of the common carotid artery (CCA) where the most accurate measurement can be made (41).

Assessment of IMT shows high reproducibility when being measured at the CCA. IMT has consistently shown a good predictive ability for future CVD events, but a recent meta-analysis showed that there is little added predictive value above traditional risk assessment, such as the Framingham Risk Score (42). As a result, IMT was downgraded to class III evidence (no benefit): not recommended in clinical practice for risk assessment for a first atherosclerotic CVD event in the 2013 ACC/AHA Guideline on the assessment of cardiovascular risk (43). This relatively weak predictive ability for CVD events may be attributed to two drawbacks of IMT. First, because IMT measurement cannot distinguish the intima from the media, increased IMT does not necessarily reflect atherosclerotic changes which mainly occur in the intima. Media hypertrophy or adaptive intimal thickening caused by ageing and shear stress can also cause an increase in IMT (44). Second, progressive atherosclerotic changes are more likely to occur at the location of nonlaminar turbulent flow such as the bifurcation or internal carotid artery (ICA) than at the CCA where progressive changes are rare (44). Thus, the CCA is not the best site to observe atherosclerotic changes in the carotid artery.

Carotid plaque assessed by ultrasound: carotid plaque is also detected using ultrasound. The definition of carotid plaque in the ultrasound examination varies in the literature (45). However, the Mannheim consensus definition is increasingly used as the standard recently: it is defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or

50% of the surrounding IMT value or demonstrates a thickness > 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (41). While IMT adds little predictive value for future CVD events beyond traditional CVD risk factors, carotid plaque appears to be more powerful predictor (15). A meta-analysis showed that carotid plaque predicts future CVD events more accurately than IMT. This higher predictive value of carotid plaque over IMT is understandable from the pathological point of view: as discussed above, IMT may increase due to age-related thickening of intimal and hypertensive thickening in the medial layers of the CCA which can occur without atherosclerosis (44, 47) whereas carotid plaque represents a later stage of atherosclerotic change. Currently, the guidelines of the European Society of Cardiology categorise ultrasound examination of carotid plaque as Class IIb: carotid plaque assessment may be considered as a risk modifier in CV risk assessment whereas IMT is not recommended for additional risk stratification (Class III) (12).

Although many of previous studies with carotid plaque use the presence of plaques (Yes/No) as an outcome, quantitative carotid plaque variables such as plaque height, number of plaques, and plaque area may be more informative than the binary presence of plaques (14). For example, the number of plaques is simple to assess, yet may be a useful measure of carotid plaque burden. Stork et al. showed that the number of plaques predicted cardiovascular mortality beyond traditional CVD risk factors in 367 elderly men (48). A more sophisticated quantitative plaque measure, total plaque area (TPA), entails the evaluation of two-dimensional plaque area. It is obtained by outlining the longitudinal image of the plaque using dedicated software. Previous studies have shown that subjects in the top tertile of TPA had an increased risk of myocardial infarction and ischemic strokes compared to those without plaque while the association between IMT and these events was weaker than that with TPA (49, 50).

Furthermore, TPA measurement may be useful in detecting the progression of the plaque burden because plaque grows along the axis of an artery 2.4 times faster than it changes in thickness (44). Thus, TPA could be a good candidate as a CVD surrogate endpoint in large-scale epidemiological studies. In addition, in spite of its simplicity, number of plaques may also be a good marker: a recent study showed that the number of plaques correlated with TPA well and showed the similar predictive ability for future cardiovascular events to TPA (24).

Total plaque volume, three-dimensional measurement of carotid plaque, may be even more accurate and sensitive to the progression of atherosclerosis than TPA: plaques typically have

a complex three-dimensional structure which two-dimensional measurement cannot capture. Three-dimensional volumetric measures allow the detection of progression of plaque in all directions. However, three-dimensional carotid ultrasound is not as widely available as two-dimensional ultrasound and generally involves time-consuming three-dimensional reconstruction of swept two-dimensional imaging. Thus, a carotid plaque variable using two-dimensional ultrasound is currently more practical in large epidemiological studies.

The limitation of carotid plaque measurements mentioned above is that they cannot assess the quality of the plaque which characterises the vulnerability of the plaque such as intraplaque haemorrhage and lipid-rich necrotic core. This is due to the relatively low frequency of the ultrasound along with acoustic shadowing. Other imaging modalities such as CT and MRI are more suitable for this purpose.

CT, MRI: MRI is the gold standard imaging modality: It not only evaluates plaque burden accurately, but it also detects plaque neovascularity, necrotic core, and fibrous tissue, the characteristics of which are related to plaque vulnerability when used with gadolinium-based contrast (51). CT can also evaluate plaque morphology as well as the precise plaque burden in spite of some weaknesses (51). Although MRI and CT are desirable tools to examine carotid atherosclerosis in individuals with some CVD risks in a clinical setting, it is often unrealistic to use them for large studies of general population samples or apparently healthy individuals due to their cost and, in the case of CT, exposure to radiation.

Non-invasive assessment of coronary artery atherosclerosis

Atherosclerotic change in a coronary artery is extremely important as it can lead to angina pectoris and myocardial infarction. Invasive angiography is the gold standard for clinical diagnosis of severe coronary artery disease (52). However, other invasive methods such as fractional flow reserve and instantaneous wave-free ratio have recently been receiving attention as they can be used to assess the functional severity of carotid lesions with stenosis beyond angiography (53, 54). However, these methods are only indicated for symptomatic patients with an intermediate-high probability of significant disease (52). Other non-invasive technologies are also available to detect coronary atherosclerosis in individuals with lower risk.

The rapid technical development of cardiac CT and MRI technology has enabled us to evaluate coronary atherosclerosis non-invasively and reliably. Coronary computed

tomographic angiography and magnetic resonance angiography can accurately reveal coronary regions responsible for symptoms (55). Tarkin et al. review other promising imaging techniques in addition to CT and MRI thoroughly (55). However, regarding the improvement of CVD risk stratification, a great deal of attention has been paid to coronary artery calcium (CAC) scanning. CAC is a non-contrast, limited chest computed tomography scan which can assess the plaque burden in the coronary artery. Coronary calcium is defined as a lesion above a threshold of 130 Hounsfield units: scores of 0 mean the absence of calcified plaque and scores>400 means severe plaque (56). Among novel risk markers, CAC seems the strongest predictor of future CVD events (57). Although it is an attractive option, the cost, exposure to radiation, and limited access to CT are issues, especially for large epidemiological studies.

2.2 Adiposity and obesity

2.2.1 Defining overweight, obesity, and adiposity

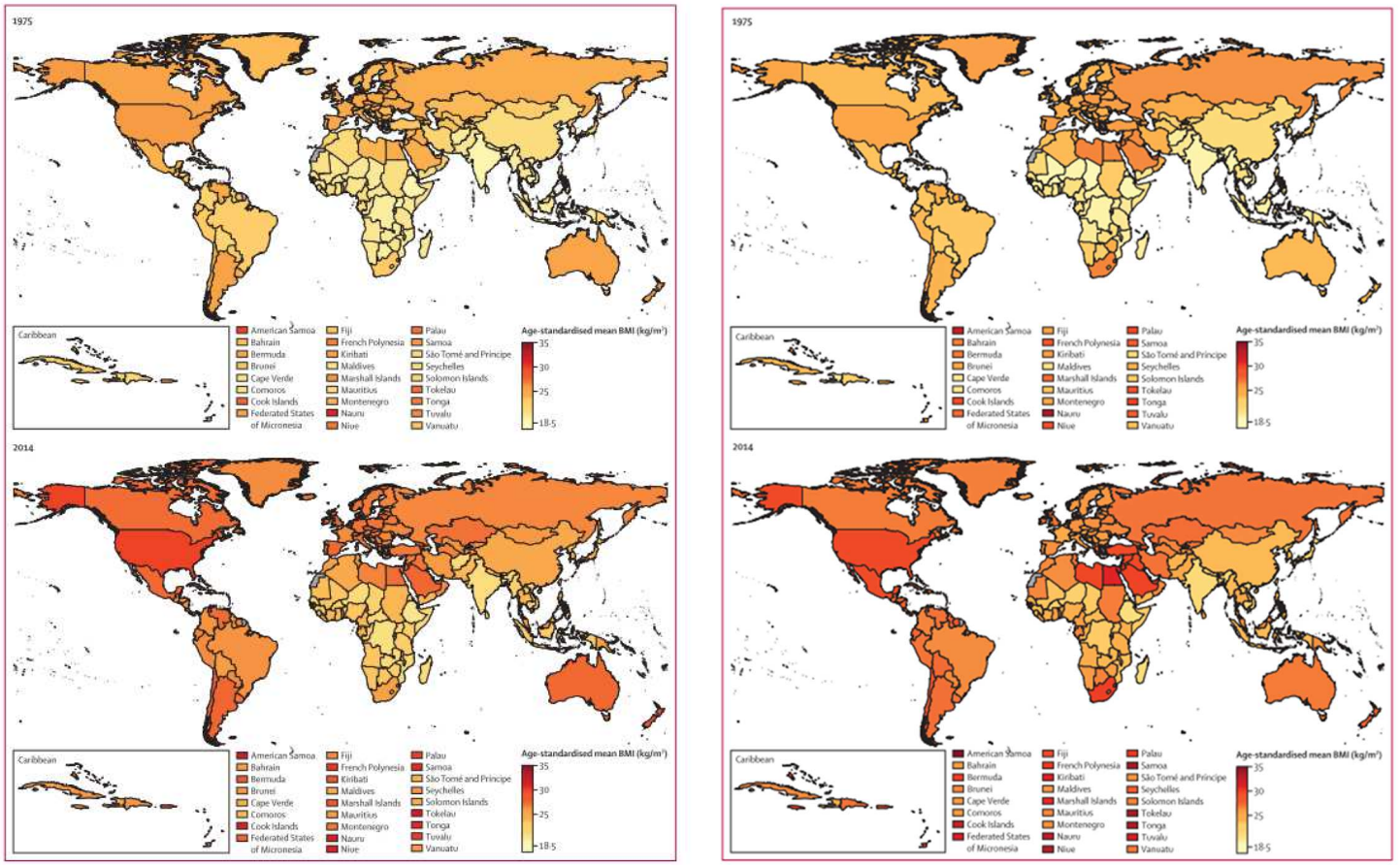
In this section, I define the terms “overweight”, “obesity”, and “adiposity” as used in this thesis. Conventionally, body mass index (BMI) cut-offs are used to define overweight (BMI 25-29.9 kg/m^2) and obesity (BMI>=30 kg/m^2) in the adult population (58). The 2013 AHA/ACC/TOS Guideline for the management of overweight and obesity in adults recommends using the BMI cut-off to identify patients with overweight and obesity based on a thorough review of available evidence (59). On the other hand, the term “adiposity” is used to express the state of fatness, but there is no strict definition in the literature. Therefore, in this thesis, I define overweight and obesity (or general obesity) using the BMI cut-off points (overweight: BMI 25-29.9 kg/m^2, obesity or general obesity: BMI>=30 kg/m^2). Similarly, abdominal obesity is defined using the WHO cut-off points of waist circumference (WC) (>102 cm for men, >88 cm for women) or waist-to-hip ratio (WHR)(>=0.9 for men, >=0.85 for women). However, the term adiposity is used to describe the extent of adipose/fat tissue in absolute or relative terms.

2.2.2 Global burden of obesity

Obesity is a worldwide epidemic. According to WHO data, 39% of adults were overweight, and 13% were obese in 2016. Obesity has various medical consequence such as CVD, diabetes, hypertension, hyperlipidemia, sleep apnoea, osteoarthritis, chronic kidney diseases, some cancers, and musculoskeletal disorders (60). In 2015, 4.0 million deaths and 120 million DALYs were attributed to high BMI (26). Among them, the most concerning

consequence is CVD. CVD was the leading cause of death and DALYs related to high BMI, followed by diabetes, with 2.7 million deaths and 66.3 million DALYs being associated with high BMI (26). It can be said that obesity is now the number one health problem in high- and middle-income countries.

The general population is getting more and more obese. The prevalence of obesity is almost three times higher than 30 years ago and continues to increase (61). Figure 2-2 shows age-standardised BMI in 1975 and 2014 (30). As can be seen from the figure, not only high-income countries but also middle- and low-income countries have experienced a substantial increase in age-standardised BMI during the past three decades. For instance, in 2014 China had the highest number of people with obesity and the second-highest number with severe obesity (BMI>=35 kg/m^2) followed by the US, while India moved from 16th place for both sexes in 1975 to 5th place for men and 3rd place for women in 2014 regarding the ranking of the number of people with obesity (30). It is expected that a rise in obesity in middle- and low-income countries will contribute to a sharp rise in obesity in the future. The global prevalence of obesity has already surpassed that of underweight, and it is expected that the prevalence of severe obesity will surpass that of underweight by 2025 (30).



Figures taken from NCD Risk Factor Collaboration (30)

Figure 2-2 Age-standardised mean BMI in 1975 (upper panels) and 2014 (lower panels) (Left: men, Right: women):

In general, obesity affects women more than men, and the middle-aged are the most affected. Figure 2-3 shows the prevalence of obesity in 195 countries according to age and sociodemographic index quintile, which is a proxy for the national level of development (26). High sociodemographic index means the country is highly developed. As can be seen in Figure 2-3, overall patterns are similar regardless of the development level of the country. The prevalence of adult obesity constantly rises from adolescence and reaches a peak at 60-64 years of age in women and 50-54 years in men, declining thereafter.

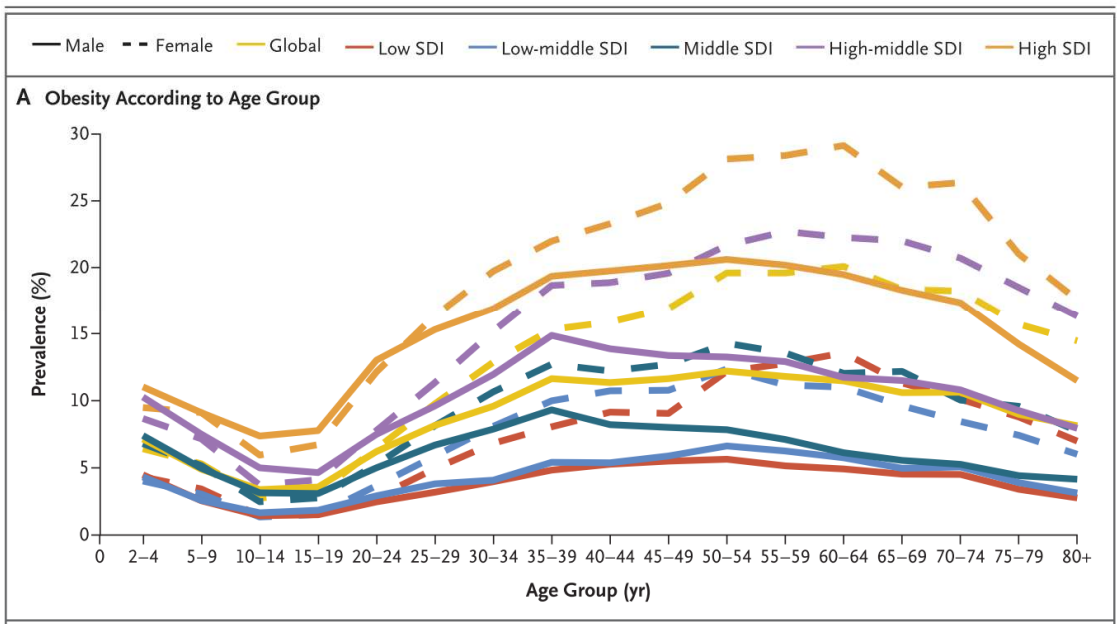


Figure taken from Afshin et al. (26)

Figure 2-3 Obesity prevalence according to age group by sociodemographic index quintile

2.2.3 The burden of obesity: Western Europe and Russia

In this section, I review the available evidence on obesity in Western Europe and Russia. Europe is a region with a heavy burden of obesity. According to 2016 WHO data, Europe had the second-highest prevalence of overweight (male 63.1% female 54.3%) and obesity (male 21.9% female 24.5%) among WHO regions, after Americas (62). The population-attributable fraction for all-cause mortality due to overweight or obesity in Europe was 14%, following North America (19%) and Australia/New Zealand (16%) (63).

As in other countries, in Russia, there is a sex difference in obesity burden, although the differences are more pronounced than in many other parts of Europe. Russian women have a higher burden of obesity than Western European women while it is similar or lower in

Russian men. The prevalence of obesity based on WHO data was 20.3% and 27.4% in men and women, respectively, in Russia, while it is 22.9% and 23.1% in Western European countries (20).

The prevalence of obesity in Russia does not seem very problematic compared to Western Europe for men. However, it should be noted that Russia is the tenth most populous country in the world and the most populous in Europe, which means Russia makes a major contribution to the absolute numbers of people with obesity in Europe. In 2014, Russia was ranked fourth in the world for the number of people with obesity for both sexes, and in third place for the number of people with severe obesity for both sexes (30). Further, it seems that high BMI is a greater contributor to health risk in Russia than in Western Europe. In 2015, Russia had the highest rates of BMI-related death (>140 deaths per 100,000 population) and DALYs among the world's 20 most populous countries (26).

Like the rest of the world, both Russia and Western Europe have experienced a significant rise in the burden of obesity in the past three decades. The prevalence of obesity increased steadily in high-income European countries for both men and women during this period (30). On the other hand, as shown in Figure 2-4, the prevalence of obesity in Russian women was relatively stable although it was constantly higher than in Western European women, and the prevalence of severe obesity (BMI>=35 kg/m^2) increased. Regarding Russian men, the prevalence of obesity showed a steady increase (Figure 2-4). If this trend continues, the increase in obesity in men and severe obesity in women could result in a further burden of CVD in Russia.

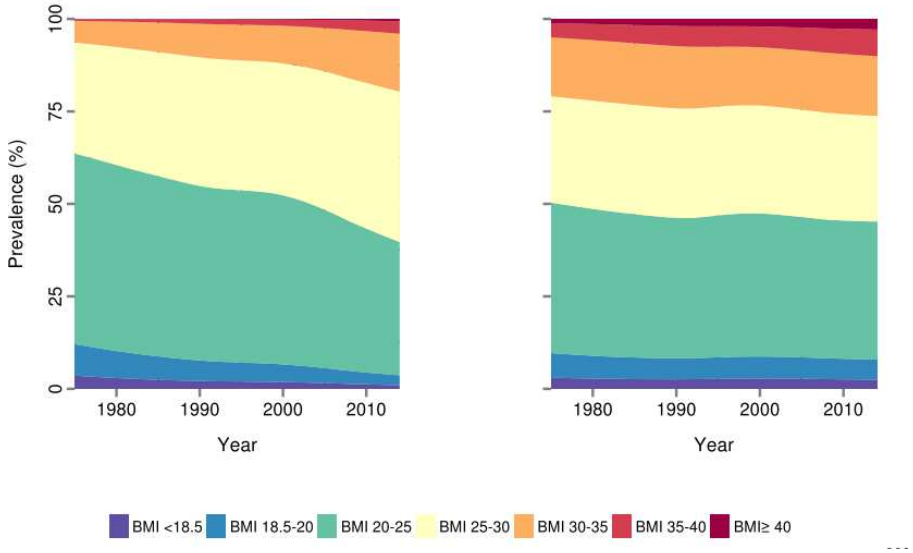


Figure taken from NCD Risk Factor Collaboration (30)

Figure 2-4 Trends in age-standardised prevalence of BMI categories in Russia (1980-2010) (left panel: men, right panel: women)

Thus far, I have described the burden of obesity in Europe and Russia. However, it is important to note that there are two limitations to the data presented above. First, Russia does not have an established monitoring system of obesity and other CVD risk factors, which makes it difficult to accurately capture the prevalence of obesity and trends in the country. Only limited data are available on obesity in the general population (6, 64, 65). As a result, the data for Russia may not be as reliable as that from Western European countries which have an established system for monitoring CVD risks. Second, overweight and obesity are defined using BMI cut-off points, and this has some important limitations for assessing adiposity, which may result in the underestimation of adiposity-related health risks. The limitations of BMI and other adiposity measures will be discussed in detail in the next section.

2.2.4 Complexity of adiposity assessment

In the history of obesity, it is not so long since humankind predominantly suffered from food shortage and undernutrition. Historically, the term "obesity" originated from Latin obesus 'having eaten until fat' and gained usage in the mid-17th century, but obesity was not recognised as a cause of illness until the mid-19th century (66). In the late 20th century, a large shift in diet and physical activity occurred, which triggered a global epidemic of obesity.

As mentioned before, overweight and obesity are conventionally defined using BMI cut-off points. BMI is very convenient and easily available in a wide setting. In clinical guidelines, assessment of BMI is the first step in obesity management (59). The assessment of adiposity level with BMI is undoubtedly important. However, adiposity is too heterogeneous and complex to understand based on BMI alone.

The heterogeneity of obesity has confused clinicians for a long time: at the individual level, the health status of those in the same BMI category varies widely. Some people with obesity have multiple CVD risk factors such as hypertension and diabetes, while others appear to be healthy without any CVD risk factors. An extreme example is Japanese Sumo wrestlers. Although Sumo wrestlers are highly obese according to their BMI category, most of them have normal plasma glucose and low total-cholesterol levels (67). The key to understanding this heterogeneity of obesity is visceral adipose tissue, i.e. adipose tissue located in the abdominal cavity.

Since the mid-1980s, it has become increasingly clear that regional adipose tissue plays a more important role in metabolic abnormalities than mere excess of the total body adipose tissue. In 1984, the Gothenburg group in Sweden sparked interest in fat distribution by showing that abdominal adiposity assessed by WHR was more strongly associated with CVD events and deaths than BMI (68, 69). Subsequently, the development of imaging technologies such as CT and MRI enabled us to distinguish visceral adipose tissue from subcutaneous adipose tissue. Visceral adipose tissue appears to be strongly associated with a wide range of disorders including insulin resistance, hyperglycaemia, lipid disorder, hypertension and all-cause mortality (70-72) and is thus considered as a key player to link adiposity with CVD (72). Even among people who are equally obese in terms of BMI, increased visceral adiposity is associated with increased CVD risks, whereas an increase in subcutaneous adipose tissue is not (73). The previously mentioned Sumo wrestlers with favourable metabolic profiles have relatively low visceral fat despite having high BMI, which reinforces the importance of the role of visceral adipose tissue (74).

Visceral fat is considered as dysfunctional adipose tissue. Normal adipose tissue is not just a storage space for excess energy; it is also an active endocrine organ and releases bioactive substances: adipokines (74). These adipokines are involved in the regulation of glucose level, lipid metabolism, blood pressure, inflammation, and oxidative stress. An increase in dysfunctional adipose tissue leads to the dysregulation of these adipokines, which disturbs

this highly controlled system and contributes to the development of CVD through atherosclerosis (75). Dysfunctional adipose tissue affects the cardiovascular system through multiple direct and indirect pathways: direct pathways involve various factors such as adipokine dysregulation and inflammation, and indirect pathways are mediated by hyperlipidemia, hyperglycaemia, and high blood pressure (76).

A growing body of evidence suggests that the assessment of visceral adiposity is important to assess obesity risk. However, the assessment of visceral adiposity is challenging: CT and MRI can provide accurate information on visceral adiposity, but the cost, availability, and the radiation exposure for CT, make them unrealistic for use for a large general population or among healthy participants. Therefore, in the following section, I describe alternative adiposity measures that are both more cost-effective and widely-available and discuss whether they are suitable for large-scale epidemiological studies. The alternative measures are broadly categorised into two groups: anthropometric measures of adiposity and body composition.

Anthropometric measures of adiposity

BMI is easy to obtain in epidemiological studies as it is based on measurements of height and weight. As previously mentioned, BMI is highly useful for the screening of obesity and has been used in numerous clinical and epidemiological studies. However, BMI is a relatively crude measure of excess fat and has certain important limitations. First, it cannot distinguish fat mass from lean mass. In fact, a meta-analysis evaluated the ability of BMI to assess excess body fat and showed that BMI could not identify half of the people with excess body fat (77). This can be especially problematic in the older population because lean mass decreases and fat mass increases in accordance with age, even if the BMI does not change (78). Second, BMI does not reflect body fat distribution. In short, it cannot differentiate general obesity from abdominal obesity. Abdominal obesity may be more strongly associated with various health issues such as hypertension and diabetes (79). Finally, BMI cannot distinguish between visceral adipose tissue and subcutaneous adipose tissue. One study showed that although BMI showed a good correlation with total fat mass (0.91-0.94) and subcutaneous adipose tissue (0.86-0.93), its correlation with visceral adipose tissue was weaker (0.61-0.69) (80). Here, the use of other adiposity measures in combination with BMI may be helpful in terms of compensating for the weakness of the latter.

Indeed, several anthropometric measures of abdominal adiposity may provide additional information on adiposity when used with BMI. Waist circumference (WC), waist-to-height ratio (WHtR) and waist to hip ratio (WHR) are examples of such abdominal adiposity measures. They are easy to obtain in a wide variety of study settings. While WC is a simple and crude way to assess abdominal adiposity, several guidelines recommend its use in estimating the risk of adiposity-related diseases (81, 82). WHR and WHtR are other widely used measures. These abdominal adiposity measures may be advantageous to BMI because they can estimate fat distribution. However, it should be noted that these measures cannot distinguish subcutaneous adipose tissue from visceral adipose tissue, and generally reflecting total fat mass rather than visceral adipose tissue (83). For example, WC is correlated with visceral adipose tissue only moderately better than BMI (83). In fact, it has been suggested that a less common anthropometric measure of abdominal adiposity, namely, sagittal diameter, predicts visceral adipose tissue better because, in the supine position, hard visceral fat contributes to sagittal diameter, while soft subcutaneous adiposity tissue falls aside. However, whether sagittal diameter does indeed predict visceral adipose tissue better than WC remains unclear (84, 85). Overall, none of the anthropometric measures of abdominal adiposity described above would appear to be perfect in terms of predicting visceral adipose tissue. Nevertheless, some studies have suggested that the combination of BMI and WC predicts visceral adipose tissue better than a single adiposity measure (83, 86). Furthermore, only a tape measure is needed for the measurement, which does not require special skills or qualifications. Overall, it may be informative to use WC, WHR, and WHtR to complement BMI in large epidemiological studies.

One example of how abdominal adiposity measures can be more informative than the single-use of BMI is the trend in obesity prevalence shown using US NHANES data. Flegal et al. implied that the increase in obesity appeared to have slowed down after 2003 in the US based on BMI using NHANES survey data (87). However, analysis of the same data using WC showed that the prevalence of abdominal obesity was still increasing over the same period (88). Indeed, several contemporary prospective studies suggest the increase in WC/abdominal obesity is more substantial than in BMI/general obesity. This trend is of concern because the larger increase in WC level/abdominal obesity than BMI level/general obesity may reflect a negative change in fat distribution in the population. For example, an analysis of the population-based cohort study, the Tromsø Study, showed a dramatic increase in abdominal obesity in every gender and age group, and especially among young groups between 1994

and 2008 (89). Other prospective studies conducted in Norway (1984-2008), Scotland (1998-2008) and China (1993-2009) have also shown that populations experienced a greater increase in WC than BMI, suggesting a change in fat distribution (90-92). Assessment based on BMI alone might lead to misunderstanding of adiposity-related risks in the contemporary population by ignoring the fat distribution.

Body composition

While the simple adiposity measures described above cannot estimate fat mass directly, body composition analysis can do this by breaking down the human body into several compartments such as fat mass and lean mass. Here, since fat mass includes both visceral fat and subcutaneous fat, it may be not very informative compared to anthropometric measures of adiposity discussed above. However, lean mass, which is a proxy of skeletal muscle mass, may play an important role in a wide range of health conditions. Like visceral adipose tissue, skeletal mass is considered to be an endocrine organ, affecting metabolism through various complicated pathways (93). It is suggested that a decrease in muscle mass or sarcopenia – which is characterised by low muscle mass and function – is associated with decreased insulin sensitivity and metabolic syndrome and induces an increase in visceral fat (93, 94). In addition, it would appear that obesity has a negative impact on skeletal muscle mass. Proinflammatory adipokines produced by visceral fat may reduce skeletal muscle mass and its function (95). Thus, adiposity and muscle mass are closely intertwined. Body composition, especially lean mass and its ratio to fat mass, could be an informative variable in adiposity-related studies.

To assess body composition in large-scale epidemiological studies, realistic approaches would include the use of bioelectric impedance analysis (BIA) and dual-energy X-ray absorptiometry (DXA). Although measurement using MRI and CT shows excellent accuracy, the use in large studies is not realistic due to the costs. BIA estimates body composition on the basis of a two-compartment model. Based on the electrical conductivity of the human body, total body water is estimated, and fat and fat-free mass are calculated using a specific equation based on the assumption of standard levels of tissue hydration (96). Muscle mass can be also predicted using an equation. The device is non-invasive, portable and relatively inexpensive. However, the alternation of hydration can make the estimation inaccurate. In addition, the selection of the estimation equation is extremely important because the validity of the estimation equation is affected by numerous factors such as age and ethnic group. In

short, it is necessary to validate the equation in accordance with the study population being investigated (96), which will make the comparison of body compositions among different populations complicated. Overall, while BIA could be useful for assessing body composition in large studies or in low-cost settings, its estimation is not as reliable as those of DXA, CT, and MRI. Caution is required when interpreting body composition data obtained using BIA.

DXA can estimate body composition based on the attenuation of calibrated X-ray beams. The degree of attenuation can be used to divide the body into three compartments: fat mass, lean mass, and bone mineral. The precision of the fat mass determined by DXA would appear to be good within the existing literature (97), while DXA can also provide reliable estimates of lean mass (98). Furthermore, it is possible to estimate visceral adipose tissue and total body skeletal muscle mass using DXA. It is also relatively low-cost and less time-consuming compared to the measurement using MRI or CT. These advantages make the estimation of body composition using DXA an attractive option. However, DXA also has certain limitations. For one, as previous research has shown, DXA underestimates fat mass in lean subjects (97), while another limitation lies in the considerable measurement variation among different machines and different software.

None of the adiposity measures discussed above is ideal for large-scale epidemiological studies. However, simple anthropometric measures of adiposity have clear advantages in that they are easily measured in a wide variety of study settings with no additional expenditure in terms of money or time. Although they are crude measures of adiposity, the use of abdominal adiposity measures combined with BMI will provide complementing information that will allow for a more accurate understanding of the adiposity level compared to a single adiposity measure. Thus, it would appear to be good approach to use multiple anthropometric measures in large epidemiological studies of an apparently healthy population. Furthermore, if a DXA machine is available, the body composition data estimated using DXA could prove to be highly informative.

2.2.5 Risk factors for obesity

To prevent obesity, an understanding of its determinants is important. In this section, I briefly summarise the current understanding of obesity determinants. Fundamentally, obesity is caused by an energy imbalance between calories consumed and calories expended (99). There are three major factors that determine obesity in adults. The first is lifestyle: dietary factors, lack of physical activity, sedentariness, lack of sleep, and night shift-work result in

weight gain. These lifestyle factors are modifiable. Secondly, genetic factors interact with lifestyle factors and accelerate weight gain, but susceptibility can be attenuated by an improvement in lifestyle factors. Third, built environments, such as urban sprawl and walkability, are also important, to improve which upstream intervention would be needed (100, 101).

Smoking also affects adiposity level, but it is likely to affect general and abdominal adiposity differently. Previous studies consistently show that smokers have lower BMI than non-smokers (102-105). However, increased WC/WHR seems more common in smokers than in non-smokers (103, 106). In particular, WHR is higher in smokers (103). The link between smoking and adiposity is complicated and unclear. One potential mechanism is the negative effect of smoking on appetite. Unhealthy lifestyle accompanied by smoking might explain abdominal adiposity in spite of low BMI. Alternatively, a high adiposity level might increase nicotine dependence, resulting in a high prevalence of smoking in the population with high adiposity levels. A recent Mendelian Randomisation study supported this hypothesis by showing that genetic disposition to high BMI and WC was associated with an increased probability of being a smoker or a former smoker (107).

Socioeconomic status and its proxy, education, also contribute to obesity. One systematic review shows that there is an inverse association between educational attainment and obesity in high-income countries while middle- and low-income countries are more likely to show a null or positive association (108). Regarding income, another proxy for socioeconomic status, a meta-analysis based on studies from the US, the UK, and Canada showed that low income was associated with obesity. At the same time, obesity was associated with low income, suggesting the role of reverse causality through stigma and discrimination (109). A recent Mendelian Randomisation study also supported this reverse causality: people with a genetic disposition to high BMI and short stature had low income, low education, and low job class (110).

Alcohol is another factor which might affect adiposity level. The link between alcohol consumption and adiposity seems extremely complicated, and results from observational studies are inconsistent. However, results from contemporary cross-sectional studies suggest that while moderate alcohol intake is not associated with obesity, this is not the case for heavy drinking and binge drinking which do increase the risk of obesity (111). A recent study

showed a negative genetic correlation between genes related to alcohol consumption and high BMI, high WC, and obesity (112).

2.3 The association between adiposity and CVD

2.3.1 The association between adiposity and CVD: evidence from observational studies

Numerous observational prospective studies support the effect of adiposity on CVD. I briefly summarise recent evidence of association of general or abdominal adiposity with hard CVD endpoints, mainly from large pooled analysis or meta-analysis.

Regarding BMI, previous observational studies have consistently shown that general obesity based on BMI categories is associated with CVD. For example, a recent pooled analysis of 163 cohorts with 2.43 million participants showed that an increase in BMI of 5 kg/m^2 among those aged 55-64 years was associated with higher relative risks for ischemic heart disease (1.44 95%CI: 1.40-1.48) and diabetes (2.32 95%CI:2.04-2.63)(113).

Abdominal adiposity, when assessed by simple anthropometric measures such as WC, WHR, and WHtR, is consistently associated with CVD events in observational studies. However, it is still controversial whether abdominal adiposity is more strongly associated with CVD events than general adiposity assessed using BMI. Some argue that abdominal adiposity is no different from general adiposity in terms of CVD risk. For example, the Emerging Risk Factors Collaboration analysed data from 221,934 individuals from 58 cohort studies which showed that increases in all three adiposity indexes, BMI, WC, and WHR, were significantly associated with high hazard ratios for CVD. However, the authors concluded that the predictive ability for CVD is similar for the different adiposity indices (114).

On the other hand, some observational studies suggest abdominal adiposity may be more strongly associated with CVD than general adiposity. For instance, the INTERHEART study, a multinational case-control study of 15,152 myocardial infarction cases, showed that abdominal adiposity assessed by WHR was a stronger predictor of myocardial infarction than general adiposity (115). Furthermore, another multinational case-control study, INTERSTROKE, found that high WHR had a stronger association with the occurrence of all stroke and ischemic stroke than high BMI (116). Interestingly, the same study showed that the association between WHR and stroke was strongest in Western Europe, North America, and Australia while it was weakest in Eastern and Central Europe. Moreover, a recent prospective study among 479,610 individuals from the UK Biobank showed that abdominal

adiposity, especially WHR, was more strongly associated with future myocardial infarction than BMI, especially among women (117).

2.3.2 The association between adiposity and CVD: evidence from Mendelian Randomisation

The conventional observational studies reviewed above have potential limitations such as residual confounding and reverse causation. This makes it difficult to infer causality between adiposity and CVD as well as comparing the strength of the association between different anthropometric measures. Mendelian Randomisation studies might help to overcome these limitations and contribute to a better understanding of the causal relationship between adiposity and CVD. Briefly, Mendelian Randomisation studies use the genetic variant as a proxy of exposure in observational studies to make causal inferences between exposure and outcomes (118). The genetic variant needs to satisfy the following three assumptions: 1) the genetic variant is associated with the exposure of interest; 2) the genetic variant is not associated with confounders; and 3) the genetic variant is associated with the outcome of interest only via exposure. Mendelian Randomisation studies using appropriate genetic variants are less susceptible to confounders and reverse causality, being considered as an analogue to a randomised controlled trial (119).

Recently, several Mendelian Randomisation studies have investigated the causal link between adiposity and CVD. Earlier Mendelian Randomisation analysis showed inconsistent results (120-122). Although an association between heart failure and BMI was suggested, results regarding the association between BMI and ischemic heart diseases were inconclusive. These inconsistent findings may have been caused by scores based on a small number of single-nucleotide polymorphisms (SNPs) and a relatively small number of CVD events. Recent Mendelian Randomisation studies tend to use scores based on a larger number of SNPs than earlier studies in an attempt to overcome weak instrument bias. Hagg et al. investigated the association between a genetic score derived from 32 SNPs related to BMI and cardiovascular outcomes in up to 22,193 individuals from nine prospective studies and assessed the causal association between obesity and several CVDs (123): 3062 coronary heart disease (CHD) cases, 1652 heart failure cases, and 1500 ischemic stroke cases were included. Their findings suggested a causal link between adiposity and ischemic stroke for the first time. Furthermore, it also replicated the causal association between adiposity and heart failure, and a causal association between adiposity and CHD was suggested.

Mendelian Randomisation studies might not only provide additional information on the causal relationship between adiposity and CVD but also help us to understand whether abdominal adiposity is more strongly associated with CVD than general adiposity. Recently, two such studies have assessed the causal association between abdominal adiposity and CVD (124, 125). Emdin et al. investigated the association of genetic disposition to increase in WHR adjusted for BMI derived from 48 SNPs with CHD (124). The findings suggested the causal association of increased WHR with CHD and type 2 diabetes independent of BMI, indicating that fat distribution can explain these events beyond BMI.

The largest Mendelian Randomisation study of this topic to date compared the association of three cardio-metabolic diseases (CHD, stroke, and type 2 diabetes) with a genetic score of BMI derived from 97 SNPs with that with a genetic score of WHR adjusted for BMI derived from 49 SNPs (125). This study used data from 14 cohort studies with 66,842 CHD cases and 12,389 stroke cases. It found an association of CHD with both BMI and WHR adjusted for BMI. However, only the genetic disposition for high WHR adjusted for BMI was associated with stroke. This study also showed that IMT was more strongly associated with a genetic disposition to high WHR than with high BMI, although both were causally associated with increased IMT.

In summary, evidence from contemporary Mendelian Randomisation studies supports: 1) the causal association between adiposity and CVD; and 2) a stronger association between abdominal adiposity and CVD than general adiposity. Mendelian Randomisation studies may contribute to further understanding the causal pathway between CVD and general and abdominal adiposity, including the role of mediators. However, it is important to recognise that this approach is not without its limitations.

Here, the validity of the genetic variant is vital. The potential causes of the invalid genetic variant include the biological mechanism, the pattern of inheritance of the genetic variant, and population effect (119, 126, 127). The biological mechanism includes pleiotropy – a genetic variant associated with other risk factors in addition to the exposure on interest – and canalisation, the effect of the genetic variant being compensated through different pathways. The pattern of inheritance can also be problematic. For example, linkage disequilibrium, the distribution of variants being correlated due to the physical proximity, may cause the violation of the second or third assumption. Finally, population effects include population stratification and ascertainment effects. Population stratification occurs when the study

population is divided into subpopulations with a different frequency of the genetic variant and distribution of exposures. This can result in a spurious association. Ascertainment effects arise when the study population recruited has a different frequency of the genetic variant compared to the general population (119, 126, 127). While these violations of assumptions lead to biased results, it is difficult to know whether the genetic variant is valid because the biological knowledge is not comprehensive. However, despite these limitations, Mendelian Randomisation analysis provides an important alternative approach to establishing how far an association may be causal. In combination with conventional observational evidence, this approach helps to provide a better evidence base concerning potential causality.

2.3.3 Role of mediators

Among CVD risk factors, three cardio-metabolic risk factors, blood pressure, cholesterol, and blood glucose, are considered to act as mediators between adiposity and CVD. Bioactive substances released from adipose tissue influence insulin resistance, diabetes, lipid levels, and blood pressure, accelerating atherosclerotic change in arteries, leading to the occurrence of CVD. However, it is still unclear to what extent the association between adiposity and CVD is explained by these indirect pathways and how much is independent of these three mediators.

Several observational studies have investigated the role of mediators in the association between adiposity and CVD. The Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration analysed 1.8 million individuals from 97 prospective cohort studies and estimated how much the association between increased BMI and CVD was mediated by three metabolic risk factors: blood pressure, cholesterol, and glucose (128). The three risk factors explained 46% and 76% of the excess risk of BMI for CHD and stroke, respectively. In this analysis, blood pressure played the most important role among the three metabolic risk factors.

Findings from smaller studies are broadly consistent with this result regarding the role of cardio-metabolic risk factors as mediators. However, the extent to which the association is mediated varies according to the study. One meta-analysis with 302,206 individuals showed that the additional adjustment for blood pressure and cholesterol explained 45% of the association between overweight and CVD with adjustment for age, sex, smoking, and physical activity (129). Another pooled analysis by Wormser et al. showed a larger

contribution of three cardio-metabolic risk factors, which explained 60% of the association between obesity and CHD and 70% with ischemic stroke (114).

Mendelian Randomisation studies have also contributed to a better understanding. A study with 90,000 individuals and 13,945 ischemic heart disease cases investigated the role of mediation of these cardio-metabolic risk factors (130). The authors concluded that excess ischemic heart disease risk was partly mediated by non-fasting chylomicron remnant, low-density lipoprotein cholesterol, and blood pressure.

2.3.4 The association between adiposity and atherosclerosis

Thus far, I have summarised recent evidence to support the association between general/abdominal adiposity and CVD events. Turning now to the main cause of CVD, atherosclerosis, how strong is the evidence of the association between atherosclerosis and adiposity? In the next chapter, I describe my systematic review of the literature to investigate the association between adiposity and carotid atherosclerosis, which will provide the context of my thesis.

3 Systematic literature review

To fully justify my choice of PhD topic, it was necessary to investigate what is already known about the association of adiposity measures (my exposure) with carotid atherosclerosis assessed by ultrasound (my outcome) in a general population of adults. To ascertain this, I conducted a systematic literature review, described in this section. Although the main focus of my own analysis is on carotid plaques, in this systematic review, I have also included studies where intima-media thickness (IMT) is the outcome. This is because the bulk of the literature that has looked at the association between adiposity and carotid atherosclerosis assessed using ultrasound has focussed on IMT as the primary outcome. Therefore, the inclusion of IMT as an outcome will make a review of this topic more comprehensive.

3.1 Aim

To conduct a systematic review of the literature that provides quantitative estimates of the direction and strength of the association of various measures of adiposity with carotid atherosclerosis, assessed by ultrasound, in adult general population samples.

3.2 Conceptual issues

Before describing the literature review in detail, I will address a number of important conceptual issues that have influenced how I conducted the review.

3.2.1 Causal Diagram

I constructed a causal diagram of the possible association between adiposity and carotid atherosclerosis to make explicit my assumptions about the causal relationships between variables related to adiposity. First, I included traditional CVD risk factors as described in 2.1.1 (age, sex, smoking, blood pressure, cholesterol, glucose), which can explain most CVD risk and are also associated with adiposity. Physical activity, diet, and the use of alcohol were excluded as the relevant data were not available for my main analysis when I obtained the dataset. Less well-established CVD risk factors were then considered. As for emerging CVD risk factors, a comprehensive review of meta-analyses and a systematic review were published recently, which investigated whether they can improve risk assessment beyond traditional CVD risk factors (43). A family history of CVD, high-sensitivity C-reactive protein and ankle-brachial index were identified as promising new CVD risk factors. I did not

include these either because they were highly clinical examinations which were not available for my analysis or because of the possibility of misclassification.

Finally, I considered the determinants of obesity as described in chapter 2.2.5 and included education because it affects both adiposity and CVD.

Blood pressure, cholesterol, and glucose were expected to be on the causal pathway between adiposity and carotid atherosclerosis and are thus considered as mediators. Other factors were considered confounders. One potential pattern of association is shown in Figure 3-1.

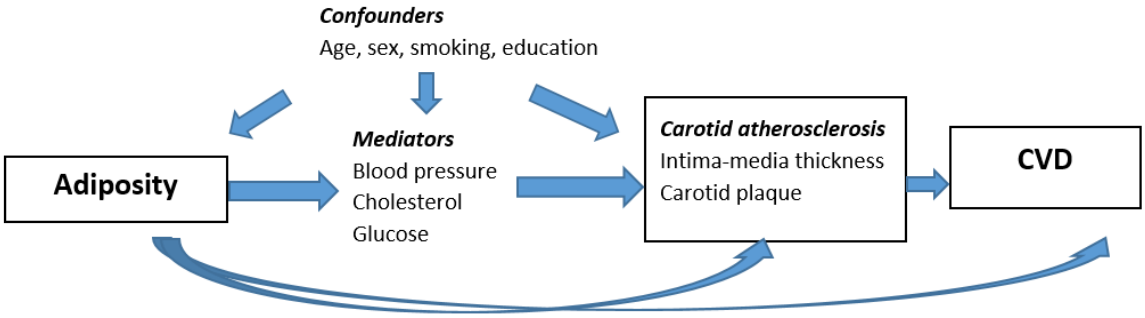


Figure 3-1 Simplified causal diagram: One potential pattern of association between adiposity and carotid markers

3.2.2 Adjustment

To ensure the study results in the systematic review were comparable, I divided studies into three groups: those that adjusted for confounders (at least age and sex), those that adjusted for at least one potential causal mediator (such as blood pressure or cholesterol), and those with a crude association. Confounders and mediators here refer to those used in my main analysis.

3.2.3 IMT assessment (carotid segment, calculation) and the definition of carotid plaque

Studies varied according to the anatomical site of IMT assessment. The simple anatomy of the carotid artery is shown below (Figure 3-2). Guidelines recommend assessing IMT at the common carotid artery (CCA), where it is easy to capture an ultrasound image, and the value is more reliable (41, 131). Because atherosclerotic changes are more likely to occur at the carotid bulb and internal carotid artery (ICA), comparison between IMT assessed at the CCA and at other segments is not valid. However, some studies have assessed IMT at non-CCA segments or used composite IMT derived from several carotid segments. Thus, to reduce

heterogeneity across studies, I summarised the association between adiposity and IMT after removing studies using non-CCA segments or composite IMT.

Studies also varied widely in terms of the definition of carotid plaque. As noted previously, the Mannheim consensus defines a plaque as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness > 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (41). However, I summarised the association between adiposity and carotid plaque regardless of the definition of plaque.

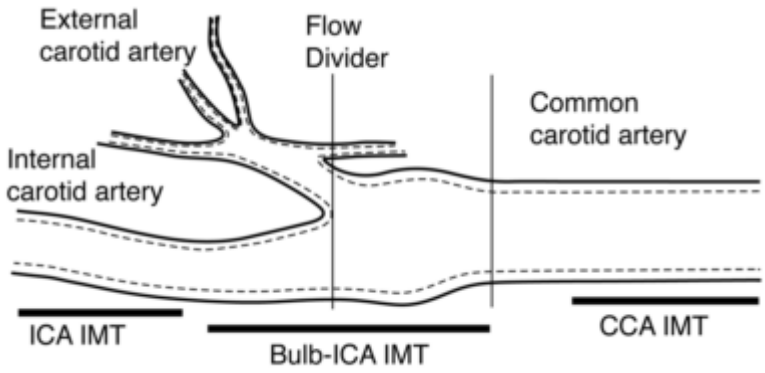


Figure 3-2 Carotid artery segment

3.2.4 Studies excluding subjects with a history of CVD, hypertension, hyperlipidemia, and diabetes

Many studies have excluded people with existing diagnoses of CVD and cardio-metabolic diseases because the authors were principally interested in the clinical question of the value of IMT/ burden of carotid plaque as a predictor of CVD risks in an asymptomatic population without known CVD risks. However, from my perspective of wishing to estimate the association between adiposity and IMT/carotid plaque, these exclusions may have led to bias. Thus, I excluded these studies for the reasons described below:

a. Causal pathway: Pre-existing hypertension, hyperlipidemia, and diabetes are on the causal pathway between adiposity and IMT/carotid plaque, so removing participants with these ailments can result in underestimation of the association. Furthermore, these conditions are common in an adult population. If participants with cardio-metabolic diseases are excluded, the sample may not be representative of a general population.

b. Collider bias: Excluding participants with existing CVD can introduce collider bias (132). CVD is a common effect of both obesity and carotid atherosclerotic changes. Excluding subjects with CVD means conditioning on CVD status as a collider, which may bias the true association between adiposity and IMT/carotid plaque in the population as a whole.

3.3 Systematic review method

I searched Embase, Pubmed, and Global Health for relevant publications. I combined 20 search terms related to adiposity and eight search terms related to carotid atherosclerosis (Appendix A1).

Studies were included if all the following criteria were met: (1) the direction, correlation or magnitude of the association between adiposity and IMT or carotid plaque was reported; (2) the study was based on a community/population-based sample; (3) the sample included an adult population; and the adult population was analysed separately.

Studies were excluded if one of the following criteria was met: (1) the full-text was not available in English; (2) the paper was a conference abstract; (3) the study only included or presented analyses for subjects without a history of CVD, hypertension, hyperlipidemia, or diabetes; (4) the study subjects were hospital or clinic-based patients; (5) subjects were included because they had a diagnosis of a specific condition (other than obesity); (6) the association between adulthood adiposity and adulthood IMT or plaque status was not reported.

The following information was abstracted for each study that included full-text review: author, year, country, study design, sample size, age, sex, the measure of adiposity used in the analysis, the method of measuring IMT and carotid plaque, the association between adiposity and IMT/plaque and the statistical method. The data extraction sheet and quality assessment sheet are presented in Appendixes A2 and A3.

3.4 Results summary

I screened 2644 articles, and 69 studies were included. The study selection process is summarised in Figure 3-3.

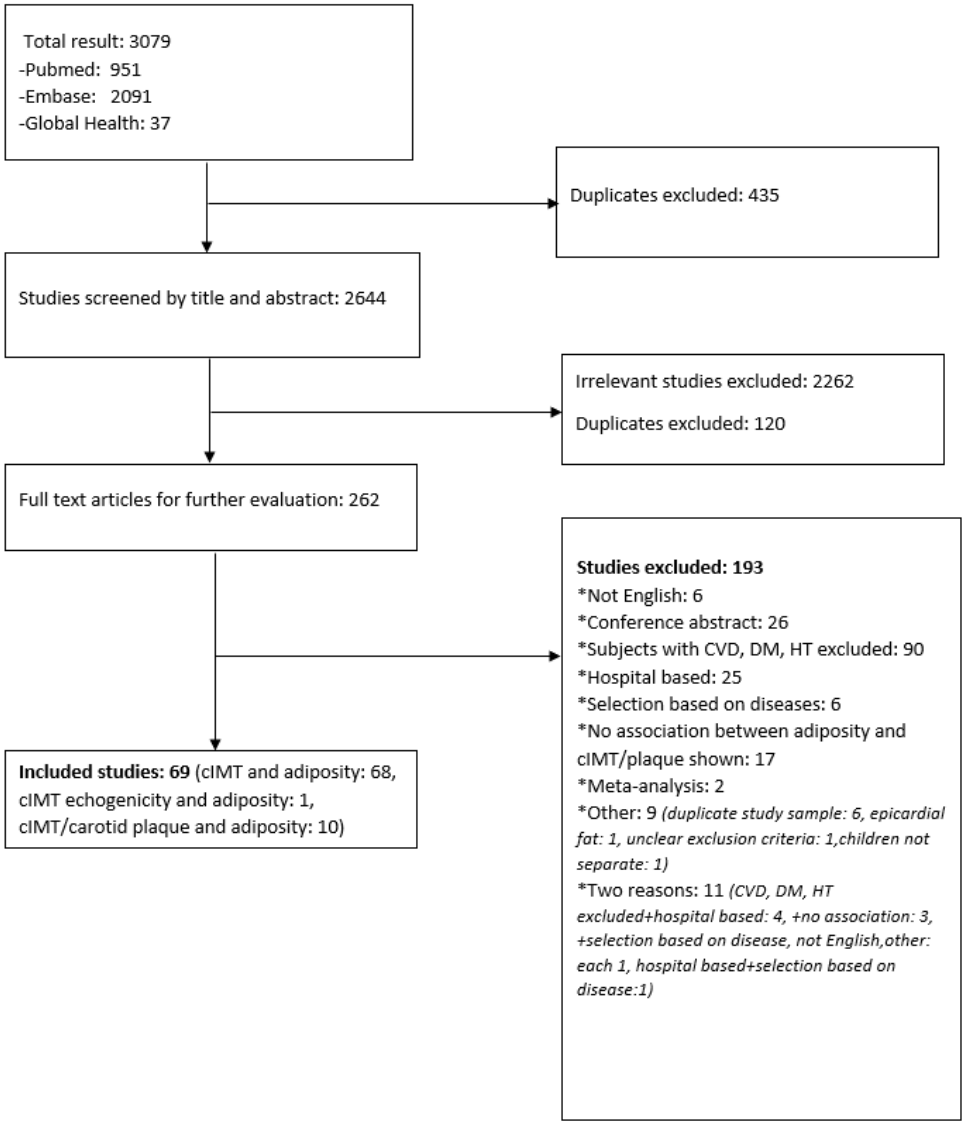


Figure 3-3 Flowchart of study selection process

Study characteristics: IMT was used as an outcome in 69 studies (n=96632), whereas both IMT and carotid plaque were outcomes in 10 studies (n=16032). About 36% of samples were derived from Western European countries, while 1% were from Central and Eastern Europe and the former Soviet Union (CEE/FSU). There were no Russian studies. There was considerable variation in the IMT assessment method and carotid plaque definition. Among adiposity measures, BMI was the most frequently used (85.5%), followed by WC (53.6%) and WHR (30.4%). Body composition was assessed in 11 studies using bioelectric impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA), CT, or MRI. The definition of carotid plaque differed from study to study, and only one study followed the definition recommended by the Mannheim consensus.

Findings: Table 3-1, Table 3-2, and Table 3-3 summarise the association between BMI and IMT assessed at CCA according to the statistical and adjustment methods. Figure 3-4 shows a forest plot for regression coefficients of studies that only use similar IMT assessments. Overall, BMI was positively associated with IMT in most studies, regardless of adjustment and statistical method. WC and WHR showed similar trends, but less consistently. Fat mass seems to be associated with IMT, but the number of studies was limited, and conclusions were difficult to draw.

Table 3-4 and Table 3-5 summarise the association between adiposity and carotid plaque. Results for this association were mixed. Because most results were adjusted for multiple mediators, and no result was adjusted for appropriate confounders, it is difficult to estimate the un-mediated association between adiposity and carotid plaque. Furthermore, differences in plaque definition and statistical methods between studies make the interpretation complicated. Most studies used plaque presence (Yes/No) as an outcome, while two studies used quantitative measures: total plaque area and plaque height (133, 134).

Table 3-1 Association between BMI and IMT adjusted for confounders

Author	Number of participants	β (95%CI) IMT(μm) per BMI unit*	IMT measurement site	IMT assessment	Adjustment
Chow 2008 (135)	India (303)	2.6 (1.2, 3.9)	Both CCA	mean	Age, sex
	Australia (1111)	1.8 (1.1, 2.6)			
Macharia 2014 (136)	491	4 (1.5, 6.5)	Both CCA	mean	Age, sex
Masley 2015 (137)	592	4.2 (2.5, 5.9)	Both CCA	mean	Age, sex
Pitha 2013 (138)	699	2.2 (1.0, 3.4)	Both CCA	mean	Age (all female samples)
Youn 2011 (139)	1716	7.4 (5.4, 9.4)	Either CCA,	mean	Age, sex
Raitakari 2003 (140)	2229	2.5 (1.62, 3.38)	Left CCA	max	Age, sex
Ferreira 2004 (141)	336	2.8**	Not clear	not clear	Sex and height (all same age)

CCA: common carotid artery, IMT: carotid intima-media thickness *Units are converted to IMT (μm) per BMI unit regardless of units used in original papers. When only p-value was reported, 95% confidence interval was calculated. **Only regression coefficient reported in the original article.

1. Studies included in this table are those which measured IMT at CCA and provided a regression coefficient adjusted only for confounders.

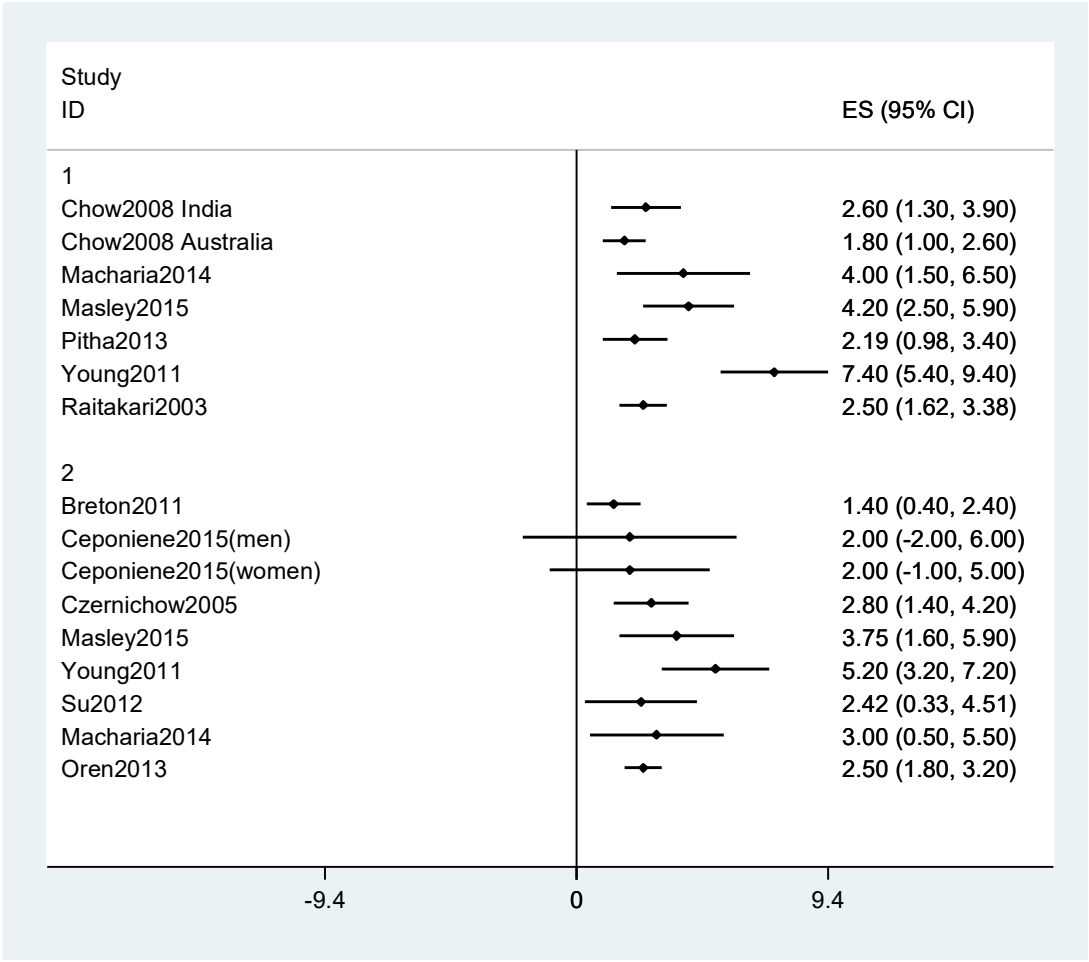
Table 3-2 Association between BMI and IMT adjusted for mediators

Author	Number of participants	β (95%CI): IMT(μm) per BMI unit*	IMT measurement site	IMT assessment method	Adjustment
Breton 2011 (142)	768	1.4 (0.3, 2.4)	Right CCA	mean	Age, sex, ethnicity, SBP
Ceponiene 2015 (143)	Male 168	2.0 (-2.0, 6.0)	Both CCA	mean	Childhood SBP, BMI, sexual maturity score. Adulthood SBP, HDL, LDL. Smoking, education level
	Female 212	2.0 (-1.0, 5.0)			
Czernichow 2005 (144)	1014	2.8 (1.4, 4.2)	Both CCA		Age, sex, MAP, smoking, DM, HL, height, heart rate
Macharia 2014 (145)	491	3.0 (0.5, 5.5)	Both CCA	mean	Age, sex, DM
Masley 2015 (137)	592	3.75 (1.6, 5.9)	Both CCA	mean	Age, sex, SBP, DBP, waist, %BF, BMI, TC/HDL ratio, Tri/HDL ratio, glucose, fitness markers, Fish, Fibre, Zing
Youn 2011 (139)	1716	5.2 (3.0, 7.2)	Either CCA	mean	Age, sex, SBP, DBP, triglycerides, HDL, LDL, hs-CRP, DM, smoking
Oren 2013 (146)	524	2.5 (1.7, 3.2)	Both CCA	mean or mean-max	Age, sex,
Su 2012 (147)	2690	2.42 (0.32, 4.51)	Both CCA	max	Age, sex, SBP, medication history (hypertension, DM), fasting glucose, smoking, alcohol, HDL, LDL, LVH on ECG

*Units are converted to IMT (μm) per BMI unit regardless of units used in original papers. When only p-value was reported, 95% confidence interval was calculated.
BMI: body mass index, CCA: common carotid artery, IMT: carotid intima-media thickness, DBP: diastolic blood pressure, DM: diabetes, ECG: electrocardiogram, HDL: high density lipoprotein, LDL: low density lipoprotein, LVH: left ventricular hyperplasia, MAP: mean arterial pressure, SBP: systolic blood pressure
1. Studies included in this table are those which measured IMT at CCA and provided a regression coefficient adjusted for at least one mediator

Table 3-3 Association between BMI and IMT (correlation coefficient)

	n	r	effect	p-value	statistical method
Azarpazhooh 2014	431	0.12	+	<0.05	Pearson correlation
Besir 2011	2230	0.208	+	<0.001	Partial correlation analysis r
Bradshaw 2007	602	0.17	+	<0.001	Spearman correlation
Chang 2007	280	0.202	+	0.001	Partial correlations
Ferreires 1999 (men)	536	0.28	+	<0.001	Spearman correlation
Ferreires 1999 (women)	477	0.22	+	<0.001	Spearman correlation
Hung 1999	1111	0.21	+	<0.0001	Spearman correlation
Huynh 2013 (men)	1150	0.097	+	<0.01	Spearman correlation
Huynh 2013 (women)	1178	0.082	+	<0.01	Spearman correlation
Kim 2008	160	0.203	+	<0.05	Pearson correlation
Koshkinen 2009	1809	0.19	+	<0.0001	Pearson correlation
Lu 2012 (men)	74	0.436	+	<0.001	Pearson correlation
Scuteri 2012	6148	0.373	+	<0.0001	Pearson correlation
Sipila 2009 (men)	607	0.045	+	0.267	Pearson correlation
Sipila 2009 (women)	746	0.171	+	<0.001	Pearson correlation
Takami 2001	849	0.138	+	0.0001	Spearman correlation
Yang 2014	472	0.125	+	<0.01	Spearman correlation
Wright 2001 (men)	233	0.19	+	<0.05	Standardised regression coefficients
Wright 2001 (women)	293	0.14	+	<0.05	



1. **Association between BMI and IMT adjusted for confounders (upper)**
2. **Association between BMI and IMT adjusted for mediators (lower)**

***Studies included in this figure are those which assessed IMT at CCA**

Figure 3-4 Forest Plot: Association between BMI and IMT according to adjustment

Table 3-4 Association between adiposity and carotid plaque: studies using logistic regressions with odds ratios

Author Date	Age (range or mean±SD) Number of participants	Carotid plaque variable used in analysis	Prevalence of plaque presence (%)	Adiposity measure	OR1(crude)	OR2 (adjusted for confounders or mediators or both)	Adjustment
Bonithon-Kopp 1996 (148)	59-71, N=1282	Plaque presence	male25.6% female 16.5% at ICA or bulb	BMI per unit	-	1.01(0.97-1.05)	sex, age, hypertension, hypercholesterolemia, diabetes, and ever smoking
Chaubey 2010 (149)	56-77, N=792	Plaque presence	men; both CCA 32%, only left 11.5%, only right 12% Women: both CCA 30%, only left 12.5%, right 13.9%	WHR per0.1	-	Left CCA 1.33 (1.04-1.71) Right CCA 1.28 (1.00-1.64)	HbA1c, smoking status, low-density lipoprotein cholesterol levels, hypertension, sex, and age
Czernichow 2005 (144)	59.4±4.7, N=1014	Plaque presence	32.0%	Per BMI tertile	-	T1 ref T2 1.33 (0.94–1.89) T3 1.27 (0.89–1.83)	age, gender, mean arterial pressure, smoking status, diabetes, hypercholesterolemia, height and heart rate
				Per FM tertile		T1 ref T2 1.34 (0.94–1.93) T3 1.12 (0.74–1.70)	
				Per FFM tertile		T1 ref T2 1.14 (0.77–1.68) T3 1.06 (0.65–1.74)	
				Per %BF tertile		T1 ref	

						T2 1.31 (0.93–1.86) T3 1.33 (0.93–1.90)	
				Per WC tertile		T1 ref T2 1.33 (0.93–1.90) T3 1.19 (0.82–1.73)	
				Per HC tertile		T1 ref T2 1.13 (0.80–1.61) T3 0.86 (0.59–1.25)	
				Per WHR tertile		T1 ref T2 0.91 (0.64–1.31) T3 1.35 (0.94–1.91))	
Debette 2008 (150)	65-85, N=6265	Plaque presence	41.5, 44.9, 50.2, 51.2% according to WC quartile	Per CC quartile	-	Q1 reference Q2 1.00 (0.86–1.17) Q3 0.91 (0.78–1.06) Q4 0.79 (0.68–0.93)	age, gender, smoking habits, alcohol consumption, systolic blood pressure, antihypertensive treatment, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides (log-transformed value), lipid-lowering drugs, diabetes, history of vascular disease.
				WC per quartile		Q1 reference Q2 1.03 (0.88–1.20) Q3 1.13 (0.97–1.32) Q4 1.01(0.85–1.19)	
				WHR per quartile		Q1 reference	

						Q2 1.16 (1.00–1.36) Q3 1.15 (0.98–1.35) Q4 1.15 (0.98–1.35)	
Hung 1999 (151)	27-77, N=1111	Plaque presence	27.4%,24.5%,25.9% according to ACE genotype	WHR (>0.91) binary	-	2.1 (1.3-3.3)	age, systolic blood pressure, pack-years of smoking, low-density lipoprotein cholesterol level, waist/hip ratio, and history of antihypertensive treatment
Khalil 2013 (152)	33-38, N=600	Plaque presence		BMI per z score	1.14 (0.95 - 1.36)	-	Age, sex
				WC per z score	1.27 (1.06-1.52)		
Terzis 2012 (153)	40.5 ± 1.1, N=106	Current plaque presence	22%	Current BMI per unit	1.14 (1.04–1.25)	1.153 (1.031–1.289)	OR1: none age, gender, BMI change, waist, BMI, change from normal weight since adolescence to over-weight status in adulthood, systolic and diastolic blood pressure, and smoking
				Current WC per unit	1.04 (1.009–1.07)		
		plaque thickness			2.54(1.22) vs 2.60(1.00),NS		

BMI; body mass index, CC; calf circumference, FM; fat mass, FFM; fat-free mass, %BF; percent body fat, WC; waist circumference, HC; hip circumference, OR: odds ratio, Q; quartile, WHR; waist-hip-ratio

Table 3-5 Association between adiposity and carotid plaque: studies in which effect estimates were not presented

Author Date	Age (range ormean±SD) Number of participants	Carotid plaque variable used in analysis	Prevalence of plaque presence (%)	Adiposity measure	Findings	Adjustment
Herder 2012 (133)	Baseline men 55.8 (9.08) Baseline women 56.6 (10.2) N=2743	Total plaque area follow-up, total plaque area change, (linear regression)		BMI		smoking habits, prevalent diabetes, angina pectoris, previous myocardial infarction, stroke, and current use of antihypertensive- and lipid-lowering drugs **at baseline**
Bradshaw 2007 (154)	18-74, N=602	Plaque presence	>40%	BMI binary: high vs low	High BMI group: 81% had carotid plaque Low BMI group: 78% had carotid plaque Difference: not significant	
				WHR binary: high vs low	High WHR group: 91% had carotid plaque Low WHR group: 78% had carotid plaque Difference: 2p<0.001	
Holewijn 2010 (134)	50-70, N=1517	Plaque presence	Men 40.7% - 46.8%, Women 26.7% - 34.8	Lowest and highest quartile of WC	Prevalence of carotid plaque was significantly different between the top WC quintile and the bottom quintile (top vs bottom: men +3.3% NS, women +9.3 % NS)	Age
		Plaque thickness			Plaque thickness was higher in the top WC quintile than the bottom quintile in women by 16.6% while it was not significantly different among men	

BMI; body mass index, CC; calf circumference, FM; fat mass, FFM; fat-free mass, %BF; percent body fat, WC; waist circumference, HC; hip circumference, Q; quartile, WHR; waist-to-hip ratio

3.5 Gaps in knowledge and conclusion

This systematic review had three major findings. First, a number of studies investigated the association between BMI and IMT. The results were generally consistent with a positive association between BMI and IMT, regardless of adjustment and statistical methods. Secondly, measures of abdominal adiposity such as WC and WHR were less commonly-used than BMI. The association between abdominal adiposity measures and IMT broadly showed similar patterns to BMI, although the results were less consistent than BMI. Finally, the association between adiposity and carotid plaque showed mixed results.

This systematic review identified five gaps in the existing knowledge. Firstly, although the association between adiposity and IMT was broadly consistent, no population-based studies with standard IMT assessment investigated the association between adiposity and IMT using appropriate confounder adjustment (age, sex, smoking). Second, only 11 studies investigated body composition among the exposure variables. Third, evidence regarding the association between adiposity and carotid plaque was scarce (10 studies) although carotid plaque is expected to predict CVD risk better than IMT. Only two studies used quantitative plaque assessment, which may perform better than the binary plaque variable (presence: Yes/No) as it provides continuous information. Fourth, the IMT assessment method and plaque definition differed widely across studies, making comparison of results difficult. Finally, no Russian studies were found, and studies from CEE/FSU regions were scarce. More research in this area is needed in order to investigate the association of IMT/carotid plaque with a range of measures of adiposity further in Russia and elsewhere.

Given the relative paucity of evidence about the association of adiposity with carotid plaque compared to the association with IMT, I concluded that a focus on the carotid plaque as my primary outcome in this thesis would address a gap in the existing evidence. Furthermore, this review has highlighted the importance of appropriate adjustment. In particular, as most of the studies investigating the association between simple anthropometric measures and carotid plaque adjusted for various mediators and confounders altogether, it was difficult to determine the unmediated association between these simple adiposity measures and carotid plaque. This provides an additional rationale for looking at the association between simple adiposity measures and carotid plaque with an appropriate adjustment in the current study.

4 Study setting and design

This chapter describes the setting and study designs and methods of the Know Your Heart study (KYH) and the Tromsø Study, the two data sources which I use in this thesis. The first section describes the Know Your Heart study, followed by the Tromsø Study. Finally, the rationale for comparing the two studies is provided.

4.1 Know Your Heart study

4.1.1 Study setting

Novosibirsk and Arkhangelsk are cities in the Russian Federation. Novosibirsk is the third-most populous city in Russia, with a population of nearly 1,500,000. It is situated in south-western Siberia. Arkhangelsk is a smaller city located in northern European Russia, 1100 km to the north of Moscow. It has a population of about 350,000. These two cities were chosen because large-scale epidemiological studies had been conducted there previously (155, 156), and it was expected that they would have the capacity for comprehensive epidemiological studies to be carried out. The location of the two cities is shown on the map below (Figure 4-1). The location of Tromsø, where the Tromsø Study was conducted, is also shown on the map. The protocols of the main examinations of KYH were designed to align with the Tromsø Study seventh survey so that the two studies would be comparable. Details of this are provided in a later section.

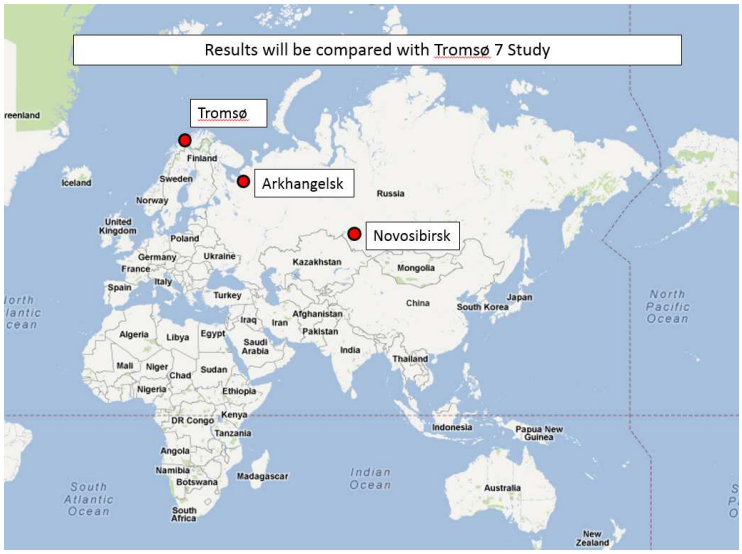


Figure 4-1 Location of Arkhangelsk, Novosibirsk, and Tromsø

4.1.2 Study design

KYH is a part of the International Project of Cardiovascular Disease in Russia (IPCDR). The objective of IPCDR is to understand why Russia has one of the highest CVD mortality rates in the world (2). Briefly, IPCDR is a comprehensive project consisting of the following four themes. The first is to investigate the differences in coding of cause of death between Russia and other countries, and how the differences in coding affect CVD mortality rates; the second is to create an updated trend of CVD mortality rates in Russia; the third is to investigate the potential role of the health care system and treatment in Russia; the last theme is to conduct a cross-sectional study to investigate the contemporary distribution of various behavioural and medical risk factors for CVD. This thesis will use data from this cross-sectional study (known as the Know Your Heart study: KYH).

Details of the rationale and design of the KYH study have been provided elsewhere (2). One of the main aims of this cross-sectional study was to compare the findings with those from another European country with low CVD risk to facilitate the understanding of the causes of high CVD mortality in Russia. In particular, the study was designed to be comparable with the seventh survey of the Tromsø Study (Tromsø 7), the population study from a low CVD risk country, Norway. To allow a reasonable comparison, the protocols for the main medical examinations in KYH, such as carotid and cardiac ultrasound examinations, were designed to align with those of Tromsø 7 which was conducted over the same period.

The data collection in KYH comprised two stages: a baseline interview in the home and a medical examination at a polyclinic. Figure 4-2 shows a simplified study design. Briefly, 4500 men and women aged 35-69 years were recruited from four districts in each city, the two largest city centre districts and two suburban districts, between November 2015 and December 2017. The four districts were selected to represent a range of socio-demographic and mortality levels. A random sample stratified by age and gender was derived from the list of the Territorial Health Insurance Funds in each district.

Recruitment was conducted by Levada, a survey company. Trained interviewers from Levada visited the addresses on the list of a randomised sample and identified residents with the appropriate age and gender. If the participant was not available at the first visit, the interviewers visited on at least two more occasions, including weekends. If the person agreed to participate in the survey, and oral informed consent was obtained, the interview was

conducted to collect data including CVD risk factors, potential confounders, and potential mediators.

At the end of the interview, the person was invited to attend a comprehensive health examination at a polyclinic at a later date. If participants accepted the invitation, an appointment was made. Data collection at the polyclinic included an interview on medical history and medication, anthropometric measurements, vital signs, spirometry, and cardiac and carotid ultrasound. Blood, urine, and faecal samples were also collected. Blood samples were frozen and transferred to Moscow for analysis.

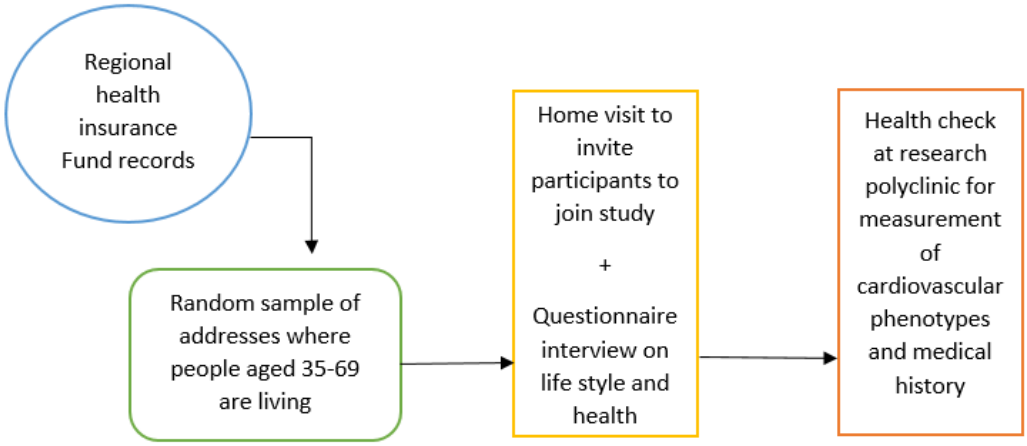


Figure 4-2 Design overview of Know Your Heart study

5089 individuals were recruited for the baseline interview. A total of 4542 individuals attended a medical examination at a polyclinic, of these 2381 (52.4%) were from Arkhangelsk, and 2161 (47.6%) were from Novosibirsk. Fewer males were included than females with 41.5% and 42.0% in Arkhangelsk and Novosibirsk respectively.

The overall response rate was markedly different between the two cities. Three types of response percentages were calculated using three types of the denominator. The type 1 response percentage was the most conservative: all the addresses approached were used as the denominator, even if an address was invalid. The type 2 response percentage excluded invalid addresses or those at which no one in the household was of the appropriate age or sex from the denominator. Addresses were usually found to be invalid because the original list was outdated or inaccurate. Finally, the type 3 response percentage restricted the denominator

to addresses with an eligible participant of the appropriate age and sex. This third type reflects a willingness to participate.

Table 4-1below summarises the three types of response percentages by sex and city. More detailed data have been made available elsewhere (2). Overall, the response percentages were much higher in Arkhangelsk than in Novosibirsk. Although the reason for the low response rate in Novosibirsk is unclear, it is possible that since it is a much larger city than Arkhangelsk, people might be more suspicious of being contacted by strangers. The response rate was particularly low among men at the lower end of the age distribution. Among those who completed a baseline interview, 96% and 83% subsequently attended the medical examination at the polyclinic in Arkhangelsk and Novosibirsk, respectively. Younger males with lower educational attainment were less likely to attend the medical examination, whereas those with a medical history of CVD risk factors were more likely to attend.

Table 4-1 Response percentages by sex and city

	Arkhangelsk			Novosibirsk		
	Men	Women	Total	Men	Women	Total
Type 1 response	36.7%	51.0%	43.9%	17.0%	25.6%	20.9%
Type 2 response	45.7%	60.1%	53.1%	22.1%	31.5%	26.5%
Type 3 response	60.4%	75.2%	68.2%	35.4%	48.0%	41.1%

Table modified from Cook et al. (2)

4.2 Tromsø Study

4.2.1 Study setting

Tromsø is located in Northern Norway, ~400km north of the Arctic Circle. It is the ninth most populous city in Norway with a population of about 67,000. In spite of its location within the Arctic Circle, the climate is relatively mild for its latitude due to the warming effect of the Gulf Stream.

4.2.2 Study design

The Tromsø Study is a population-based prospective cohort study started in 1974 to investigate causes of CVD mortality; it was eventually broadened to include other chronic diseases. An overview of its design has been published elsewhere (157). It consists of seven

surveys: Tromsø 1 (1974), Tromsø 2 (1979-80), Tromsø 3 (1986-87), Tromsø 4 (1994-95), Tromsø 5 (2001-02), Tromsø 6 (2007-08), and Tromsø 7 (2015-16). Details of the Tromsø Study are described elsewhere (157). Most of the participants are Caucasian. Tromsø surveys 4-7 have two stages: following the first visit (visit 1), the pre-identified population was invited to a second visit (visit 2) with a more extensive examination. Carotid ultrasound examination was performed in visit 2 in Tromsø 4-7.

Tromsø 4 was conducted in 1994-95; it was the largest survey, and all residents of the municipality of Tromsø aged over 25 years were invited based on the official population registry. Data were collected through a self-administered questionnaire, physical examination, and blood sample. Information on CVD risk factors, lifestyle, and medical history was obtained through a questionnaire. Furthermore, everyone aged 55-74 years and a 5-10% random sample of other age groups were eligible for visit 2 where extensive studies, including carotid ultrasound examination, were conducted. All participants who underwent carotid examination were invited to Tromsø 5 visit 2 as well as Tromsø 6 visit 2 where participants underwent follow-up carotid examination. In summary, participants in Tromsø 4-6 visit 2 mainly comprised Tromsø 4 visit 2 participants regardless of the sampling methods for visit 1 participants. The attendance rate was high at >75% for Tromsø 1-5 and 66% for Tromsø 6.

The most recent Tromsø survey (Tromsø 7) was conducted in 2015-16. All residents aged over 40 years living in the municipality of Tromsø were invited. A total of 21,083 men and women attended (response rate 65%). A random sample including previous participants was invited to visit 2.

4.2.3 Why compare the Tromsø Study with the Know Your Heart Study?

The selection of the city in the two studies

In this thesis, I compared the burden of adiposity, the burden of carotid atherosclerosis, and their associations in a general population in Russia with that in Norway, using data from KYH and Tromsø 7. The ultimate aim is to elucidate the reason for the excess CVD burden in Russia. To justify this comparison, it is necessary to show that CVD mortality in the two Russian cities was not significantly different from that in the whole of Russia and that in Tromsø was not significantly different from the whole of Norway. The CVD mortality rate in each city compared to the whole nation is discussed below.

KYH

Table 4-2 below compares CVD and all-cause mortality rates for the whole of Russia, Arkhangelsk, Novosibirsk, Tromsø, and the whole of Norway between 2012 and 2016. The mortality rate from all circulatory diseases in Novosibirsk was slightly lower than the national average for both sexes while that in Arkhangelsk was higher than the national average. Regarding mortality from ischemic heart disease, both cities showed a higher rate than the national average. Nevertheless, the mortality rate from all circulatory diseases and ischaemic heart disease in both cities was relatively similar to the national average, which was markedly higher than the Norwegian national average and that of Tromsø.

To check whether Novosibirsk and Arkhangelsk are considered as typical Russian cities, age distribution and educational attainments were compared between Novosibirsk, Arkhangelsk, and the whole of Russia. The age and education distributions of Novosibirsk and Arkhangelsk were reasonably similar to those of the total Russian urban population, although the prevalence of higher education was slightly higher in Novosibirsk than the national average (2). It can be said that the two cities are not very different from typical Russian cities.

Table 4-2 Mortality rates by sex and cause for population aged 35-69 (2012-16)

Cause of death	Men					Women				
	Russia	Arkhangelsk (urban)	Novosibirsk (urban)	Norway	Tromsø*	Russia	Arkhangelsk (urban)	Novosibirsk (urban)	Norway	Tromsø*
All circulatory diseases**	735	821	711	88	*77*	239	245	236	33	*19*
Ischaemic heart disease***	407	517	447	49	*37*	111	125	124	12	*7*
All causes	1755	1852	1772	408	*393*	619	612	610	264	*156*

Notes:

Mortality rates by sex and cause (age standardized/100,000) for Russia and the urban populations of Arkhangelsk and Novosibirsk oblasts and Norway as a whole and the municipality of Tromsø, Norway, aged 35–69 for years 2012–16

Rates age standardized to 1976 Standard European Population

Data for Russia, Arkhangelsk and Novosibirsk from the Russian Fertility and Mortality database of the Centre of Demographic Research of the New Economic School http://www.demogr.nes.ru/index.php/en/demogr_indicat/data

Data for Norway and Tromsø provided by Section of Health Data and Digitalisation, Norwegian Institute of Public Health

* Rates for municipality of Tromsø (90% of population living in the urban area of the city) are based on only 318 all cause deaths for men and 184 all cause deaths for women. Numbers of deaths from Ischaemic Heart Disease are 30 for men and 8 for women. To indicate their associated imprecision they are shown in italics.

** ICD 10 codes I00-I99 (Diseases of the circulatory system)

***ICD 10 codes I20-I25 (Ischaemic Heart Diseases)

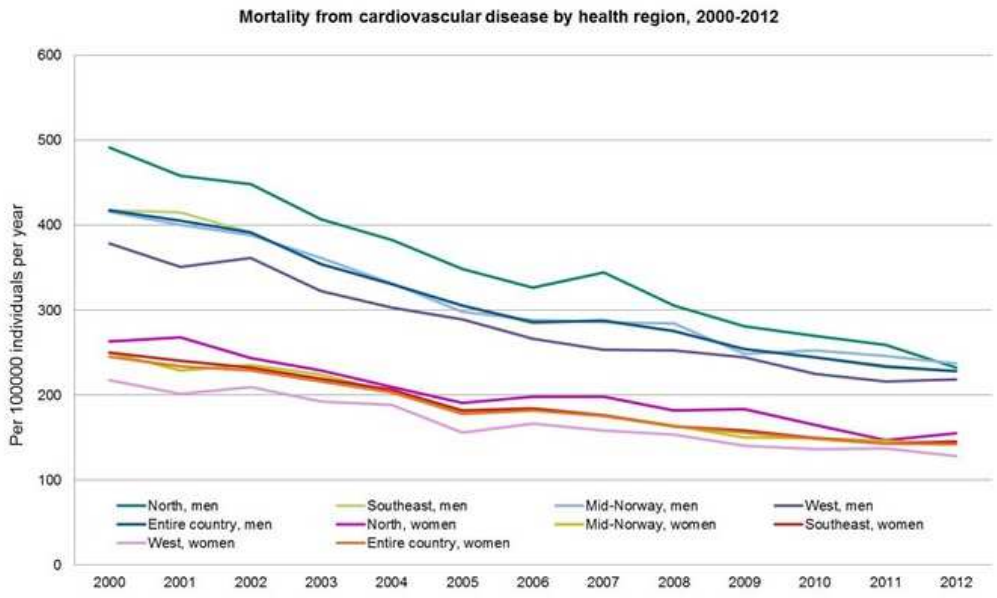
Table taken from Cook et al. (2)

Tromsø Study

Regarding Tromsø, Table 4-2 above shows that the mortality rate from all circulatory diseases and ischemic heart disease in Tromsø was lower than that for the whole of Norway

during this period. However, the mortality rate in Tromsø in this table is based on a relatively small number of deaths (2).

Figure 4-3 shows the CVD mortality rate by Norwegian regions between 2000 and 2012. Historically the CVD mortality rate in northern Norway, where Tromsø is located, was higher than the national rate (158). However, CVD mortality rates from different regions in Norway have converged over the last decade. The difference in CVD mortality rate between Northern Norway and the whole nation is now small. Thus, it is safe to say that the CVD mortality rate in Tromsø is reasonably similar to the whole of Norway and considerably lower than that in Russia. Therefore, I concluded that the selection of two Russian cities and Tromsø was appropriate for comparison of the CVD burden in a general population between Russia and Norway.



Graph taken from public health report from the Norwegian Institute of Public Health (158)

Figure 4-3 Age-adjusted mortality from cardiovascular disease by different Norwegian regions (2000-2012)

Comparison between KYH and Tromsø Studies

In the 1970s, Norway had a higher CVD burden than most Western European countries (159). However, the CVD mortality rate in Norway has declined in the past four decades and

is now similar to other Western European countries (158). The decrease in CVD risk factors, especially blood pressure and cholesterol levels, is likely to have contributed to this decline in CVD burden (158). However, like other countries, an increase in other CVD risk factors such as general and abdominal obesity and type 2 diabetes is a concerning trend in Norway (89). Because Norway once suffered a high CVD burden, like Russia, followed by a sharp decline in CVD mortality, comparison between Norway and Russia in terms of the distribution of CVD risk factors might allow us to identify CVD risk factors which explain the higher CVD burden in Russia and provide insight into effective intervention.

In addition to the similar protocols for medical examinations in KYH and Tromsø 7, there are several factors that enable us to compare the two studies. First, due to its geographical proximity to Russia, Tromsø has a relatively similar climate to the two Russian cities. Furthermore, the ethnic composition is also similar to the Russian cities, which mainly consist of people with European ancestry. These factors help us to make a reasonable comparison of adiposity level, carotid plaque burden, and the impact of adiposity on carotid atherosclerosis between the two populations from two countries with very different CVD burdens.

4.2.4 Ethical approval and confidentiality

KYH

Ethical approval for the study was obtained from the ethics committees of the London School of Hygiene & Tropical Medicine (approval number 8808 received 24/02/2015), Novosibirsk State Medical University (approval number 75 approval received 21/05/2015), the Institute of Preventative Medicine (no approval number; approval received 26/12/2014), Novosibirsk and the Northern State Medical University, Arkhangelsk (approval number 01/01-15 received 27/01/2015).

The KYH dataset I received was anonymised and did not include any information on personal identifiers (name, initials, postcode) which was consistent with the London School of Hygiene and Tropical Medicine guidelines for good research practice.

Tromsø Study

Ethical approval was obtained from the Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics. Written informed consent for using information in future research projects was introduced in the Tromsø Study fourth survey.

Data access to Tromsø Study 4-7th surveys for this PhD project was requested and approved by the Data and Publication Committee at the Arctic University of Norway. The dataset I received was anonymised.

5 Main exposure, outcome and other variables

This chapter summarises the main variables pertinent to my main analysis. The first section describes methods of measurement of the main anthropometric exposures, followed by consideration of potential measurement error. Then the method to measure the main outcome carotid plaque is explained followed by those for the main confounders and mediators. Finally, CVD risk factors which I considered including in my main model but ultimately omitted are described.

5.1 Key exposures: adiposity measures

5.1.1 Assessment of anthropometric measures in the two studies

The key exposures in my main models were four simple anthropometric measures: body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR).

In the Know Your Heart study (KYH), height was measured using a Seca® 217 portable stadiometer (Seca Ltd) to the nearest millimetre. Weight was measured with a TANITA BC 418 body composition analyser (TANITA, Europe GmbH) with participants barefoot and wearing one layer of thin clothing. WC was measured at the smallest part of the trunk using a tape measure to the nearest millimetre followed by measurement of the hip at the widest part. When it was difficult to locate the natural indent of the trunk, the measurement was conducted at the level of the umbilicus. The tape was kept horizontal during the measurement, which was repeated twice. The protocol for the assessment of anthropometric measures is found in Appendix A4.

In the Tromsø Study, participants were measured in light clothing. Height was measured to the nearest centimetre in Tromsø 4 and to the nearest millimetre in Tromsø 5-7. Weight was measured to the nearest kilogram in Tromsø 4 and to the nearest 100 grams in Tromsø 5-7. WC was measured at the level of the umbilicus to the nearest centimetre using a tape measure. Hip circumference was measured at the widest part.

It should be noted that the measurement of WC was made at different sites in the two studies: the smallest part of the trunk in KYH and the level of the umbilicus in Tromsø 7. Previous studies have shown that WC measured at different sites differs significantly, especially among women (160, 161). Therefore, I converted WC at the level of the umbilicus in Tromsø

7 to the minimal WC using the equation derived by Mason et al. (161). The mean value of WC before and after the conversion and the conversion equation used are presented in Appendix A5. This equation was derived from 542 healthy volunteers aged 20-67 years who were predominantly white. Considering the relatively similar age and ethnic structures to Tromsø 7 participants, using this equation was reasonable in the Tromsø 7 population.

5.1.2 Measurement error

Random measurement error in continuous exposure variables could cause regression dilution bias and underestimation of the regression coefficient between adiposity and carotid plaque burden in my analysis (162). Therefore, I used data from the repeat study of KYH and investigated the reproducibility of the anthropometric measures. The details of the repeat study are described elsewhere (2). Briefly, two hundred participants from each Russian city were invited back one year after the first examination to reduce the effect of seasonality. They were subsequently re-interviewed and underwent a second health check. The primary objective of this repeat study was to estimate the correction factors to correct for measurement error. Meanwhile, the secondary objective was to investigate the reproducibility of each measurement.

I estimated the intra-class correlation coefficients (ICC) for height, WC, hip circumference, BMI, WHR, and WHtR. Table 5-1 shows the results. The ICCs for height was 0.99. However, the ICCs for other measurements were not as high as that for height. However, it should be noted that the repeat measurement was made one year after the first measurement, during which time, weight, WC, and hip circumference are highly likely to have undergone true changes, with the magnitude of true changes differing among the individuals. As an illustration of this, one American study followed 1,145 middle-aged adults aged between 50 and 75 years. After one year, mean weight increased by 1.72 kg, while mean WC increased by 1.76 cm (163). Therefore, it is possible that the correction of regression dilution based on the ICCs from this result does not necessarily improve the estimates because the ICCs may partially reflect heterogeneity in the true changes among individuals, in which case correcting for this as measurement error may over-correct, introducing bias rather than removing it.

Table 5-1 Intra-class correlation coefficients (ICC) of various anthropometric measures: Know Your Heart Study

	Individual ICC	95% CI
Height		
1st height measurement	0.99	0.99, 0.99
2nd height measurement	0.99	0.99, 0.99
Mean height measurement	0.99	0.99, 0.99
Weight		
Tanita weight	0.97	0.97, 0.98
Weight	0.97	0.97, 0.98
Waist circumference		
1st waist measurement	0.93	0.92, 0.95
2nd waist measurement	0.94	0.92, 0.95
Mean waist measurement	0.94	0.92, 0.95
Hip circumference		
1st hip measurement	0.93	0.91, 0.94
2nd hip measurement	0.88	0.86, 0.90
Mean hip measurement	0.92	0.91, 0.94
Adiposity measures		
BMI	0.97	0.96, 0.97
WHR	0.91	0.89, 0.92
WHtR	0.94	0.92, 0.95

Then I conducted a literature review of existing studies investigating the reproducibility of anthropometric measurements. One literature review showed excellent intra- and inter-observer reliability of height and weight (164). As a result, BMI is unlikely to be materially affected by measurement error. However, in the same literature review, WC and hip measurement showed lower inter-observer reliability, although intra-observer reliability was excellent (164). However, this result is based on old studies conducted more than 20 years ago. Recent studies have shown higher inter-observer reliability. For example, Mason et al. investigated the inter-observer reliability of WC measurement at four different measurement sites (161). The intraclass correlation coefficient (ICC) of WC at the level of the umbilicus and minimal waist in women was 0.96 and 0.98, respectively. For men, the ICC for WC at the level of the umbilicus and minimal waist was even better with 0.98 and 0.99, respectively. Similarly, Chen et al. reported excellent intra-observer and inter-observer ICC for both WC and WHR (intra-observer ICC: WC 0.987, WHR 0.970, inter-observer ICC: WC 0.988, WHR0.969) (165). Although this reproducibility from contemporary studies presents higher ICCs than the present study, it is very likely that ICCs in KYH are affected by heterogeneity in the true biological changes among individuals.

Training has been shown to improve the reliability of anthropometric measurement (166). Intra- and inter-observer reliability of several anthropometric measures was investigated in a study involving 12 physicians whose knowledge of appropriate measurement technique was poor. Without training, inter-observer reliability of the assessment by these physicians was unsatisfactory regarding WC (the coefficient of reliability 0.92), hip circumference (0.70), and WHR (0.51) although that of BMI was excellent. However, after a one-hour training session, inter-observer reliability showed substantial improvement (WC 0.97, hip circumference 0.92, WHR 0.89). In both the KYH and Tromsø 7 studies, appropriate training was delivered to improve the reliability of anthropometric measurements, and the detailed method was explained in the protocol.

In summary, the reproducibility of anthropometric measures in KYH seems acceptable, and the corrections of regression dilution using ICCs obtained might be problematic due to the true biological change between first and second measurements. Furthermore, intra- and inter-observer reliability of anthropometric measures, including WC and WHR, in contemporary studies seems very good. In addition, examiners in KYH and Tromsø 7 were provided with appropriate training in advance of the measurement process. Therefore, I did not conduct corrections for the effects of measurement error on the assumption that our reproducibility is comparable to that from recent studies. However, the association between carotid plaque and adiposity may be underestimated.

5.2 Key outcomes: carotid plaque

5.2.1 Assessment of carotid plaque in the two studies

Key outcomes in this thesis are two carotid plaque variables assessed using ultrasound: the presence of carotid plaques and carotid plaque score based on the presence of plaques in each carotid segment. In Chapters 6 and 7 using the Tromsø Study, total plaque area (TPA) is used as well.

Carotid ultrasound examination was performed using a comparable protocol in KYH and Tromsø 7. The protocol can be found in Appendix A6. Both carotid arteries were scanned for carotid plaques using Vivid q (GE Health care) with a linear 6~13 MHz transducer in KYH and Vivid 7 (GE Health care) with a linear 12 MHz transducer in Tromsø 7. Carotid plaque was defined as a focal structure encroaching into the arterial lumen at least 0.5 mm or 50% of the surrounding intima-media thickness (IMT) value, or demonstrates a thickness > 1.5 mm

as measured from the media-adventitia interface to the intima-lumen interface based on the Mannheim consensus in both studies (41). Images were stored, digitalised, and converted to Digital Imaging and Communications in Medicine (DICOM) digital records for offline reading.

There was a minor difference in the reading process for carotid plaque between the two studies. In Tromsø 7, the presence of carotid plaques and the plaque score, based on the presence of plaques in each carotid segment, were determined during the ultrasound examination by experienced sonographers. The longitudinal still image of every plaque was digitally documented using DICOM files for the offline reading of TPA. In KYH, offline readings were made by a team of experienced cardiologists to determine the presence of plaques and the actual number of plaques based on cine-loop of the carotid artery and still images of plaques using EchoPAC software (v. 113, GE-Vingmed AS, Norten, Norway). However, it is unlikely that this difference between the online and offline reading of the presence and number of plaques causes a meaningful difference since the assessment was made by experienced ultrasonographers /physicians in both studies. Another difference was how carotid plaques were counted, which is described in detail in the next section.

5.2.2 Measurement error

Random measurement error in the carotid ultrasound variables will not bias the regression coefficients between carotid variables and adiposity because they are outcome variables, but will affect the precision. Regarding the Tromsø Study, reasonable reproducibility of carotid plaque measurement has been previously reported (167). Briefly, they investigated the inter- and intra-observer agreements on plaque detection using the Tromsø Study fourth (1994-95) and fifth surveys (2001-02). Inter-observer agreement in plaque detection was good with kappa values of 0.72 and 0.67 in the fourth survey and the fifth survey, respectively. The intra-observer agreement was excellent (kappa values: 0.76 in the fourth survey, 0.80 in the fifth survey). Reproducibility of TPA was also investigated. The inter-observer reproducibility of TPA was satisfactory with a mean arithmetic difference -1.0 (95% CI: -1.4, -0.6) although the difference between the two observers tended to increase as TPA increased. The Tromsø Study used a consistent protocol of carotid examination in all the surveys. Overall, it is expected that my main outcomes, the presence of plaques and plaque score, will have reasonable reproducibility.

The burden of plaque was assessed slightly differently between the two studies, which may result in systematic measurement error. In both studies, plaques were sought in each segment (common carotid artery (CCA), bifurcation, internal carotid artery (ICA)) of both carotid arteries, a total of six carotid segments). The Tromsø 7 protocol explicitly further divided each segment into far and near wall locations, giving a total of twelve sites. However, only one plaque could be counted at each site because of concerns about differentiating between a single large plaque and two discrete plaques. This means each individual in Tromsø 7 can contribute to the maximum plaque number of twelve (maximum of two plaques for each of the six segments). In KYH, however, the protocol did not involve recording the near or far wall location, and there was no restriction on how many plaques could be recorded for each of the six segments. This protocol difference could lead to an artefactually higher plaque burden in KYH than Tromsø 7. Appendix A7 shows the number of plaques in KYH and in Tromsø 7 originally recorded by each carotid segment.

To ensure comparability of plaque burden in the two studies, I created a more comparable total plaque score using the following rule: when three plaques (the maximum seen) were recorded for any one of the six carotid segments in KYH this was converted to a score of two. Otherwise, the number of plaques for the six segments were converted to plaque score without changes. A total plaque score was then created by adding the plaque score from each carotid segment in KYH and Tromsø 7, giving a maximum plaque score of twelve. However, because KYH did not provide information on wall side location, the plaque scores were not completely comparable between the two studies. For example, if there were two plaques at the right bifurcation in KYH, it was not possible to determine if the appropriate score should be one (if both plaques were on the same wall side) or two (if on different sides).

To reduce the risk of the overestimation of plaque score in KYH, I also conducted a sensitivity analysis by using a simpler plaque score, which was independent of information about wall side. A score of one was assigned for the presence of one or more plaques in each of the six carotid segments (CCA, bifurcation, ICA of each carotid artery). This resulted in a consistent approach for both studies, with a maximum possible score of six for each individual. As reported in Chapter 9, the results of this sensitivity analysis were substantively the same as found using the full plaque score with a maximum of twelve.

5.3 Other CVD risk factors

5.3.1 Main confounders (age, sex, smoking, educational attainment)

Age, sex, smoking, and educational attainment were considered a priori as main confounders throughout all my analyses. Information on age, smoking (current smoker, ex-smoker, never-smoker) and educational attainment was obtained through face-to-face interview in KYH and self-administered questionnaires in Tromsø 7. The education variable in KYH was a categorical variable with seven levels (1: incomplete secondary, 2: complete secondary, 3: professional school, 4: professional school and secondary, 5: specialised secondary, 6: incomplete higher education, 7: higher education). Tromsø 7 had three levels (1: primary education, 2: secondary education, 3: tertiary education). Due to differences in the education system between Western European countries and Russia, it was difficult to convert the educational variable in KYH to the categorical variable with three levels that could be compared with Tromsø 7. Therefore, the education variables in the two studies were converted to the binary variables (higher education: Yes/No). The KYH questionnaire is available online (https://wellcomeopenresearch.org/articles/3-67) as supplementary material in Cook et al.(2). The Tromsø Study questionnaire is available on the Arctic University of Norway website (http://tromsoundersokelsen.uit.no/tromso/).

5.3.2 Main mediators (SBP, cholesterol level, glucose/HbA1c)

As can be justified from my literature review in Chapter 3, systolic blood pressure (SBP), cholesterol level, glucose (or glycosylated haemoglobin (HbA1c)) were considered as main mediators throughout my analyses. In KYH, SBP was measured three times, seated, using OMRON 705 IT automatic blood pressure monitors (OMRON Healthcare). Non-fasting venous blood samples were frozen within 2 hours of collection and stored at -20 degrees. Within three weeks, they were transferred to -80-degree freezers and eventually shipped to the laboratory in Moscow where all samples were analysed based on a standardised method.

In the Tromsø Study, SBP was recorded three times with Dinamap (CARESCAPE V100 Monitor, GE Healthcare). Both Dinamap and OMRON (used in KYH) have been validated to British Hypertension Society standards. Non-fasting venous blood samples were obtained, and fresh serum was analysed at the University Hospital laboratories.

5.3.3 CVD risk factors not included in the main model (physical activity, alcohol)

Two variables I considered including in the main model but ultimately omitted were physical activity and alcohol consumption. I did not include physical activity in the main model because the assessment methods differed markedly between the two studies. In KYH, the total physical activity index and Cambridge physical activity index were derived based on a detailed questionnaire regarding recreational and occupational physical activity. However, in Tromsø 7, recreational physical activity was categorised using a simple question (Describe your exercise and physical exertion in leisure time over the last year. 1: reading, watching TV/screen or other sedentary activity?, 2: Walking, cycling, or other forms of exercise at least 4 hours a week? (including walking or cycling to place of work, Sunday-walking, etc.), 3: Participation in recreational sports, heavy gardening, snow shovelling, etc at least 4 hours a week?, 4: Participation in hard training or sports competitions, regularly several times a week?).

Furthermore, although physical activity is a very important CVD risk factor, regarding the association between adiposity and carotid atherosclerosis, it cannot be simply considered as a confounder: physical activity is more likely to be located upstream of this association, with adiposity behaving as a mediator. Since physical activity is not on the causal pathway between adiposity and carotid atherosclerosis, it is not considered as a mediator either. For this reason, it seems reasonable to exclude it from my main model, although I used it in one analysis using the Tromsø Study (Chapter 7).

Alcohol consumption is considered an important CVD risk factor in Russia (168-170). However, when I obtained the dataset, comparable alcohol variables in the two studies were still unavailable. The available alcohol variable in KYH was a categorical variable with six levels based on ethanol consumption per year which was derived from questions about frequency and usual volume of consumption of beer, wine, and spirits (not drinker, >0 <2 litre/year, 2-4 litre/year, 4-9 litre/year, 10-19 litre/year, 20+ litre/year). On the other hand, Tromsø 7 had a categorical variable with five levels (How often do you usually drink alcohol? 1: never, 2: monthly or less frequently, 3: 2-4 times a month, 4: 2-3 times a week, 5: 4 or more times a week).

Furthermore, it is unclear whether alcohol should be considered as a confounder in the association between adiposity and carotid atherosclerosis; alcohol may affect adiposity as described in Chapter 2. However, whether either the volume of alcohol consumption or

pattern of drinking is associated with carotid atherosclerosis is unclear. In addition, alcohol consumption is not on the causal pathway between adiposity and carotid atherosclerosis, and thus cannot be considered as a mediator of this association.

6 The contribution of obesity to carotid atherosclerotic plaque burden in a general population sample in Norway: The Tromsø Study (Paper 1)

6.1 Introduction

Following two chapters (Chapters 6 and 7) use the Tromsø Study fourth (1994-95) and fifth (2001-02) surveys, and Chapters 8 and 9 use both the Know Your Heart Study and the Tromsø Study seventh survey.

In this chapter, I describe my investigation of the cross-sectional associations between general and abdominal adiposity and carotid plaque burden (the presence of carotid plaques, total plaque area (TPA)) using four anthropometric adiposity measures (body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR)) in a general population in Norway. I used data from the Tromsø Study fifth survey.

As described in my systematic literature review (Chapter 3), in contrast to extensive evidence on the associations between adiposity and CVD, there is little evidence on the associations between adiposity and carotid plaque, even in high-income Western countries. The limited evidence available does not adjust for confounders separately from mediators. Therefore, it is unclear whether this pathway is similar to that between adiposity and CVD, or whether it is similarly mediated by cardio-metabolic CVD risk factors. For this reason, in advance of my main analysis, a comparison between the Know Your Heart Study and the Tromsø Study seventh survey, I conducted an exploratory analysis using a previous survey from the Tromsø Study, and examined whether a potential causal pathway (Figure 3-1 in Chapter 3) is a reasonable assumption.

6.2 Published paper

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Student ID Number	1300239	**Title**	Dr.
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The contribution of obesity to carotid atherosclerotic plaque burden in a general population sample in Norway: The Tromsø Study

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ABSTRACT

Background and aims: Few studies have investigated the association of different measures of adiposity with carotid plaque. We aimed to investigate and compare the associations of four measures of adiposity: body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) with the presence of carotid plaque and total plaque area (TPA) in the right carotid artery.
Methods: We included 4906 individuals aged 31–88 years who participated in a population-based study with ultrasonography of the right carotid artery. Adiposity measures were converted to sex-specific SD units to allow comparison of effect sizes. TPA was log transformed due to its skewed distribution. Logistic and linear regression models were used respectively to investigate the association of each adiposity measure with the presence of plaque and with log-transformed TPA. Estimates were adjusted for potential confounders and mediators such as blood pressure and lipids.
Results: After adjustment for age, sex, smoking, and education level, there was strong evidence of an association between all adiposity measures and log-transformed TPA, whereas only WHR was weakly associated with presence of plaque. WHR showed the largest adjusted effect size for both log-transformed TPA (beta 0.055, 95%CI 0.028–0.081) and the presence of plaque (OR 1.07, 95%CI 1.01–1.15). Adjustment for mediators led to appreciable attenuation of observed effects.
Conclusions: Adiposity is more consistently associated with extent of plaque burden than with whether an individual does or does not have any plaque. There was evidence that established biomarkers mediate much of this association. Abdominal adiposity appears to show the strongest effect.

1. Introduction

Obesity is a global epidemic, affecting an increasing proportion of the world's population [1]. In 2014, approximately 40% of adults worldwide were estimated to be overweight, and 13% obese [2]. This trend is alarming because it might be expected to result in a steep increase in non-communicable diseases, particularly cardiovascular disease (CVD) which is the leading cause of death worldwide. Obesity contributes to CVD through a variety of pathways including hypertension, hyperlipidemia, and diabetes [3]. A better understanding of the pathways through which obesity affects CVD risk may contribute to developing interventions to mitigate the effect of this modifiable risk. Furthermore, it is known that to achieve sufficient weight-loss and to sustain it for a long-term is

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relatively difficult while the control of hypertension, hyperlipidemia, and diabetes are well-established [4,5].

Many studies have investigated the association between various adiposity measures and subclinical carotid atherosclerosis [6]. However, most of these have focussed on carotid intima-media thickness (IMT). Studies using carotid plaque as an outcome are scarce, and most of them use the binary indicator of presence or absence of plaque [7–15]. Although studies have consistently shown that increased IMT predicts future CVD events, increased IMT does not necessarily reflect atherosclerotic changes of the carotid artery and can be caused by other mechanisms [16]. In contrast, the presence of carotid plaque is characteristic of a later stage of atherosclerosis and thus, not surprisingly, predicts future CVD events better than IMT [17]. Beyond this, a quantitative measure of carotid plaque burden, such as total plaque area (TPA), has also been shown to be strongly predictive of future CVD events [18,19].

A small number of studies have investigated the association of body mass index (BMI) with quantitative measures of carotid plaque burden, although they failed to demonstrate a significant association [13,20]. However, BMI has limitations as a measure of adiposity [21]. It neither differentiates between fat and lean mass nor does it reflect body fat distribution. Accumulating evidence suggests that abdominal obesity may be correlated with CVD risks more strongly than general obesity, reflecting a more important role of visceral adipose tissues in the development of CVD [22]. Waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) are easily evaluated in a routine clinical setting and reflect abdominal obesity better than BMI. To our knowledge, associations of plaque burden with these alternative measures of adiposity have not been investigated.

The aim of our study was to evaluate the associations between four adiposity measures (BMI, WC, WHR, WHtR) and plaque presence (yes/no) as well as TPA in a population-based sample. In addition, we investigated the extent to which established CVD risk factors mediated any such associations. We used the Norwegian Tromsø Study which is one of few population-based studies that have quantitative plaque measures as well as multiple measures of adiposity.

2. Materials and methods

2.1. Participants

Subjects in this analysis participated in the fifth survey of the Tromsø Study (Tromsø 5) conducted in 2001-02. The Tromsø Study is a large population-based study with repeated health surveys conducted in the municipality of Tromsø, Norway, which started in 1974. Details of the Tromsø Study have been published previously [23].

The study consisted of two parts: a first visit that collected data via questionnaires, interviews, physical examinations and biological samples, and a second visit at which more extensive clinical examinations were conducted, including carotid ultrasonography. A total of 10353 people aged 30–89 years were invited to the first visit, and 8130 people attended [23]. A subset of 6969 participants among those who attended the first visit were invited to the second visit, of whom 5952 attended. The Regional Committee for Research Ethics approved the study, and The Norwegian Data Inspectorate licensed the data.

2.2. First visit measurements

Height, weight, waist and hip circumference were measured using standard methods. Blood pressure was measured using Dinamap Vital Signs Monitor 1846l. After a 2-min rest, three measurements were taken with 1-min intervals, with the participant in a sitting position. The mean of the two final readings was used in the analysis. Smoking habit, length of education (years), and medical history (hypertension, diabetes, CVD) were collected from self-administered questionnaires. A non-fasting blood sample was obtained. Serum total cholesterol and triglycerides were analyzed with enzymatic colorimetric methods. Serum high-density lipoprotein (HDL) cholesterol was measured after separating apoB containing lipoproteins by using heparin and manganese chloride [24]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation.

2.3. Ultrasound examination

By design, the carotid plaque examinations excluded those who had not had a carotid ultrasound examination in the Tromsø 4 study (1994-95). This meant that of those who attended the second visit (N = 5952), carotid ultrasound examinations in Tromsø 5 were conducted on only 5453 participants, of whom data was available for analysis for 5423.

Carotid plaque was assessed by recording ultrasonographic images of the right carotid artery using an Acuson Xp10 128 ART ultrasound scanner equipped with a linear array 5–7 MHz transducer. Participants were in a supine position during the examination.

The plaque was defined as a localized protrusion of the vessel wall into the lumen of at least 50% compared with the adjacent intima-media thickness. The near and far wall of the common carotid artery, bifurcation, and internal carotid artery were examined to seek plaques, and a score was calculated based on the presence of plaque in each location up to a maximum of six. For each plaque identified, a longitudinal still plaque image was recorded. Subsequent offline reading was undertaken to measure plaque area using a semi-automatic border detection software Arterial Measurement System (AMS) that involved the operator outlining each plaque [25]. For those with more than one plaque, all plaque areas were added together to calculate TPA. Details of the ultrasound examination have been described in a previous publication [26].

2.4. Statistical methods

Characteristics of the study population were summarised using means with standard deviations (SD) or medians with the interquartile range for continuous variables, or numbers and percentages for categorical variables.

The distribution of TPA was highly skewed and zero-inflated, which led us to use a combination of two models. A logistic regression model was used with presence of plaque (yes/no) as an outcome to explore the association of adiposity with having at least one plaque. Secondly, log-transformed TPA was used as the outcome in a linear regression model to explore the association between adiposity and plaque burden excluding those with no recorded plaque. TPA was log-transformed to improve the approximation to the normal distribution. These two models in conjunction are equivalent to using a hurdle model [27], with the logistic regression modelling the distribution of zero plaque versus at least one plaque, and the linear regression modelling the distribution of the non-zero measures.

For both outcomes, we investigated the association with four different measures of adiposity: BMI, WC, WHR, WHtR. To directly compare the strength of association of each adiposity measure, taking account of the different distributions in men and women, we calculated sex-specific standardized adiposity scores by subtracting the sex-specific mean of each adiposity measure from the observed

value and then dividing by the sex-specific SDs. We fitted a sequence of three models to each of the adiposity measures in turn. Model 1 was adjusted for age as a continuous variable and sex. Model 2 was further adjusted for potential behavioural confounders; smoking (a categorical variable of three levels: current smoker, ex-smoker, never-smoker), and years of education (as a continuous variable). Model 3 was additionally adjusted for potential biological mediators of the association between adiposity and plaque as follows; systolic blood pressure (SBP), HDL-cholesterol, LDL-cholesterol and glycated hemoglobin (HbA1c), all as continuous variables. These analyses were restricted to participants with all four adiposity measures and all covariates recorded to compare effect sizes.

Previous research on adiposity in relation to cIMT has suggested that there may be different associations seen in men and women [28]. We therefore checked for interactions with sex in the associations of adiposity with TPA, by adding interaction terms for all three models.

Statistical analyses were performed using Stata statistical software (version 14: Stata Corp) [29].

3. Results

Of the 5423 subjects for whom we had carotid ultrasound data, we used the subset of 4906 who had complete data on all relevant variables. Missing data on education (5%) and HbA1c (2%) were the main reasons for subjects being dropped from the analyses. The characteristics of the subset analysed were very similar to that of all subjects who had undergone carotid ultrasound examination (data not shown).

The prevalence of carotid plaque and median TPA are shown in Table 1. The prevalence of having at least one plaque was 60%. Table 2 shows the means of each adiposity measure by category of plaque burden. There was a tendency for the adiposity means to increase across categories of plaque burden except for BMI.

Table 3 shows the odds ratios for the presence of plaque for each of the four adiposity measures. In models 1 and 2 only WHR showed some evidence of an association with presence of plaque. This was attenuated and became non-significant on adjustment for mediators in model 3.

Table 4 shows the associations of log-TPA with each adiposity measure. There was evidence of positive associations with all four measures in model 2 after adjustment for potential confounders, with WHR showing the strongest effect. All associations were attenuated on further adjustment for potential mediators, with WHR remaining the only one showing (weak) evidence of an association. Tests for interactions between adiposity and sex and adiposity and age on log-TPA were non-significant (data not shown).

Table 1
Participant characteristics of study population. The Tromsø Study 2001-2002.

	Participants included in the analysis[a] (n = 4906)	Participants included in the analysis with plaque[b](n = 2906)
Age (years) median (IQR)	66 (60–72)	68 (63–74)
Sex (% of women)	2722 (55.5)	1450 (49.9)
Current smoker (%)	1249 (25.5)	794 (27.3)
Ex-smoker (%)	1999 (40.8)	1234 (42.5)
Education (year)	9.9 (3.6)	9.4 (3.3)
SBP (mmHg)	143.0 (21.6)	147.3 (21.5)
Anthropometry measures		
Height (cm)	167.4 (9.3)	167.3 (9.5)
Weight (kg)	75.2 (13.9)	75.1 (13.8)
BMI (kg/m^2)	26.8 (4.2)	26.8 (4.1)
WC (cm)	90.3 (12.2)	91.1 (12.0)
WHR	0.89 (0.09)	0.90 (0.09)
WHtR	0.54 (0.07)	0.55 (0.07)
Blood sample		
HDL cholesterol (mmol/l) median(IQR)	1.43(1.19–1.72)	1.42 (1.18–1.70)
LDL cholesterol (mmol/l)	4.11 (1.07)	4.16 (1.09)
Triglycerides (mmol/l) median (IQR)	1.34 (0.96–1.90)	1.39 (1.00–1.94)
HbA1C (%)	5.50 (0.80)	5.58 (0.83)
Comorbidities (self-report)		
Diabetes (%)	222 (4.6)	152 (5.4)
Myocardial infarction (%)	378 (7.9)	311 (11.0)
Angina pectoris (%)	501 (10.5)	398 (14.1)
Stroke (%)	191 (4.0)	148 (5.2)
Current medication (self-report)		
Anti hypertensive drug (%)	1194 (24.9)	869 (31.7)
Lipid lowering drug (%)	708 (15.0)	541 (19.5)
Carotid variable		
Plaque presence (%)	2906 (59.2%)	2906 (100%)
Number of plaque (%)		
1	1372 (28.0)	1372 (47.2)
2	931 (19.0)	931 (32.0)
3	427 (8.7)	427 (14.7)
4	134 (2.7)	134 (4.6)
5	36 (0.7)	36 (1.2)
6	6 (0.1)	6 (0.2)
TPA (mm^2)		17.6 (10.0–31.3)

Data are mean values with standard deviations for continuous variables except for age, triglycerides, HDL cholesterol, and TPA which is shown with median and interquartile range.
BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-hight ratio, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HbA1c: glycated haemoglobin, TPA: total plaque area.
[a] Participants are restricted to those with all main variables and non-missing TPA variable.
[b] Participants are restricted to those with all main variables and at least one plaque.

Table 2
The association between adiposity and TPA quartiles (crude). The Tromsø Study 2001-2002.

	Total number[a]	BMI (kg/m^2)	WC (cm)	WHR	WHtR
Persons without plaques	2000	26.8 (4.3)	89.0 (12.5)	0.87 (0.09)	0.53 (0.07)
Persons with plaques					
TPA quartile 1 (lowest)	727	26.7(4.2)	89.3 (11.9)	0.88 (0.09)	0.54 (0.07)
TPA quartile 2	726	27.1 (4.3)	91.2 (12.3)	0.89 (0.09)	0.55 (0.07)
TPA quartile 3	727	26.6 (3.9)	90.8(11.7)	0.90 (0.09)	0.54 (0.07)
TPA quartile 4	726	26.7 (4.0)	93.2 (11.7)	0.92 (0.08)	0.55 (0.07)

Data are mean values with standard deviations.
BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-hight ratio, TPA: total plaque area.
[a] Only the participants with all adiposity variables and covariates are used in this table.

Table 3
The association between sex-specific standardized adiposity scores and the presence of plaque (Yes/No) using logistic regression: this analysis is restricted to participants with non-missing presence of plaque variable and all variables in the model (n = 4906). The Tromsø Study 2001-2002.

	Model 1 OR (95%CI)	*p*-value	Model 2 OR (95%CI)	*p*-value	Model 3 OR (95%CI)	*p*-value
st BMI	0.98 (0.92, 1.04)	0.45	1.03 (0.96, 1.09)	0.45	0.95 (0.88, 1.01)	0.11
st WC	0.98 (0.92, 1.05)	0.58	1.01 (0.95, 1.08)	0.79	0.92 (0.86, 0.99)	0.03
st WHR	1.08 (1.01, 1.15)	0.02	1.07 (1.01, 1.15)	0.03	1.01 (0.94, 1.08)	0.87
st WHtR	1.02 (0.96, 1.08)	0.57	1.04 (0.98, 1.11)	0.21	0.96 (0.90, 1.03)	0.28

St BMI:standardized body mass index, st WC: standardized waist circumference, st WHR: standardized waist-to-hip ration, st WHtR: standardized waist-to-height ratio, OR: odds ratio, 95% CI: 95% confidence interval, SD: standard deviation.
Model 1: adjusted for age and sex, Model 2: adjust for variables in Model 1 plus other confounders (smoking and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, LDL-cholesterol, glycated hemoglobin).

Table 4
The association between sex-specific standardized adiposity scores and log-transformed TPA using linear regression: this analysis is restricted to participants with plaque and all variables in the model (n = 2906). The Tromsø Study 2001-2002.

	Model 1 β (95%CI)	*p*-value	Model 2 β (95%CI)	*p*-value	Model 3 β (95%CI)	*p*-value
st BMI	0.012 (−0.015, 0.039)	0.38	0.032 (0.004, 0.059)	0.02	0.002 (−0.026, 0.031)	0.87
st WC	0.031 (0.005, 0.058)	0.02	0.042 (0.015, 0.068)	0.002	0.012 (−0.017, 0.040)	0.42
st WHR	0.056 (0.029, 0.083)	<0.001	0.055 (0.028, 0.081)	<0.001	0.030 (0.002, 0.058)	0.04
st WHtR	0.038 (0.011, 0.065)	0.005	0.046 (0.019, 0.073)	0.001	0.017 (−0.012, 0.045)	0.26

St BMI: standardized body mass index, st WC: standardized waist circumference, st WHR: standardized waist-to-hip ratio, st WHtR: standardized waist-to-height ratio, β: regression coefficient, 95% CI: 95% confidence interval, SD: standard deviation.
Model 1: adjusted for age and sex, Model 2: adjust for variables in Model 1 plus other confounders (smoking and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, LDL-cholesterol, glycated hemoglobin).

4. Discussion

To our knowledge, this is the first study to investigate the burden of carotid plaque and multiple adiposity measures in a large population-based study. Our study had three major findings. First, all adiposity measures were associated with log-TPA, but the evidence for an association with plaque presence was weak except for WHR. Second, the associations between adiposity and carotid plaque appeared to be at least partially mediated by traditional CVD risk factors. Thirdly, our main measure of central obesity (WHR) showed the strongest and most consistent association with plaque presence and burden.

Previous studies investigating the association between adiposity and the presence of plaque are scarce, and the results are inconsistent [7–12] in part reflecting differences in statistical methods and the extent to which adjustments were made separately for potential confounders and mediators. Czernichow et al. showed that BMI, WC, and WHR were not associated with carotid plaque occurrence after adjustment for traditional CVD risk factors in 1014 healthy adults [9]. Several other studies however had results that were broadly consistent with our analyses. A French study with 6265 participants aged 65-years and older showed that frequency of carotid plaque increased as WC and WHR increased, but the association became non-significant after the adjustment for traditional CVD risk factors [10]. On the other hand, WHR had significant associations with the presence of plaque in two cross-sectional studies. Chaubey et al. showed that the odds of having plaque increased by approximately 30% as WHR increased by 0.1 after adjustment for traditional CVD risk factors [8]. Finally, an Australian population study showed that those with WHR larger than 0.91 had two-fold increased odds for having plaque compared to those with smaller WHR [11].

The particularly weak association between BMI and the burden of carotid plaque in our analysis after adjustment for traditional CVD risk factors is in line with the only two previous study results [13,20]. Herder et al. showed that baseline BMI was not associated with future TPA nor change in TPA in a 13-year follow-up study [13]. Selwaness investigated determinants of plaque burden assessed using MRI, and BMI was not significantly associated with the burden of plaque [20]. We were unable to find any other studies that had examined plaque burden in relation to any of our three other measures of adiposity.

The fact that our study has found evidence for adiposity being related to extent of plaque burden rather than the presence/absence of plaque per se is intriguing. This may indicate that adiposity contributes to the process of the development of existing plaque more than the process of plaque initiation. However, because TPA is a continuous measure and contains more information than the binary plaque (present/absent) variable this may make an association easier to detect.

Several studies investigated the association between multiple adiposity measures and IMT and compared the effect size. However, their results are inconsistent. After adjustment for traditional CVD risk factors, some studies showed abdominal obesity was more strongly associated with IMT [30,31] while in one study BMI showed the largest effect size [9]. In another study, BMI and WC showed the same effect size [32]. It seems which anthropometry measure is the most strongly associated with IMT is still inconclusive.

The potential importance of our finding that TPA is associated with adiposity is underlined by the fact that it has been found to be predictive of CVD events. Spence et al. investigated 1686 patients from an atherosclerosis prevention clinic and showed that those in the top TPA quartile had nearly three times the odds of stroke and myocardial infarction compared to those in the first TPA quartile after 5-year follow up [18]. The Tromsø Study compared the associations between myocardial infarction and stroke with IMT and TPA. TPA was a strong predictor of future myocardial infarction and stroke, while IMT at the common carotid artery was not associated with future events [19,34]. Sillesen et al. showed that plaque burden was more strongly associated with coronary artery calcium score than IMT [35].

One of our most striking results is that the association of adiposity with the burden of carotid plaque is strongly attenuated by adjustment for biological factors that are plausible mediators of this association. Because our study is cross-sectional it is not possible to describe this as definitive evidence of mediation, but it is consistent with such an interpretation. Looking at the larger literature on mediators of the association between obesity and CVD events, a recent pooled-analysis using 97 cohort studies showed that half of the risk of coronary heart diseases and stroke was mediated by blood pressure, cholesterol, and glucose [36]. A more recent report from the ARIC study suggested that the association of obesity with coronary heart disease was fully explained by traditional mediators [37]. These results taken overall suggest that strict control of traditional CVD risk factors including lipids and blood pressure could substantially mitigate the harmful effect of obesity on carotid atherosclerosis.

When comparing the effect sizes of four different adiposity measures, WHR showed the largest effect size with a significant association with log-TPA followed by WHtR. While these estimated effects have wide and overlapping confidence intervals and thus any differences need to be treated cautiously, our results do suggest that central (visceral) obesity may play a more important role than obesity as measured by BMI. Visceral adipose tissue is known to pose a greater risk for CVD with its stronger association with insulin resistance and dyslipidemia than total fat and subcutaneous adipose tissue [38,39]. It has also been suggested that the visceral adipose tissue contributes to the progress of atherosclerotic change [40].

Our study has some limitations. Firstly, the cross-sectional design does not allow conclusions concerning causality. Secondly, TPA is essentially a 2-dimensional proxy for the volume of a 3-dimensional plaque structure. While this may result in measurement error relative to a better assessment of plaque volume, using 3-dimensional ultrasound or MRI for example, this is unlikely to bias the strength of association with plaque size as long as this is a random measurement error in the outcome variable [47]. Thirdly, we only had data available for the right carotid artery. However, this is unlikely to have generated bias as there is no reason to believe that the strength of association between adiposity and plaque will differ between right and left carotid. Fourthly, the prevalence of plaque was relatively high in our population. This may be because participants were relatively old and the study was conducted in 2001-02 when the CVD event rate in the population was appreciably higher [48]. To this extent, whether the strength of association we report here is generalizable to contemporary populations is a matter for further investigation. Finally, a particular strength of our study is that the data are from a large population-based study with a high response rate.

In summary, our results suggest that adiposity measures reflecting abdominal obesity such as WHR may be more closely associated with plaque burden than the traditional measure of BMI, and that these effects may be mediated by established biological risk factors such as blood pressure and lipids. Although further studies are needed to investigate the usefulness of these adiposity measures as predictors for an individual in a clinical setting assessment of these measures may help to identify a high-risk group with advanced carotid plaque. Further research is desirable to investigate the prospective association between adiposity and carotid plaque burden. It has been suggested that TPA could capture the progress of atherosclerosis more sensitively than IMT because longitudinal plaque growth is faster than its growth toward the lumen [13,51]. This would make TPA suitable for prospective studies.

4.1. Conclusion

Adiposity is more consistently associated with extent of plaque burden than with whether an individual does or does not have any plaque. Abdominal adiposity appears to show the strongest effects. There was evidence that established biomarkers mediate much of this association. It would be useful to examine the prospective effect of abdominal obesity on the progress of carotid atherosclerosis in a cohort study.

Conflicts of interest

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

Author contributions

YI undertook the analyses and drafting of the manuscript. KM advised on statistical methods and analysis. KM, DAL, EM, LAH, and AH provided ongoing guidance during drafting. All authors commented on drafts of the paper and approved the final manuscript.

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6.3 Summary

- There was strong evidence of an association between adiposity and carotid plaque burden (TPA) after adjustment for potential confounders (sex, smoking, education).
- The associations between adiposity and carotid plaque burden appeared to be mediated by cardio-metabolic CVD risk factors to a large extent.
- Abdominal adiposity seemed more strongly associated with plaque burden than general adiposity. In particular, waist-to-hip ratio showed the largest effect size among four adiposity measures.

7 How does obesity in adults affect the progression of carotid atherosclerosis in the general population? (Paper 2)

7.1 Introduction

In this chapter, I present a prospective association between four anthropometric adiposity measures and the progression of carotid atherosclerosis (new plaque formation, number of plaques, total plaque area (TAP)) in a general population in Norway. Data from the Tromsø Study fourth (1994-95), fifth (2001-02), and sixth (2007-08) surveys were used in this analysis.

In the previous chapter, based on the Tromsø Study fifth survey, I reported evidence of an association between adiposity, especially abdominal adiposity, and carotid plaque burden, which was largely mediated by cardio-metabolic CVD risk factors. In this chapter, I will extend this analysis to strengthen the findings observed in the previous chapter.

7.2 Submitted paper

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SECTION A – Student Details

Student ID Number	1300239	**Title**	Dr.
First Name(s)	Yume		
Surname/Family Name	Imahori		
Thesis Title	How does obesity in adults affect the progression of carotid atherosclerosis in the general population?		
Primary Supervisor	David Leon		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where is the work intended to be published?	International Journal of Obesity
Please list the paper's authors in the intended authorship order:	Yume Imahori, Ellisiv B. Mathiesen, Katy E. Morgan, Chris Frost, Alun D. Hughes, Laila A. Hopstock, Stein Harald Johnsen, Nina Emaus, David A. Leon
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualized, conducted the analyses, wrote the first draft, coordinated all comments by co-authors, and was primarily responsible for the final draft..

SECTION E

Student Signature	[redacted]
Date	8 Mar 2019

Supervisor Signature	[redacted]
Date	7/3/2019

Title: How does obesity in adults affect the progression of carotid atherosclerosis in the general population?

Running title: Obesity and the progression of carotid atherosclerosis

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Author contributions: YI undertook the analyses and drafting of the manuscript. KM and CF advised on statistical methods and analysis. CF, DAL, EM, AH provided ongoing

guidance during drafting. All authors commented on drafts of the paper and approved the final manuscript

Abstract

Background/Objectives: Few reports are available on the contribution of general and abdominal obesity to the progression of carotid atherosclerosis in late adulthood. This study investigated the impact of four simple anthropometric measures of general and abdominal obesity on the progression of carotid atherosclerosis and to what extent the association between adiposity and the progression of plaque burden is mediated by metabolic markers.

Methods: 4345 adults (median age 60) from the population-based Tromsø Study were followed over 7 years from the first carotid ultrasound screening to the next. The progression of carotid atherosclerosis was measured in three ways: incidence of plaques in previously plaque-free participants, change in the number of plaques, and total plaque area (TPA). We used generalized linear models to investigate the association between each adiposity measure (body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR)) and each outcome. Models were adjusted for potential confounders (age, sex, smoking, education, physical activity). The pathways through which any associations observed might operate were investigated by further adjusting for physiological metabolic mediators (blood pressure, blood lipids and HbA1c**).**

Results: There was little evidence that adiposity was related to the formation of new plaques during follow-up. However, abdominal adiposity was associated with TPA progression. WHtR showed the largest effect size (mean change in TPA per 1 SD increase in WHtR of 0.665 mm^2, 95% confidence interval 0.198, 1.133) while BMI showed the smallest. Effect sizes were substantially reduced after the adjustment for potential mediators.

Conclusions: Abdominal obesity seems more strongly associated with the progression of carotid atherosclerosis than general obesity. These associations appear largely mediated by known physiological -metabolic markers.

Introduction

Obesity is a global epidemic. In 2016, 39% of adults worldwide were estimated to be overweight, and 13% obese (99). The global prevalence of obesity has almost tripled over the last 30 years and continues to increase (99). This trend is of substantial concern, as obesity is associated with an increase in cardiometabolic disorders and cardiovascular disease (CVD), the leading cause of death worldwide. Paradoxically, over this period, the burden of CVDs has been decreasing in industrialised countries (21, 22). However, the current increase in obesity could offset or reverse the downward trend of CVD mortality in spite of the decrease in the prevalence of other traditional CVD risk factors such as smoking and hypertension (22). A recent analysis of US data has suggested that if BMI had not increased, life expectancy in 2011 at age 40 would be 0.9 years higher than was actually the case (171), which in part would be due to a slowdown in the rate of decline of CVD mortality (172).

Atherosclerosis is a major cause of CVD. Carotid atherosclerosis is easily and non-invasively detected using the ultrasound. Carotid intima-media thickness (IMT) consistently predicts future CVD events, but carotid plaque outperforms IMT regarding its predictive ability of future CVD (14). Most previous studies on the association between obesity and carotid atherosclerosis in adults have been cross-sectional. Relatively few studies have attempted to look at this relationship prospectively. One population study investigated the association between lifetime BMI and carotid intima-media thickness (IMT) in late adulthood and showed that reduction in BMI category was associated with a decrease in IMT (173). Several others have investigated determinants of carotid plaque progression including obesity as one of the potential factors (133, 174-176).

If there is evidence of obesity influencing progression, this is of direct relevance to clinical management in adulthood. Also, compared to general obesity (the exposure in most previous studies), abdominal obesity might play a more important role in progression because of its stronger association with cardio-metabolic diseases (177).

In an earlier investigation of cross-sectional data from the population-based Tromsø Study, we have shown that abdominal obesity was more closely associated with carotid plaque burden assessed by total plaque area (TPA) than general obesity (178). We also found that physiological cardio-metabolic risk factors such as hyperlipidemia, glucose intolerance, and hypertension mediated much of this association. The aim of the current analysis is to extend

these investigations to determine whether progression of carotid plaque burden over a 7-year period is related to different measures of obesity in the Tromsø Study, and how far any indication of such an association is mediated by the same set of cardio-metabolic risk factors.

Method

Study design and participants

The Tromsø Study (157) is an on-going population-based prospective cohort study based on the population of the municipality of Tromsø in North Norway, consisting of seven surveys from 1974-2016 (Tromsø 1-7).

Baseline (1994-95): The Tromsø Study the fourth survey (the 4th survey) conducted in 1994-95 is the largest survey so far inviting everyone aged 25 years and above living in the Tromsø municipality. Among the 4th survey participants, all those aged 55-74, plus sampling fractions between 5 and 10% from other age groups, were eligible for a second visit with extensive clinical examinations including carotid ultrasound, with 76% (n=6727) attending. (179). All the 4th survey second visit participants were invited to the 5th (2001) and 6th (2007-08) survey for the follow-up ultrasound examination.

7-year follow-up at the 5th Survey (2001): All 4th survey second visit participants, except those who died (n=499) or moved from Tromsø (n=372), were invited to the 5th survey ultrasound examination (n=5856), with 83% attending (n=4858), i.e., 72% of the participants from the 4th survey second visit were rescanned. After excluding participants without valid written consent (n=29) and with missing data on the main covariates (n=484), 4345 participants were included in the current analysis of the7-year follow-up (1994-2001) data.

14-year follow-up at the 6th Survey (2007-08): All the 4th survey second visit participants except those who died (n=1515) or moved from Tromsø (n=468) were also invited to the 6th survey ultrasound examination (n=4744), with 63% attending (n=2975), i.e., 44% of the participants from the 4th survey were rescanned. After excluding those who withdrew their consent (n=1) or had missing covariates (n=289), 2685 participants with complete repeated measurements were eligible for the current analysis of 14-years follow-ups (1994-2008) data.

The Regional Committee for Research Ethics and The Norwegian Data Inspectorate have approved the Tromsø Study study. All participants included in the present study have given written informed consent.

Measurement of obesity and other CVD risk factors: the baseline 4th survey (1994-95)

Height and weight were measured with light clothing and without shoes using standard methods. Waist circumference (WC) was measured at the level of the umbilicus while hip circumference was measured at the widest part of the thigh to the nearest 0.5 cm. BMI was calculated by dividing body weight by height squared. Waist-to-hip-ratio (WHR) and waist-to-height-ration (WHtR) were calculated by dividing WC by hip and height, respectively. Categorical adiposity variables were created with three BMI levels (normal: BMI<25 kg/m^2, overweight: 25<=BMI<30 kg/m^2, obesity: 30 kg/m^2≤MI), two WC levels (abdominal obesity: men>102 cm, women>88 cm), and two WHR levels (abdominal obesity: men>=0.9, women>=0.85) according to WHO cut-off points (180).

Information on current smoking (yes/no), education level (primary education, vocational/high school, university/college), physical activity (hours spent on leisure physical activity (sweating/out of breath) per week in leisure time: none, less than 1, 1-2, 3 or more) and medical history (myocardial infarction, angina pectoris, stroke, diabetes) were obtained by self-administered questionnaires. Non-fasting serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were determined using standardised methods. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. HbA1c was measured using the Cobas Mira instrument. Blood pressure was measured seated three times with one-minute interval between readings after 1 min seated rest using an automatic device (Dinamap Vital Signs Monitor 1846; Critikon Inc., Tampa, FL, USA). The average of the last two blood pressure measurements was used in the analysis.

Ultrasound examination: the 4th -6th survey (1994-2008)

The right carotid artery was scanned in the far and near wall of the common carotid artery, bifurcation, and internal carotid artery to seek for carotid plaques using an Acuson Xp 10 128 ART ultrasound scanner equipped with a 7.5 MHz linear-array transducer in the 4th and 5th surveys, and a GE Vivid 7 scanner with a linear 12 MHz transducer in the 6th survey, as previously described (167, 181). The same protocol was used at all surveys. Carotid plaque was defined as a focal structure encroaching into the arterial lumen at least 0.5 mm or 50% of

the surrounding intima-media thickness (IMT) value or IMT >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface. Cine loops and still images of each carotid plaque in the longitudinal plane were stored and digitalised for offline analysis. All plaque images were outlined manually to assess plaque areas using the Adobe Photoshop imaging-procession programme (ver. 7.0.1). When there were multiple plaques, all plaque areas were summarised to calculate total plaque area (TPA). Inter-equipment variability and intra-reader and inter-reader re-reproducibility of plaque detection and measurement of plaque area were acceptable (167, 181).

Statistical analysis

Primary analysis: changes from the baseline 4th survey to the 5th survey (1994-2001):

Baseline characteristics were summarized as means with standard deviations (SD) (or medians with the inter-quartile range if markedly non-normally distributed) for continuous variables, and counts and frequencies (percentages) for categorical variables.

We used three outcomes to assess the progression of carotid atherosclerosis: new plaque formation (binary yes/no) in those without plaque at baseline, change in the number of plaques from baseline to follow-up, and change in TPA over the same period.

We converted each adiposity measure at baseline to sex-specific standardised adiposity scores by subtracting the sex-specific mean of each measure from the observed value and then dividing by the sex-specific SD.

The association between new plaque formation and each adiposity measure was examined using a series of logistic regression models. The effect of each adiposity measure on the change in the number of plaques/TPA was estimated using analogous linear regression models. Due to concerns over normality assumptions, bias-corrected and accelerated bootstrap confidence intervals (CI) were estimated from 10000 bootstrap resamples for all linear regression models. However, the differences between these and standard CIs tended to be relatively small, and since bootstrapping precludes reporting of p-values, we present standard CIs in the results section.

Separately for each outcome variable and each adiposity measure, we fitted a sequence of three models. We selected potential confounders and mediators a priori based on evidence of the

association between obesity and CVD. Model 1 was adjusted for age (categorical 5-year interval) and sex. Model 2 was further adjusted for the other potential behavioural and socio-demographic confounders: smoking, physical activity, and education, this being our main model for estimating the association between adiposity and the progression of atherosclerosis. To assess the extent to which any observed associations in model 2 might be mediated by physiological cardio-metabolic risk factors, model 3 further adjusted for the following potential mediators systolic blood pressure, HDL-C, LDL-C, and glycated haemoglobin as continuous variables, and antihypertensive- and lipid-lowering drug use and existing diabetes as binary variables.

All the above models were refitted separately adding interactions with age and sex. None of the interactions was statistically significant (results not reported). STATA version 14 (Stata Corp) was used for all analysis.

Secondary analyses: changes from the baseline 4th survey to the 6th survey (1994-2008):

We conducted parallel analyses to those above using the 6th survey follow-up data (2008). In planning this study, we had intended to focus equally on this analysis. However, having undertaken the analysis, the sample size was appreciably smaller after 14-year follow-up, and effect estimates markedly less precise than those from our 5th survey analysis. Therefore, we focus our presentation on the 5th survey analysis. As our initial intention was to include analyses from the 6th survey data and to avoid publication bias, analyses involving the 6th survey after 14-year follow-up are reported only in supplementary tables, although the results were largely uninformative.

Results

Baseline characteristics

Table 1 shows participant characteristics at baseline. The median age of participants was 60 years (interquartile range 55-66), and 51% were women. Compared to the 4th survey second visit participants who did not attend the 5th or 6th survey, the attending population was younger with a more favourable CVD risk profile (Tables S1-2). Adiposity measures were approximately normally distributed. According to the BMI classification, 44.4% and 13.4% of the participants were categorised as overweight and obesity, respectively. Increased WC

(men>=102 cm, women>=88 cm) was observed in 28.3% of the participants while 42.7% had increased WHR (men>=0.9, women>=0.85). Lipid-lowering drug use prevalence was 2.6%.

At baseline, 46.2% of participants had at least one plaque. The median TPA in subjects with plaques was 15.7 mm^2 in men and 12.7 mm^2 in women.

Carotid plaque burden at 7-year follow-up

Table 2 shows carotid plaque burden at the 5th survey by category of adiposity 7 years earlier at the 4th survey. At the 7-year follow-up, 62.2% of the participants had at least one plaque. Among the 2337 participants without plaque at the baseline 4th survey, 40% developed at least one new plaque. Median TPA increased from 0 mm^2 at baseline to 8.9 mm^2. Participants who belong to higher adiposity category were more likely to have higher plaque burden and more progression of plaque burden compared to those in lower categories.

New plaque formation among participants without plaque at baseline (baseline – the 5th survey)

Table 3 shows the odds ratios for developing at least one plaque at 7-year follow up among the 2337 participants without plaque at baseline per 1 SD increase in each adiposity index. After adjustment for confounders (model 2), all estimated odds ratios were small and non-significant.

Progression of plaque number (baseline – the 5th survey)

Table 4 shows the estimated changes in the number of plaques per 1 SD increase in each adiposity measure at baseline. After adjustment for confounders (model 2), no adiposity measure except for WHR was significantly associated with changes in the number of plaques.

Progression of TPA (baseline – the 5th survey)

Table 5 shows the estimated changes in TPA per 1 SD increase in each adiposity measure at baseline. After adjustment for confounders (model 2), there were statistically significant associations between the progression in TPA and all adiposity measures except for BMI. WHtR showed the largest regression coefficient, followed by WHR, and BMI showed the smallest. After further adjustment for potential mediators (model 3), all effect sizes decreased substantially, and all became non-significant.

14-year follow-up (baseline - the 6th survey)

Analogous tables to those above for the 6th survey follow-up data are shown in Tables S3-5. These analyses included markedly fewer people than those for the 5th survey follow-up and effect estimates were consequently considerably less precise with wide CIs.

Discussion

In this 7-year follow-up of a population-based sample of women and men in late adulthood, we found that abdominal obesity was more strongly associated with progression of carotid plaque burden than general obesity. New plaque formation among participants without plaque at baseline was however not associated with any adiposity measures. Furthermore, all significant associations observed were in part mediated by cardio-metabolic risk factors.

The progression of carotid plaque burden and obesity

Our results suggest that abdominal obesity in late adulthood might contribute to the progression of carotid plaque burden with larger effect estimates than general obesity, imposing an excess risk on the progression of atherosclerosis. This finding concurs with our previous findings from a cross-sectional analysis, although considerable overlap of CIs prevents us from drawing definitive conclusions (178).

Several previous studies have investigated the effect of general obesity on the progression of carotid atherosclerosis (133, 174, 175). Herder et al. investigated the determinants of the progression of IMT and TPA after 13 years follow-up; in this study, BMI at baseline did not predict progression of either (133). Similarly, van der Meer et al. showed that BMI was not associated with an increase in plaque number over an average follow-up of 6.5 years (174). Molino-Lova et al. showed that overweight/obesity according to BMI category was not associated with the new formation of plaque in 486 elderly participants without plaque at baseline over a 3 year follow-up period (175). All these findings agree with the relatively weak association between BMI and the progression of atherosclerosis in our analysis.

On the other hand, not much has been done to clarify the influence of abdominal obesity on the progression of carotid atherosclerosis. One prospective study (n=1894) with 4-year follow-up showed that an increase in WC was one of the determinants of the new formation of plaque among 462 participants without plaque at baseline after adjustment for age, sex,

and follow-up time while BMI was not (176). However, neither was a determinant of the progression of TPA. In the Rotterdam Study, the determinants of the progression of the number of plaques were analyzed in 3409 participants after the 6.5-year follow-up. An increase in WHR was associated with an increase in the number of plaques after adjustment for traditional CVD risk factors. Again, BMI was not associated with increases in the number of plaques (174). Although a direct comparison of their findings with ours is difficult due to differences in statistical methods and adjustments, the potentially stronger effect of abdominal obesity compared to general obesity is consistent with our results.

Other prospective studies

The objectives of the studies mentioned above were to investigate the determinants of the progression of atherosclerosis (133, 174, 176) or the association between other main exposures than obesity and atherosclerosis (175). When restricted to studies directly investigating the association between obesity and atherosclerosis, there is some evidence that IMT in adulthood may partly reflect childhood obesity (141, 143, 182-185). However, the question of whether or not the development or progression of carotid plaque in adult life is related to obesity in early life has not been investigated.

General and abdominal obesity

Most previous studies have used BMI to assess obesity. However, it does not provide information about the fat distribution. Abdominal obesity, reflecting excess visceral adipose tissue, has a stronger association with insulin resistance and dyslipidemia than general obesity (177). Regarding whether abdominal obesity is more strongly associated with CVD than general obesity, evidence from observational studies is inconsistent. The Emerging Risk Factors Collaboration analysed 221,934 individuals from 58 cohorts and found that BMI, WC, and WHR were all associated with CVD risk, and the authors concluded that their effect sizes were similar (63).

On the other hand, some studies suggest a stronger effect of abdominal obesity than general obesity. The INTERHEART Study, a large multicentre case-control study with 12,461 myocardial infarction cases, suggested that increased WHR was more strongly associated with the occurrence of myocardial infarction than BMI (115). Further, the recent INTERSTROKE Study showed a stronger association of WHR than BMI with stroke (116).

Recently, two large Mendelian randomisation studies have shed light on the effect of abdominal obesity on CVD (124, 125). Dale et al. analysed data from 14 prospective studies with 66,842 coronary heart disease cases and 12,389 ischemic stroke cases, and compared associations of genetic risk scores for BMI and WHR adjusted for BMI with various cardio-metabolic risks. The results showed that WHR might have a stronger effect on coronary heart disease and stroke than BMI. In particular, only WHR was associated with increased risk of ischemic stroke (125). Another study with 111,986 participants from UK Biobank showed that a genetic disposition to higher WHR adjusted for BMI was associated with type 2 diabetes and coronary heart disease, supporting causal relationships (124). These findings emphasise the important role of abdominal obesity. Using BMI may lead to underestimation of the true risk of obesity for CVD.

TPA and the number of plaques

In the present study, associations were more statistically significant in the analyses using TPA as an outcome than those using the number of plaques. It is expected that continuous plaque variables such as TPA and total plaque volume (TPV) can capture the small change in plaque over time more easily than a simple categorical plaque variable, requiring smaller sample sizes and potentially shorter follow-up time to detect significant changes. Further, it has been suggested that TPA is likely to be more sensitive to the progression of atherosclerosis than the more commonly studied outcome of IMT because plaque grows along the axis of the artery 2.4 times faster than it changes in thickness (44). One study with 349 atherosclerotic patients compared the predictive ability of future CVD events among TPV, TPA, and IMT after 5-year follow-up (186). Progression of TPV was significantly associated with CVD events after the adjustment for traditional CVD risk factors. Although the predictive ability of TPA was inferior to TPV, TPA performed better than IMT. While TPV is more sensitive to the progression with its three-dimensional information, TPA would be sensitive enough to detect the progression of plaque burden within a reasonable timeframe, which makes TPA an attractive outcome in large population-based studies.

The formation of new plaque among participants without plaque at baseline

New plaque formation was not associated with any adiposity measures. Considering that our sample were in late adulthood, having no plaque at baseline might mean that participants are to some extent resistant to atherosclerotic changes due to genetic or other reasons. This might

contribute to the slower progression of carotid atherosclerosis and make it difficult to detect changes in this population. Another potential explanation is lack of power: by restricting the analysis to participants without plaque at baseline, the sample size was almost halved. Besides, like the number of plaques variable, binary plaque variable provides less statistical information on the progression of plaque burden than a continuous plaque variable capturing size such as TPA.

The contribution of mediators:

All effect sizes of observed associations between obesity and the progression of plaque burden in the main analysis were substantially reduced after the adjustment for cardio-metabolic risk factors in model 3, and no associations remained significant. This finding is supported by previous studies where strong determinants of the progression of carotid plaque burden included systolic blood pressure and total cholesterol (133, 174). Our finding validates that the pathway from obesity to carotid atherosclerosis is at least in part through cardio-metabolic risk factors.

Recent studies with CVD mortality as an outcome also showed the risk of obesity for atherosclerotic CVD is largely or fully mediated by these cardio-metabolic risk factors (128, 187). These findings suggest that the strict control of cardio-metabolic risk factors might in part attenuate the risk of obesity on the progression of atherosclerosis. Interventions to bring long-term and sustained weight loss through lifestyle change have not been uniformly successful (31, 188). On the other hand, the effective treatment of cardio-metabolic risk factors is established, the strict control of which might reduce the indirect effect of obesity on atherosclerosis. Nevertheless, the treatment of obesity is vital to block any direct pathway to the progression of atherosclerosis and to control cardio-metabolic risk factors better (76). Because our main objective was to investigate the association between obesity and plaque burden with and without the adjustment for mediators as a whole, this did not require partitioning of the contribution of a specific mediator to the overall effect.

Study strengths and limitations

Our major strength is a large sample size with a relatively long follow-up period, which allowed us a reasonable estimation of the association between the progression of carotid atherosclerosis and obesity. Further, theTromsø Study is one of the few prospective studies

with repeated carotid plaque measurements. The use of the quantitative plaque variable TPA is another advantage.

Our main limitation is a loss to follow-up. This might reduce the power of the study to detect associations between adiposity and the progression of carotid plaque burden. Further, selection bias may be introduced. Thus, the association should be interpreted with caution, as should generalisability to the whole population. Another limitation is the possibility of residual confounding. For example, we did not include the statin use in the model because the statin use was not common at the baseline.

Furthermore, we did not consider subsequent changes in CVD risk factors and medications. Moreover, the abdominal obesity index that we used is a crude measure of abdominal adipose tissue. However, it is easily available in a real-world clinical setting. Further study using the reliable measurement of visceral adipose tissue would be informative. Finally, only the right carotid artery was assessed.

Conclusion

Abdominal obesity, especially WHtR and WHR, seems to be more strongly associated with the progression of carotid atherosclerosis than general obesity. These associations are largely mediated by established cardio-metabolic risk factors.

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The authors declared no conflict of interest

Table1. Participant characteristics at baseline (Tromsø Study the 4th survey: analysis restricted to participants with all covariates and outcomes at both 4th and 5th survey: n=4345)

Variable	Total (4345)	Men (2114)	Women (2231)
Age (years) median(IQR)	60 (55-66)	60 (55-65)	61 (56-67)
Female (%)	2231 (51.4)		
Anthropometric measures			
Height (cm)	168.5 (9.4)	175.6 (6.7)	161.8 (6.2)
Weight (kg)	74.0 (13.0)	80.6 (11.5)	67.8 (11.2)
BMI (kg/m^2)	26.0 (3.7)	26.1 (3.2)	25.9 (4.2)
WC (cm)	89.7 (11.0)	95.0 (9.0)	84.7 (10.5)
WHR	0.87 (0.08)	0.92 (0.06)	0.82 (0.06)
WHtR	0.53 (0.06)	0.54 (0.05)	0.52 (0.07)
Categorical obesity$_a$			
Normal (BMI<25 kg/m^2) (%)	1836 (42.3)	803 (38.0)	1033 (46.3)
Overweight (25<=BMI<30 kg/m^2)(%)	1930 (44.4)	1087 (51.4)	843 (37.8)
Obesity (BMI>=30 kg/m^2) (%)	579 (13.3)	224 (10.6)	355 (15.9)
Abdominal obesity (WC: m>102 cm, w>88 cm) (%)	1226 (28.2)	449 (21.2)	777 (34.8)
Abdominal obesity (WHR (%) m>0.9 f>0.85)	1850 (42.6)	1245 (58.9)	605 (27.1)
Potential confounders			
Smoker (%)	1290 (29.7)	657 (31.1)	633 (28.4)
Physical activity (h/week) $_b$			
None	2666 (61.4)	1077 (51.0)	1589 (71.2)
Less than 1	673 (15.5)	381 (18.0)	292 (13.1)
1-2	670 (15.4)	421 (19.9)	249 (11.2)
3 or more	336 (7.7)	235 (11.1)	101 (4.5)
Education (%)			
Primary education	2229 (51.3)	910 (43.1)	1319 (59.1)
Vocational/high school	1328 (30.6)	738 (34.9)	590 (26.5)
University/college	788 (18.1)	466 (22.0)	322 (14.4)
Potential mediators			
Systolic blood pressure (mmHg)	143.4 (21.5)	143.6 (19.4)	143.2 (23.2)
Total cholesterol (mmol/l)	6.69 (1.27)	6.51 (1.18)	6.85 (1.32)
Triglycerides (mmol/l)	1.51 (0.88)	1.62 (0.96)	1.41 (0.79)
HDL cholesterol (mmol/l)	1.52 (0.44)	1.37 (0.39)	1.66 (0.44)
LDL cholesterol (mmol/l)	4.48 (1.17)	4.40 (1.09)	4.55 (1.22)
HbA1c (%)	5.44 (0.63)	5.42 (0.57)	5.47 (0.68)
Medical history/medication			
Myocardial infarction (%)	197 (4.5)	156 (7.4)	41 (1.8)
Angina pectoris (%)	335 (7.7)	198 (9.4)	137 (6.1)
Stroke (%)	83 (1.9)	42 (2.0)	41 (1.8)
Diabetes (%)	96 (2.2)	46 (2.2)	50 (2.2)
Lipid lowering drug use (%)	114 (2.6)	64 (3.0)	50 (2.2)
Blood pressure lowering drug use (%)	515 (11.9)	248 (11.7)	267 (12.0)
TPA at T4 (mm^2)			

median (IQR)			
TPA Q1	0-0	0-0	0-0
TPA Q2	0-0	0-4.0	0-0
TPA Q3	0-12.9	4.0-16.2	0-10.4
TPA Q4	12.9-135.1	16.2-135.1	10.4-121.2
Number of plaques at T4 (%)			
0	2337 (53.8)	1027 (48.6)	1310 (58.7)
1	1268 (29.2)	655 (31.0)	613 (27.5)
2	534 (12.3)	295 (14.0)	239 (10.7)
3	147 (3.4)	95 (4.5)	52 (2.3)
4	49 (1.1)	35 (1.7)	14 (0.6)
5	9 (0.2)	7 (0.3)	2 (0.1)
6	1 (0.02)		1 (0.04)

BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HbA1c: glycated haemoglobin, TPA: total plaque area

[a]Increased waist circumference (>102 cm for men, >88 cm for women) and increased WHR (>=0.9 for men, >=0.85 for women) are categorized according to WHO cut-off points (180). [b]Hours spent on hard physical activity (sweating/out of breath) in leisure time.

Table2. Carotid plaque at the 5th survey (2001) according to adiposity category at the baseline 4th survey (1994-95) (n=4345)

	Total population	BMI categories[a]			WC categories[a]		WHR categories[a]	
Carotid variable	n/N (%)	Normal n/N (%)	Overweight n/N (%)	Obesity n/N (%)	Abdominal obesity No n/N (%)	Abdominal obesity Yes n/N (%)	Abdominal obesity No n/N (%)	Abdominal obesity Yes n/N (%)
At least one plaque at T5	2704/4345 (62.2)	1078/1836 (58.7)	1250/1930 (64.8)	376/579 (64.9)	1901/3119 (61.0)	803/1226 (65.5)	1414/2495 (56.7)	1290/1850 (69.7)
New plaques at T5[b]	938/2337 (40.1)	374/1021 (36.6)	450/1028 (43.8)	114/288 (39.6)	660/1703 (38.8)	278/634 (43.9)	521/1454 (35.8)	417/883 (47.2)
Number of plaques at T5								
0	1641 (37.8)	758 (41.3)	680 (35.2)	203 (35.1)	1218 (39.1)	423 (34.5)	1081 (43.3)	560 (30.3)
1	1230 (28.3)	478 (26.0)	578 (30.0)	174 (30.1)	861 (27.6)	369 (30.1)	675 (27.1)	555 (30.0)
2	869 (20.0)	361 (19.7)	378 (19.6)	130 (22.5)	614 (19.7)	255 (20.8)	463 (18.6)	406 (22.0)
+3	605 (13.9)	239 (13.0)	294 (15.2)	72 (12.4)	426 (13.7)	179 (14.6)	276 (11.1)	329 (17.8)
Change in number of plaques from T4 to T5								
Increased	442 (10.2)	181 (9.9)	198 (10.3)	63 (10.9)	304 (9.8)	138 (11.3)	243 (9.7)	199 (10.8)
No change	2191 (50.4)	960 (52.3)	943 (48.9)	288 (49.7)	1612 (51.7)	579 (47.2)	1349 (54.1)	842 (45.5)
Decreased	1712 (39.4)	695 (37.9)	789 (40.9)	228 (39.4)	1203 (38.6)	509 (41.5)	903 (36.2)	809 (43.7)
	Median (IQR)							
TPA at T5 (mm^2)	8.9 (1-22.6)	7.5 (0-20.7)	9.7 (0-24.6)	10.9 (0-24.3)	8.3 (0-21.7)	10.4 (0-24.4)	6.6 (0-18.8)	12.5 (0-28.8)
Change in TPA from T4 to T5 (mm^2)	0 (0-12.1)	0 (0-10.6)	1.6 (0-13.1)	0.7 (0-12.1)	0 (0-11.5)	1.7 (0-13.5)	0 (0-9.3)	3.7 (0-15.2)

Data are count and percentage for binary plaque and median and IQR for number of plaques and TPA. BMI: body mass index, T4: Tromsø Study the fourth survey, T5: Tromsø Study the fifth survey, TPA: total plaque area

WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, TPA: total plaque area

[a]BMI categories: normal <25.0 kg/m^2, overweight 25.0-29.9 kg/m^2, obesity >30 kg/m^2 , abdominal obesity WC: >102 cm for men, >88 cm for women, WHR: >=0.9 for men, >=0.85 for women (categorized according to WHO cut-off points, [b] New plaques at T5: The number of participants who developed at least one plaque among participants without plaque at T4

Table3. Odds ratios of having plaque at the 5th survey (2001) among participants without plaque at the baseline 4th survey (1994-95), by adiposity at the 4th survey (incident plaque when plaque is absent at the 4th survey) (n=2337)

	Model 1 OR (95%CI)	p-value	Model 2 OR (95%CI)	p-value	Model 3 OR (95%CI)	p-value
BMI (per 1 SD)	0.98 (0.89, 1.08)	0.75	1.03 (0.93, 1.13)	0.60	0.94 (0.85, 1.05)	0.29
WC (per 1 SD)	1.01 (0.92, 1.11)	0.87	1.04 (0.94, 1.15)	0.44	0.96 (0.86, 1.07)	0.49
WHR (per 1 SD)	1.06 (0.96, 1.16)	0.24	1.06 (0.97, 1.17)	0.20	1.01 (0.92, 1.12)	0.77
WHtR (per 1 SD)	1.04 (0.94, 1.14)	0.49	1.07 (0.96, 1.18)	0.21	0.98 (0.87, 1.09)	0.68

BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio, 95% CI: 95% confidence interval, SD: standard deviation, Model 1: adjusted for age and sex, Model 2: adjust for variables in Model 1 plus other confounders (smoking, physical activity and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated hemoglobin, diabetes, lipid and blood pressure lowering drug)

Table 4. The association between the change in the number of plaques between the baseline 4th survey (1994-95) and the 5th survey (2001) and a 1 SD increase in baseline adiposity (n=4345)

	Model 1 β (95%CI)	p-value	Model 2 β (95%CI)	p-value	Model 3 β (95%CI)	p-value
BMI (per 1 SD)	-0.016 (-0.047, 0.015)	0.32	0.004 (-0.028, 0.035)	0.82	-0.011 (-0.046, 0.023)	0.51
WC (per 1SD)	0.001 (-0.030, 0.032)	0.97	0.014 (-0.017, 0.045)	0.38	0.002 (-0.031, 0.036)	0.89
WHR (per 1 SD)	0.029 (-0.002, 0.060)	0.07	0.032 (0.001, 0.064)	0.04	0.028 (-0.005, 0.061)	0.09
WHtR (per 1 SD)	0.010 (-0.021, 0.042)	0.52	0.024 (-0.008, 0.056)	0.14	0.013 (-0.021, 0.048)	0.45

BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio, 95% CI: 95% confidence interval, SD: standard deviation, Model 1: adjusted for age and sex, Model 2: adjust for variables in Model 1 plus other confounders (smoking, physical activity and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, non-HDL cholesterol, glycated hemoglobin, diabetes, lipid and blood pressure lowering drug)

Table 5. The association between change in total plaque area (mm^2) between the baseline 4th survey (1994-95) and the 5th survey (2001) and a 1 SD increase in baseline adiposity (n=4302)

	Model 1 β (95%CI)	p-value	Model 2 β (95%CI)	p-value	Model 3 β (95%CI)	p-value
BMI (per 1 SD)	0.184 (-0.298, 0.666)	0.45	0.412 (-0.077, 0.902)	0.10	0.005 (-0.525, 0.534)	0.99
WC (per 1 SD)	0.468 (-0.014, 0.949)	0.06	0.608 (0.122, 1.094)	0.01	0.261 (-0.260, 0.782)	0.33
WHR (per 1 SD)	0.698 (0.211, 1.185)	0.005	0.697 (0.209, 1.186)	0.005	0.471 (-0.038, 0.980)	0.07
WHtR (per 1 SD)	0.614 (0.122, 1.106)	0.01	0.750 (0.252, 1.249)	0.003	0.397 (-0.138, 0.932)	0.15

BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio, 95% CI: 95% confidence interval, SD: standard deviation, Model 1: adjusted for age and sex, Model 2: adjust for variables in Model 1 plus other confounders (smoking, physical activity and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, non-HDL cholesterol, glycated hemoglobin, diabetes, lipid and blood pressure lowering drug)

Table S1: Participant characteristics at the baseline 4th survey (1994-95) in those scanned at the 5th survey (2001) vs those not scanned at the 5th survey

Continuous variable at the 4th survey	The 5th survey carotid scanned	The 5th survey carotid missing	Adjusted for age, sex β (95%CI)	p-value
Number	4829	1748		
Age (median) (year)	60 (55-66)	65 (58-70)	-0.49 (-0.59, -0.39)	<0.001
Height (cm)	168.4 (9.4)	167.9 (9.6)	0.54 (0.19, 0.89)	0.003
Weight (kg)	74.0 (13.1)	73.6 (14.5)	0.41 (-0.25, 1.06)	0.23
BMI (kg/m^2)	26.0 (3.8)	26.0 (4.5)	-0.02 (-0.24, 0.19)	0.83
WC (cm)	89.8 (11.1)	91.1 (12.3)	-0.56 (-1.12, -0.01)	<0.05
WHR	0.87 (0.08)	0.88 (0.09)	-0.008 (-0.012, -0.005)	<0.001
WHtR	0.53 (0.06)	0.54 (0.07)	-0.005 (-0.009, -0.002)	0.002
SBP (mmHg)	143.7 (21.6)	149.5 (24.5)	-2.79 (-3.93, -1.65)	<0.001
Total cholesterol (mmol/l)	6.70 (1.27)	6.72 (1.38)	-0.03 (-0.09, 0.04)	0.43
Triglycerides (mmol/l)	1.52 (0.89)	1.65 (1.06)	-0.13 (-0.18, -0.07)	<0.001
HDL cholesterol (mmol/l)	1.52 (0.44)	1.49 (0.46)	0.03 (0.00, 0.05)	0.03
LDL cholesterol (mmol/l)	4.48 (1.17)	4.49 (1.27)	0.00 (-0.06, 0.06)	0.98
HbA1c (%)	5.4 (0.6)	5.6 (0.8)	-0.08 (-0.12, -0.04)	<0.001
TPA (median)	0 (0-13.1)	7.9 (0-22.9)	-4.01 (-4.93, -3.09)	<0.001
Categorical variable at the 4th survey				
Female (%)	2490 (51.6%)	829 (47.4%)	0.79 (0.71, 0.89)	<0.001
Plaque (1+ vs 0)	2229 (46.2%)	1031 (59.0%)	0.69 (0.61, 0.77)	<0.001
Current Smokers (vs non-smokers)	1456 (30.2%)	703 (40.2%)	0.57 (0.51, 0.64)	<0.001
Medical history				
Myocardial infarction	220 (4.6%)	187 (10.8%)	0.48 (0.39, 0.60)	<0.001
Angina pectoris	377 (7.8%)	237 (13.6%)	0.68 (0.57, 0.82)	<0.001
Stroke	100 (2.1%)	81 (4.7%)	0.56 (0.41, 0.76)	<0.001
Diabetes	114 (2.4%)	95 (5.5%)	0.52 (0.39, 0.69)	<0.001

BMI: body mass index, CI: confidence interval, HbA1c: glycated haemoglobin, HDL: high-density lipoprotein, LDL: low-density lipoprotein, OR: odds ratio, SBP: systolic blood pressure, TPA: total plaque area, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio,

Table S2: Participant characteristics at the baseline 4th survey (1994-95): scanned at the 6th survey (2007-08) vs those who scanned at the 6th survey

Variable at the 4th survey	The 6th survey carotid scanned	The 6th survey carotid missing	Adjusted for age, sex β (95%CI)	p-value
Number	2974	3714		
Age (median) (year)	58 (53-63)	65 (59-70)	-1.30 (-1.39, -1.21)	<0.001
Height (cm)	168.9 (9.3)	167.7 (9.6)	0.48 (0.14, 0.82)	0.005
Weight (kg)	74.1 (12.7)	73.7 (14.1)	0.05 (-0.58, 0.67)	0.89
BMI (kg/m^2)	25.9 (3.5)	26.2 (4.3)	-0.13 (-0.34, 0.08)	0.22
WC (cm)	89.0 (10.7)	91.1 (12.0)	-0.97 (-1.50, -0.44)	<0.001
WHR	0.86 (0.08)	0.88 (0.08)	-0.009 (-0.013, -0.006)	<0.001
WHtR	0.53 (0.06)	0.54 (0.07)	-0.007 (-0.011, -0.004)	<0.001
SBP (mmHg)	139.6 (20.0)	149.6 (23.4)	-4.23 (-5.32, -3.13)	<0.001
Total cholesterol (mmol/l)	6.58 (1.24)	6.81 (1.33)	-0.10 (-0.16, -0.03)	0.003
Triglycerides (mmol/l)	1.49 (0.86)	1.61 (1.00)	-0.11 (-0.16, -0.06)	<0.001
HDL cholesterol (mmol/l)	1.51 (0.43)	1.51 (0.45)	0.02 (-0.01, 0.04)	0.14
LDL cholesterol (mmol/l)	4.39 (1.15)	4.56 (1.22)	-0.07 (-0.13, -0.01)	0.02
HbA1c (%)	5.4 (0.4)	5.6 (0.8)	-0.10 (-0.14, -0.06)	<0.001
TPA (median)	0 (0-9.6)	7.2 (0-20.5)	-3.62 (-4.50, -2.73)	<0.001
Categorical variable at the 4th survey				
Female (%)	1532 (51.5%)	1849 (49.8%)	0.80 (0.72, 0.89)	<0.001
Plaque (1+ vs 0)	1149 (38.6%)	2172 (58.5%)	0.67 (0.60, 0.75)	<0.001
Smokers	833 (28.0%)	1361 (36.7%)	0.48 (0.43, 0.54)	<0.001
Medical history				
Myocardial infarction	99 (3.3%)	315 (8.5%)	0.54 (0.42, 0.69)	<0.001
Angina pectris	153 (5.2%)	470 (12.7%)	0.59 (0.48, 0.72)	<0.001
Stroke	30 (1.0%)	153 (4.1%)	0.34 (0.22, 0.51)	<0.001
Diabetes	43 (1.5%)	170 (4.6%)	0.45 (0.31, 0.65)	<0.001

BMI: body mass index, CI: confidence interval, HbA1c: glycated haemoglobin, HDL: high-density lipoprotein, LDL: low-density lipoprotein, OR: odds ratio, SBP: systolic blood pressure, TPA: total plaque area, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio

Table S3. Odds ratios of having plaque at the 6th survey (2007-08) among participants without plaque at the baseline 4th survey (1994-95), by adiposity at the 4th survey (incident plaque when plaque is absent at the 4th survey) (n=1639)

	Model 1 OR (95%CI)	p-value	Model 2 OR (95%CI)	p-value	Model 3 OR (95%CI)	p-value
st BMI	1.05 (0.93, 1.18)	0.43	1.09 (0.96, 1.23)	0.18	0.99 (0.86, 1.13)	0.88
st WC	1.06 (0.94, 1.20)	0.31	1.08 (0.96, 1.22)	0.19	1.00 (0.88, 1.15)	0.95
st WHR	1.08 (0.97, 1.21)	0.17	1.08 (0.96, 1.21)	0.18	1.03 (0.91, 1.16)	0.67
st WHtR	1.10 (0.97, 1.24)	0.13	1.12 (0.99, 1.27)	0.08	1.02 (0.89, 1.17)	0.82

BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio, 95% CI: 95% confidence interval, SD: standard deviation, Model 1: adjusted for age and sex, Model 2: adjusted for variables in Model 1 plus other confounders (smoking, physical activity and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, non-HDL cholesterol, glycated hemoglobin, diabetes, lipid and blood pressure lowering drugs)

Table S4. The association between the change in the number of plaques between the baseline 4th survey (1994-95) and the 6th survey (2007-08)and a 1 SD increase in baseline adiposity (n=2685)

	Model 1 β (95%CI)	p-value	Model 2 β (95%CI)	p-value	Model 3 β (95%CI)	p-value
st BMI	0.005 (-0.036, 0.047)	0.81	0.019 (-0.023, 0.061)	0.39	0.014 (-0.031, 0.059)	0.55
st WC	0.004 (-0.038, 0.045)	0.86	0.012 (-0.030, 0.054)	0.59	0.008 (-0.037, 0.053)	0.74
st WHR	0.008 (-0.032, 0.048)	0.70	0.009 (-0.032, 0.049)	0.67	0.007 (-0.036, 0.049)	0.76
st WHtR	0.012 (-0.030, 0.055)	0.58	0.020 (-0.023, 0.063)	0.37	0.016 (-0.031, 0.062)	0.51

BMI:body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio, 95% CI: 95% confidence interval, SD: standard deviation, Model 1: adjusted for age and sex, Model 2: adjusted for variables in Model 1 plus other confounders (smoking, physical activity and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, non-HDL cholesterol, glycated hemoglobin, diabetes, lipid and blood pressure lowering drugs)

Table S5. The association between change in total plaque area (mm^2) between the baseline 4th survey (1994-95) and the 6th survey (2007-08) and a 1 SD increase in baseline adiposity (n=1515)

In the 6th survey, participants without plaque are categorised as TPA missing data. Consequently, this analysis includes a smaller number of participants than that using change in the number of plaques between the 4th survey and the 6th survey

	Model 1 β (95%CI)	p-value	Model 2 β (95%CI)	p-value	Model 3 β (95%CI)	p-value
st BMI	-1.009 (-2.172, 0.155)	0.09	-0.694 (-1.877, 0.489)	0.25	-0.971 (-2.233, 0.290)	0.13
st WC	-0.479 (-1.662, 0.704)	0.43	-0.269 (-1.464, 0.926)	0.66	-0.529 (-1.802, 0.743)	0.42
st WHR	-0.168 (-1.278, 0.943)	0.77	-0.122 (-1.235, 0.990)	0.83	-0.294 (-1.448, 0.859)	0.62
st WHtR	-0.801 (-2.010, 0.408)	0.19	-0.593 (-1.819, 0.633)	0.34	-0.895 (-2.193, 0.402)	0.18

BMI:body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio, OR: odds ratio, 95% CI: 95% confidence interval, SD: standard deviation, Model 1: adjusted for age and sex, Model 2: adjusted for variables in Model 1 plus other confounders (smoking, physical activity and education), Model 3: adjusted for variables in Model 2 and mediators (systolic blood pressure, HDL cholesterol, non-HDL cholesterol, glycated hemoglobin, diabetes, lipid and blood pressure lowering drugs)

7.3 Potential further directions of analysis

This paper focused on answering the clinically relevant question of whether adiposity level at baseline predicts the subsequent progression of carotid plaque burden. However, further analysis may focus on answering the slightly different question of whether any change in adiposity during the follow-up period is associated with a change in plaque burden. The use of change in adiposity will address rather an etiological question of whether an increase or a decrease in adiposity level – regardless of the baseline – is associated with the progression of plaque burden. While this is an interesting question, it is not the same as that addressed in this paper. It should also be noted that the interpretation of an association between the changes in two given variables is complex. Specifically, if the two variables are associated with each other at baseline, and if the change in each is associated with the baseline level (for example through regression to the mean), then this will induce an association between the two observed changes that is merely a consequence of these associations rather than representing a true relationship between the respective changes.

All four adiposity scores were approximately normally distributed in men and women in both populations, with means that differed markedly particularly for women, but with similar standard deviations. To make comparisons between the two populations, standard deviation scores were created using the means and the standard deviations in the Norway data.

7.4 Summary

- Findings from this prospective study with 7-year follow-up were generally consistent with findings from the previous cross-sectional analysis.
- There was little evidence of an association of adiposity with the new formation of plaque or a change in the number of plaques.
- There was evidence of an association between change in total plaque area and all adiposity measures, except BMI.
- These associations are largely mediated by cardio-metabolic CVD risk factors.

8 Comparison of adiposity in the Know Your Heart and Tromsø 7 studies

So far, I have investigated the association between carotid plaque and adiposity using data from the Tromsø Study. In the following two chapters, I report on comparisons based on data from two population-based studies: the Russian Know Your Heart Study (KYH) and the Tromsø Study seventh survey (Tromsø 7). I will present the anthropometric measures of adiposity and the prevalence of general and abdominal obesity in each study and compare them to understand the distribution of my main exposure adiposity in the two studies.

First, I describe the methods followed by participant characteristics in KYH and Tromsø 7. Finally, anthropometric measures of adiposity and the prevalence of obesity are presented, and the two studies are compared.

8.1 Method

8.1.1 Study population

Data sources reported in this chapter are KYH and the Tromsø 7 first visit. As described in chapter 4.2.2, Tromsø 7 comprises two stages: all residents over 40 years in Tromsø were invited to a first visit (visit 1) and a random sample, including participants in the previous survey, was invited to a second visit where more extensive clinical examinations including the carotid ultrasound were conducted. For this reason, in this analysis, all visit 1 participants were included, regardless of attendance at the carotid examination, to accurately determine the distribution of obesity in this population.

Tromsø 7 includes participants aged over 40 years while KYH includes participants aged between 39 and 69 years. To make the two studies comparable, participants included in the analysis were restricted to those aged 40-69 years. In total, 4640 and 17649 participants were included in KYH and Tromsø 7, respectively.

8.1.2 Adiposity and other variables

As described in Chapter 5, all the anthropometric measurement methods were comparable across the two studies except for waist circumference (WC): in Tromsø 7, WC was measured at the level of the umbilicus, while it was assessed at the minimal point of the trunk in KYH. Therefore, WC in Tromsø 7 was converted to the minimum WC using the conversion

equation, and waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) in Tromsø 7 were calculated using this converted WC (161). The mean values of the original and converted WC in Tromsø 7, by age group and sex, are presented in Appendix A5.

As described in detail in Chapter 5, three variables potentially related to adiposity and cardiovascular disease (CVD), education, physical activity, and available alcohol variables, were measured differently in the two studies, making comparison challenging. Thus, these data were presented as original variables in each study.

8.1.3 Statistical methods

Adiposity measures in each country

Since there are marked sex differences in the amount of fat and fat distribution, all results are presented according to sex. Anthropometric measures in each study were summarised using mean (SD), and the prevalence of obesity was summarised using numbers and percentages according to sex.

Comparison of anthropometric measures and the prevalence of obesity

To perform a statistical comparison of anthropometric measures of adiposity and the prevalence of obesity in two studies, the pooled data from the KYH dataset and the Tromsø 7 visit 1 dataset were analysed. Two types of outcome were used: four continuous adiposity measures of adiposity (BMI, WC, WHR, WHtR) and three binary variables of obesity using BMI, WC, and WHR cut-off points (BMI>=30 kg/m^2: Yes/No, WC men>102 cm women>88 cm: Yes/No, WHR men>=0.9 women>=0.85: Yes/No), respectively. The binary variable formed using the BMI as a cut-off is a proxy for general obesity, while those using WC and WHR cut-off are indicators of abdominal obesity. The study (KYH or Tromsø 7) was used as the main exposure.

Body size in terms of, for example, height may be positively correlated with the selected adiposity measures. For example, a tall individual may have a larger WC than a short individual even if their adiposity level is similar. If adiposity level is strongly correlated with height, it is possible that the difference in height between the two populations, if any, leads to differences in the selected four adiposity measures between the two studies, even when the adiposity level is similar. Therefore, I examined to what extent height was correlated with each of the adiposity measures using Pearson's correlation coefficient. Furthermore, I

examined correlation coefficients among the four adiposity measures and investigated to what extent general adiposity (BMI) is associated with abdominal adiposity (WC, WHR, WHtR).

The association between each anthropometric measure and study was examined using the separate linear regression models for each sex. The model was adjusted for categorical age (5-year intervals) bearing in mind the possibility that the association is not linear. Interactions between sex and study, and between age and study were investigated by adding interaction terms to models for the full dataset with age, sex, and study as predictor variables. An analogous set of logistic regression models was used to investigate associations between each binary obesity variable and study.

8.2 Results

8.2.1 Participant characteristics in the two studies

Table 8-1 shows participant characteristics of the KHY and Tromsø 7 visit 1 participants. It should be noted that the values in this table are not age-standardised. Caution is needed when comparing the two studies.

KYH participants

In KYH, the mean age was 55.6 years, and 57.1% were women. The largest age group was 60-69 years. There was a big difference in the prevalence of current smoking by sex, with 36.7% of men and 16.0% of women being current smokers. Overall, 35.5% had completed higher education. The alcohol drinking pattern differed between men and women: men were more likely to consume more litres of alcohol per year than women. Regarding cardio-metabolic CVD risk factors, men had higher systolic blood pressure (SBP) than women while women had higher total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol.

Tromsø 7 visit 1 participants

Among Tromsø 7 visit 1 participants aged 40-69 years, the mean age was 53.7 years, and 52.7% were women. The participants were slightly younger than the KYH participants. The largest age group was 40-49 years. Unlike KYH, the prevalence of current smokers was similar between men (14.3%) and women (15.5%). 52.8% had completed higher education,

which was higher than KYH. Men tended to drink alcohol more frequently than women, but drinking patterns were relatively similar between sexes. Regarding cardio-metabolic CVD risk factors, as was seen in KYH, men showed higher SBP than women. However, SBP of both men and women in Tromsø 7 was lower than KYH. In Tromsø 7, HDL cholesterol was higher in women than men, while LDL cholesterol was slightly higher in men.

Table 8-1 Participant characteristics in the two studies (KYH and Tromsø 7 visit 1)

	Know Your Heart Study			**Tromsø Study the seventh survey visit 1**			
Variable	**Total**	**Men**	**Women**	**Variable (if different to KYH)**	**Total**	**Men**	**Women**
	N=4640	**N=1994**	**N=2646**		**N=17649**	**N=8349**	**N=9300**
Age (year)	55.6 (8.7)	55.7 (8.6)	55.4 (8.8)		53.7 (8.4)	53.8 (8.5)	53.6 (8.4)
Categorical age							
40-49	1346 (29.0)	560 (28.1)	786 (29.7)		6433 (36.5)	3055 (36.6)	3378 (36.3)
50-59	1498 (32.3)	635 (31.9)	863 (32.6)		6036 (34.2)	2791 (33.4)	3245 (34.9)
60-69	1796 (38.7)	799 (40.1)	997 (37.7)		5180 (29.4)	2503 (30.0)	2677 (28.8)
Sex (female)	2646 (57.0)				9300 (52.7)		
Smoking							
Never-smoker	2081 (50.3)	443 (25.5)	1638 (68.2)		7416 (42.3)	3654 (44.1)	3762 (40.8)
Ex-smoker	1036 (25.0)	655 (37.7)	381 (15.9)		7488 (42.7)	3447 (41.6)	4041 (43.8)
Current smoker	1023 (24.7)	639 (36.8)	384 (16.0)		2615 (14.9)	1188 (14.3)	1427 (15.5)
Education*				Education*			
Incomplete secondary	186 (4.0)	115 (5.8)	71 (2.7)	Lower than secondary	3312 (19.0)	1627 (19.7)	1685 (18.3)
Complete secondary	507 (10.9)	275 (13.8)	232 (8.8)	Upper secondary	4942 (28.3)	2546 (30.8)	2396 (26.0)
Professional no secondary	173 (3.7)	82 (4.1)	91 (3.4)	Tertiary	9221 (52.8)	4093 (49.5)	5128 (55.7)
Professional & secondary	382 (8.2)	176 (8.8)	206 (7.8)				
Specialised secondary	1620 (34.9)	609 (30.5)	1011 (38.2)				
Incomplete higher	124 (2.7)	69 (3.5)	55 (2.1)				
Higher	1648 (35.5)	668 (33.5)	980 (37.0)				
Physical activity**				Physical activity**			

Inactive	268 (5.9)	102 (5.2)	166 (6.4)	Sedentary	2415 (14.0)	1249 (15.2)	1166 (12.8)
Moderately inactive	523 (11.5)	237 (12.1)	286 (11.0)	Light	10005 (57.8)	4076 (49.6)	5929 (65.2)
Moderately active	2600 (57.0)	1031 (52.6)	1569 (60.4)	Moderate	4285 (24.8)	2529 (30.8)	1756 (19.3)
Active	1168 (25.6)	589 (30.1)	579 (22.3)	Heavy	603 (3.5)	359 (4.4)	244 (2.7)
Alcohol* **				Alcohol***			
Not alcohol drinker	991 (21.4)	357 (18.0)	634 (24.0)	Never	1117 (6.4)	426 (5.1)	691 (7.5)
>0 <2 (little/year)	2214 (47.8)	557 (28.0)	1657 (62,7)	Monthly	4138 (23.5)	1610 (19.4)	2528 (27.3)
2-4 (little/year)	604 (13.1)	391 (19.7)	213 (8.1)	2-4/month	6952 (39.6)	3436 (41.3)	3516 (38.0)
5-9 (little/year)	422 (9.1)	323 (16.3)	99 (3.8)	2-3/week	4385 (25.0)	2288 (27.5)	2097 (22.7)
10-19 (little/year)	249 (5.4)	219 (11.0)	30 (1.1)	4+/week	985 (5.6)	558 (6.7)	427 (4.6)
20+ (little/year)	149 (3.2)	140 (7.1)	9 (0.3)				
Cardio-metabolic risk factors							
Systolic blood pressure (mmHg)	133.7 (20.2)	138.7 (19.8)	130.2 (19.8)		126.9 (18.3)	130.8 (17.1)	123.4 (18.7)
Total cholesterol (mmol/l)	5.6 (1.2)	5.4 (1.1)	5.7 (1.2)		5.5 (1.0)	5.5 (1.0)	5.5 (1.0)
Triglycerides (mmol/l)	1.6 (1.2)	1.7 (1.4)	1.5 (1.1)		1.5 (1.0)	1.8 (1.1)	1.3 (0.8)
HDL cholesterol (mmol/l)	1.4 (0.4)	1.3 (0.3)	1.5 (0.4)		1.6 (0.5)	1.4 (0.4)	1.7 (0.5)
LDL cholesterol (mmol/l)	3.8 (0.9)	3.7 (0.9)	3.8 (1.0)		3.6 (1.0)	3.7 (1.0)	3.6 (1.0)
Medical history/medication							
Myocardial infarction	154 (4.8)	105 (7.8)	49 (2.6)		376 (2.2)	314 (3.9)	62 (0.7)
Angina pectoris	381 (12.0)	182 (13.5)	199 (10.8)		249 (1.4)	180 (2.2)	66 (0.7)
Heart failure	(-)	(-)	(-)		182 (1.1)	129 (1.6)	53 (0.6)
Stroke	113 (3.5)	59 (4.4)	54 (2.9)		309 (1.8)	182 (2.2)	127 (1.4)
Hypertension	2283 (62.2)	928 (60.1)	1355 (63.8)		4141 (23.9)	2149 (26.2)	1992 (21.9)

Diabetes	315 (8.5)	110 (7.0)	205 (9.6)		704 (4.1)	393 (4.8)	311 (3.4)
Anti-hypertensive medication†	1886 (82.7)	738 (79.7)	1148 (84.7)		2951 (16.9)	1601 (19.4)	1350 (14.6)
Lipid-lowering medication					1881 (10.8)	1068 (13.0)	813 (

BMI: body mass index, HDL: high-density lipoprotein, KYH: Know Your Heart Study, LDL: low-density lipoprotein, PA: physical activity, Tromsø 7: Tromsø Study seventh survey, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio

**Physical activity: Physical activity in KYH is based on total physical activity index. Physical activity in Tromsø 7 is based on a simple questionnaire (1. Reading, watching TV/screen or other sedentary activity? 2. Walking, cycling, or other forms of exercise at least 4 hours a week? (including walking or cycling to the place of work, Sunday-walking, etc.), 3. Participation in recreational sports, heavy gardening, snow shovelling etc at least 4 hours a week, 4. Participation in hard training or sports competitions, regularly several times a week?

***Alcohol consumption in KYH is ethanol consumption per year calculated based on questions of frequency and usual volume of beer, wine, and spirits while in Tromsø 7 it is based on the following question (How often do you usually drink alcohol? 1: Never, 2: Monthly or less, 3:2-4 times a month, 4: 2-3 times a week, 5: 4 or more times a week)

† In KYH, the denominator is participants with hypertension while in Tromsø 7 it is all participants

8.2.2 Anthropometric measures and the prevalence of obesity in the two studies

Correlations of anthropometric measures

Table 8-2 below shows the correlation coefficients for the four adiposity measures with each other and with height in participants aged 40-69 years in KYH and Tromsø 7 visit 1. The height showed only weak correlations with all adiposity measures. WHtR showed relatively stronger correlations than other three adiposity measures.

Regarding the correlations for the four adiposity measures with each other, three abdominal adiposity measures (WC, WHR, WHtR) showed strong correlations with each other. While correlations of BMI with WC or WHtR were quite strong, its correlation with WHR was relatively weak.

Table 8-2 Correlations of anthropometric measurement (participants aged between 40-69 years in Know Your Heart and Tromsø 7 visit 1)

	BMI	WC	WHR	WHtR	Height
KYH men					
BMI	1				
WC	0.92	1			
WHR	0.66	0.82	1		
WHtR	0.92	0.95	0.83	1	
Height	0.01	0.15	-0.04	-0.15	1
KYH women					
BMI	1				
WC	0.90	1			
WHR	0.50	0.74	1		
WHtR	0.91	0.97	0.75	1	
Height	-0.17	-0.05	-0.19	-0.29	1
Tromsø 7 men					
BMI	1				
WC	0.88	1			
WHR	0.60	0.79	1		
WHtR	0.88	0.94	0.82	1	
Height	-0.05	0.11	-0.13	-0.23	1
Tromsø 7 women					
BMI	1				
WC	0.88	1			
WHR	0.47	0.74	1		
WHtR	0.88	0.96	0.75	1	
Height	-0.11	0.04	-0.12	-0.23	1

Sex-difference in the study

Table 8-3 shows anthropometric measures, the prevalence of obesity in KYH and Tromsø 7 visit1, and age-adjusted comparison between the two studies. For KYH, anthropometric measures were generally slightly higher in men than women, but BMI was higher in women. The prevalence of general obesity and abdominal obesity using WC cut-off point was higher in women than in men while men had a higher prevalence of abdominal obesity using the WHR cut-off point. Conversely, in Tromsø 7, all anthropometric measures and the prevalence of obesity were higher in men than in women.

Difference between the two studies

In terms of the comparison between the two studies, KYH men were significantly shorter and lighter than Tromsø 7 men. However, the difference in BMI was not statistically significant between the two studies. Regarding abdominal variables, while WC was significantly lower in KYH men than Tromsø 7 men, WHR and WHtR were not significantly different between the two studies. There was no significant difference in the prevalence of general obesity using BMI cut-off or of abdominal obesity using WC and WHR cut-offs in men between KYH and Tromsø 7. Overall, the difference in adiposity level and the prevalence of obesity in men between the two studies was small.

Women showed a contrasting result. KYH women were significantly shorter and heavier than Tromsø 7 women. The general adiposity measure (BMI) and all three abdominal adiposity measures (WC, WHR, WHtR) were significantly higher in KYH women than Tromsø 7 women. The prevalence of general obesity in KYH was considerably higher than in Tromsø 7. The prevalence of abdominal obesity showed a substantial difference: odds ratio for abdominal obesity using the WC cut-off point in KYH compared to Tromsø 7 was 2.8 (95%CI: 2.5-3.0) whereas that using the WHR cut-off point was 3.8 (95%CI: 3.5-4.2).

In summary, the burden of general and abdominal adiposity did not seem materially different between KYH and Tromsø 7 for men whereas the burden of adiposity, especially abdominal adiposity, seemed much higher in KYH women than in Tromsø 7 women. There was strong evidence of interactions between country and sex in all linear regression models using all adiposity measures. Regarding age, there was evidence of interactions between country and age in all linear regression models except for that using WHR. The difference between the studies tended to become larger as age increased.

Table 8-3 Anthropometric measures and the prevalence of obesity in two studies

	KYH men	**Tromsø 7 men**	**Age-adjusted comparison (95%CI) KYH vs T7**	**KYH women**	**Tromsø 7 women**	**Age-adjusted comparison (95%CI) KYH vs T7**
Variable	N=1994	N=8349	Mean difference (95%CI)	N=2646	N=9300	Mean difference (95%CI)
Age	55.7	53.8 (8.5)		55.4 (8.8)	53.6 (8.4)	
Anthropometric measures						
Height (cm)	174.7 (6.7)	178.3 (6.7)	-3.3 (-3.7, -3.0)	161.1 (6.3)	165.0 (6.4)	-3.6 (-3.8, -3.3)
Weight (kg)	84.7 (16.2)	88.9 (14.2)	-3.8 (-4.6, -3.1)	75.1 (16.4)	72.9 (14.0)	2.2 (1.5, 2.8)
BMI (kg/m^2)	27.7 (4.8)	27.9 (4.0)	-0.2 (-0.4, 0.0)	29.0 (6.3)	26.8 (4.9)	2.1 (1.8, 2.3)
WC (cm)	97.2 (12.6)	98.3 (10.8)	-1.5 (-2.0, -0.9)	90.7 (14.3)	82.1 (11.7)	8.1 (7.6, 8.7)
WHR	0.95 (0.07)	0.94 (0.07)	-0.03 (-0.00, 0.01)	0.85 (0.07)	0.79 (0.07)	0.05 (0.05, 0.06)
WHtR	0.56 (0.07)	0.55 (0.06)	0.00 (-0.00, 0.01)	0.56 (0.09)	0.50 (0.07)	0.06 (0.06, 0.07)
Prevalence of obesity						Odds ratio (95%CI)
Obesity (BMI≥30 kg/m^2)*	469 (27.0)	2140 (25.7)	1.08 (0.96, 1.21)	928 (38.7)	2044 (22.0)	2.19 (1.99, 2.41)
Abdominal obesity using WC cut-off**	569 (32.7)	2779 (33.4)	0.92 (0.82, 1.03)	1285 (53.4)	2644 (28.5)	2.75 (2.51, 3.02)
Abdominal obesity using WHR cut-off***	1320 (76.0)	6002 (72.2)	1.09 (0.96, 1.24)	1131 (47.1)	1696 (18.3)	3.81 (3.45, 4.20)

BMI: body mass index, WC: waist circumference, WHtR: waist-to-height ratio, WHR: waist-to-hip ratio
**prevalence of obesity: BMI cut-off >=30 kg/m^2
***prevalence of abdominal obesity: WC cut-off men>102 cm, women>88 cm, WHR cut-off men>=0.9, women>=0.85

8.3 Discussion

There were two main findings in this analysis. First, regarding conventional CVD risk factors, KYH participants showed an unfavourable pattern compared to Tromsø 7 visit 1 participants: men in KYH had a higher smoking prevalence than men in Tromsø 7, and both sexes in KYH had higher SBP. Second, the level of adiposity was only significantly different among women between the two studies: there was no significant difference in the burden of obesity in men between the two studies whereas the burden of both general and abdominal obesity in KYH women was considerably higher than that of Tromsø 7 women.

Participants in KYH were characterised by high smoking prevalence in men and high SBP in both sexes, which generally agrees with existing evidence on the comparison of CVD risk factors between Russia and Western Europe (shown in Chapter 2) (1). However, taking only traditional CVD risk factors into consideration, the KYH population does not seem to be at extremely high-risk for CVD compared to the Tromsø 7 population.

The higher prevalence of general obesity in KYH women is also consistent with previous findings. According to the CVD statistics report from the European Society of Cardiology, the prevalence of general obesity in Russian men was similar to or lower than that of Western European counterparts, while Russian women had a higher prevalence of general obesity than Western European women (20). Central and Eastern European countries are more likely to show higher obesity prevalence than Western European countries for both men and women (189). However, the obesity level in Russian women might be more problematic than other Central European countries. One large-scale population study, the HAPIEE Study, compared the prevalence of general obesity in Russia, Poland, and the Czech Republic (190). The prevalence of general obesity was highest in Russian women while Russian men had the lowest prevalence, with Russia showing the largest gender difference among the three countries. The authors suggested that this large gender difference might be attributable to the low Gross National Product in Russia.

Evidence on the prevalence of abdominal obesity in the general population is limited compared to the prevalence of general obesity. Only two population studies in Russia provide information on the prevalence of abdominal obesity in a general population sample; these used a different measurement site of WC (65, 191). The SAGE first wave provided prevalence estimates of abdominal obesity using the WHR cut-off among the general

population aged 50+ (men: 50-59 years 69%, 60-69 years 69.2%, women: 50-59 years 48.8%, 60-69-years 65.7%) (65). WC was assessed at the superior border of the iliac crest (192). A more recent Epidemiological Survey of cardiovascular diseases in different regions of the Russian Federation (ESSE-RF) ascertained the prevalence of abdominal obesity using the WC cut-off among 21,768 participants among aged 25-64 years. The prevalence was 24.3% in men and 38.4% in women, which were lower than in KYH(191).

More and more attention has been paid to abdominal obesity due to its stronger association with cardio-metabolic CVD risk factors. However, the protocol for WC measurement has not been standardised. One literature review reports 14 different measurement sites in previous publications (160). This difference in measurement site affects the WC value. In particular, the prevalence of abdominal obesity is affected by measurement site (160, 193). In the SAGE study mentioned above, although the prevalence of abdominal obesity was relatively similar to that in KYH, the prevalence in women was higher than KYH. This can be explained by the difference in the WC measurement site; the superior border of the iliac crest in SAGE and the minimal point of the trunk in KYH. In the ESSE-RF study, the prevalence of abdominal obesity in comparable age groups seemed higher than KYH, especially among women. This may also be caused by different WC measurement sites. This emphasises the difficulty in comparing the prevalence of abdominal obesity between studies and the importance of standardised WC measurement. Nevertheless, bearing in mind the comparison with the two other Russian studies, the high burden of abdominal obesity in women observed in KYH is unlikely to be overestimated. Although more data are available on abdominal obesity in Western Europe, unstandardized measurement protocols make it difficult to accumulate evidence to lead to a conclusion.

Another concern when comparing the adiposity level between different populations relates to the residual influence of height. The KYH participants were significantly shorter than the Tromsø 7 participants. Here, the concern is that the difference in adiposity level between the two studies is partially explained by the difference in height and does not reflect a true difference in adiposity. However, the height was only weakly correlated with the four selected adiposity measures in this study, although the correlation with WHtR did appear to be slightly stronger than the other three measures. This relatively strong correlation between WHtR and height is consistent with the previous study (194). When comparing two

populations with different heights, WHtR may be more susceptible to the residual effect of height.

I used three simple adiposity measures (WC, WHR, WHtR) as a proxy of abdominal adiposity. As is shown in Table 8-2, while WC and WHtR showed strong correlations with BMI, WHR was relatively weakly associated with BMI. When BMI is combined with abdominal adiposity measures in adiposity studies, WHR may be a good option for complementary information. In addition, WHR may be a more important measure in terms of the association with CVD burden. In my previous analysis in Chapter 6, WHR appeared to be more strongly associated with carotid plaque burden than the other two adiposity measures (WC and WHtR). Meanwhile, as was discussed in Chapter 2, it is suggested that WHR may be more strongly associated with CVD events than BMI (124, 125). It would thus appear to be reasonable to choose WHR as a proxy of abdominal adiposity when the impact of adiposity on CVD burden is explored.

To the best of my knowledge, this is the first study to compare the prevalence of abdominal obesity between Russia and Western European countries. Data used in my study were collected over the same period in both Tromsø 7 and KYH. Furthermore, the waist measurement in Tromsø 7 was converted appropriately, which allowed me to make a reasonable comparison of abdominal obesity between KYH and Tromsø 7. In our study, it was of concern that the difference in the prevalence of abdominal obesity in women in KYH compared with women in Tromsø 7 was even higher than the difference in general obesity.

9 To what extent does obesity explain differences in carotid burden in studies conducted in Norway and Russia? Comparison of two populations at very different risk of cardiovascular death (Paper 3)

9.1 Introduction

In this chapter, the association of general and abdominal adiposity (body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR)) with carotid plaque burden (the presence of carotid plaques, carotid plaque score) is examined and compared between the Know Your Heart study (KYH) in Russia and the Tromsø Study seventh survey (Tromsø 7) in Norway. I then consider whether the difference in carotid plaque burden between the two studies, if any, can be explained by the burden of adiposity.

9.2 Submitted paper

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Student ID Number	1300239	**Title**	Dr.
First Name(s)	Yume		
Surname/Family Name	Imahori		
Thesis Title	To what extent does obesity explain differences in carotid plaque burden in studies conducted in Norway and Russia? Comparison of two populations at very different risk of cardiovascular death		
Primary Supervisor	David Leon		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where is the work intended to be published?	European Journal of Epidemiology
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	A. Leon
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualized, conducted the analyses, wrote the first draft, coordinated all comments by co-authors, and was primarily responsible for the final draft..

SECTION E

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Date	7/3/2019

To what extent does obesity explain differences in carotid plaque burden in studies conducted in Norway and Russia? Comparison of two populations at very different risk of cardiovascular death

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Conflict of interest

The authors declare that they have no conflicts of interest.

Abstract

Large differences exist between Russia and Norway and other Western European countries in cardiovascular disease (CVD) mortality, the reasons for which are poorly understood. The presence and extent of carotid plaque provides a measure of atherosclerosis. There is evidence from studies of Western populations that obesity is associated with carotid plaque prevalence. We investigated whether this association was observed in a random sample of the general population of two Russian cities (Know Your Heart (KYH) Study N=3262) and compared its strength to that observed in a parallel study in Norway (Tromsø 7 study N=1800). We then investigated whether differences in adiposity between the two populations could explain differences in the burden of carotid plaque. Women in KYH had higher levels of general/abdominal adiposity than those in Tromsø 7. However, levels among men were similar. The associations of the presence of plaques (yes/no) with obesity were similar in the Norwegian and Russian studies with waist-to-hip ratio (WHR) showing the largest effect size (OR per SD in WHR 1.18 (95% CI 1.06, 1.31) for men; 1.15 (1.06, 1.25) for women). Associations were also observed between WHR and number of plaques. The prevalence of carotid plaques was higher in the Russian study than the Norwegian one (age-adjusted OR 3.2 (2.6, 4.0) for men and 1.9 (1.6, 2.3) for women). However, adiposity failed to explain these differences in carotid plaque burden. We conclude that factors other than obesity underlie the large differences in the burden of CVD between Russia and Norway.

Key words: Carotid atherosclerosis, Carotid plaque, Obesity, Russian Federation, Europe, Health inequality

Introduction

The Russian population suffers from a much higher burden of cardiovascular disease (CVD) than Western European countries. In 2015, the CVD mortality rate in the Russian Federation was four times higher than in England and Wales or Norway (2). The differences in premature CVD mortality rates are even larger: in 2012-16, the CVD mortality rate for the Russian population aged 35-69 was eight times higher than that in Norway (2). These premature deaths contribute to low life expectancy in Russia. A major cause of CVD is atherosclerosis, the detection of which in its early stages is useful to stratify CVD risk in the population. Carotid plaque can be non-invasively identified using ultrasound and predicts future CVD events independently of conventional CVD risk factors (14).

Increased obesity prevalence is a public health concern in both Russia and Western Europe. The negative impact of obesity on CVD is well established, although most of the evidence regarding this association is derived from high-income Western countries. Less is known about this association in non-Western countries, such as Russia, which may have a different distribution and structure of confounders (63, 195, 196).

The reason behind the huge gap in the burden of CVD between Russia and Western Europe has not been well understood. Historically it has been contended that differences in conventional CVD risk factors such as smoking, blood pressure, and cholesterol levels do not seem to explain this difference (4, 5). Due to the disproportionally high CVD mortality rate in Russia, the European Society of Cardiology recommends that a different version of the Systematic Coronary Risk Evaluation (SCORE) for Western Europe be used for Russia and other central European countries to estimate CVD risk more appropriately (12, 197). To the best of our knowledge, the contribution of obesity to this gap in CVD burden has not been specifically reported.

While some studies of the health impact of obesity in Russia have been conducted, most are restricted to using body mass index (BMI) which is a crude adiposity measure with important limitations. Abdominal obesity may be a more important risk factor with its stronger association with cardio-metabolic CVD risk factors. Understanding the distribution of both general and abdominal obesity, and quantifying their associations with atherosclerosis could strengthen the evidence base for improving strategies to reduce the burden of CVD in Russia.

The aim of this study was to compare the association between general and abdominal obesity assessed by four simple anthropometric measures (BMI, waist circumference (WC), waist-to-

hip ratio (WHR), waist-to-height ratio (WHtR)) and ultrasound-assessed carotid plaque measures (the presence of plaques and plaque score) between Russia and Norway ,which is one of CVD low-risk European countries.

Methods

This study compares two population-based cross-sectional studies: the Know Your Heart Study (KYH) conducted in two cities in Russia and the seventh survey of the Tromsø Study (Tromsø 7) from Tromsø, Norway. The design of the two studies is comparable in terms of the main examinations.

Study design and participants

KYH is a population-based cross-sectional study of 4500 women and men aged 35-69 years conducted between 2015 and 2017 in two Russian cities: Novosibirsk and Arkhangelsk. The details of KYH have been described elsewhere (1). Briefly, participants were recruited from a random sample stratified by age and gender, derived from the list of the Territorial Health Insurance Funds., and trained interviewers visiting the addresses on the list identified residents with appropriate age and sex, and collected information on CVD risk factors and medical history. Participants were then invited to a comprehensive examination, including anthropometric measurement, blood sampling, and carotid ultrasound. Of potential participants initially approached, 68% and 41% in Arkhangelsk and Novosibirsk, respectively, agreed to be interviewed; of these, 89% attended the medical examination.

Ethical approval for the study was obtained from the ethics committees of the London School of Hygiene & Tropical Medicine, the Novosibirsk State Medical University, the Institute of Internal and Preventative Medicine - Branch of Institute of Cytology and Genetics, the Siberian Branch of RAS (Novosibirsk), and the Northern State Medical University (Arkhangelsk).

Data from participants in Tromsø 7 are used in this analysis. The Tromsø Study is an ongoing population-based study in Tromsø municipality, North Norway, and consists of seven surveys from 1974-2016 (Tromsø 1-7) (2). All residents in Tromsø aged 40 years and older were invited to participate in the study. A total of 21083 (response rate 65%) attended the first visit during which questionnaires were applied, a physical examination was carried out, and biological samples were taken. A random sample including previous participants were invited

to a second visit to undergo more comprehensive examinations. Of this sample, 4153 participants were invited to a carotid ultrasound examination, and 2974 (71.6%) attended.

Ethical approval was obtained from the Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics.

Participants aged between 40-69 years (n=5782) were eligible for the present study. We excluded participants with missing data on all adiposity measures (n=42), and potential confounders and mediators (n=678), leaving 3262 participants from KYH (57% women) and 1800 from Tromsø 7 (55% women) for the analyses.

Assessment of anthropometric measures and other CVD risk factors

In both studies, anthropometric measures were assessed by trained staff using standard methods. Height and weight were measured without shoes in light clothing. Height was measured to the nearest millimetre using a Seca® 217 portable stadiometer (Seca limited) in KYH and an electronic stadiometer (DS-103, Dongsahn JENIX Co. Ltd) in Tromsø 7. Weight was measured to the nearest 100 g with a TANITA BC 418 body composition analyser (TANITA, Europe GmbH) in KYH and an electronic digital scale (DS-B02, Dongsahn JENIX Co.Ltd) in Tromsø 7. Body mass index (BMI) was calculated by dividing weight in kilograms by squared height in meters. Waist circumference (WC) was measured at a different site in the two studies: in KYH, WC was measured at the narrowest part of the trunk to the nearest millimetre using a tape measure, while in Tromsø 7 WC was measured at the level of the umbilicus. Hip circumference was measured at the widest part in both studies. To ensure WC was comparable between the two studies, WC in Tromsø 7 was converted to the narrowest waist using a conversion equation (161). Waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated by dividing WC by hip circumference and height, respectively. A previous study has shown excellent intra- and inter-observer reliability of height and weight (164). Recent studies also show excellent intra- and inter-observer reliability for WC and WHR when they were measured by trained staff (161, 165).

We selected potential confounders and mediators a priori, based on evidence of the association between obesity and CVD. Age, smoking, and education were considered as potential confounders while systolic blood pressure (SBP), HDL cholesterol, LDL cholesterol, glycosylated haemoglobin (HbA1c) and medical history of diabetes mellitus (DM) were considered as potential mediators. Information on age (defined as 5-year

categories), smoking (current smoker, ex-smoker, never-smoker), educational attainment (higher education: Yes/No), and medical history of DM (Yes/No) were collected through face-to-face interview in KYH and self-administered questionnaire in Tromsø 7.

Systolic blood pressure (SBP) was measured three times in the sitting position using OMRON 705 IT automatic blood pressure monitors (OMRON Healthcare) in KYH and Dinamap (CARESCAPE V100 Monitor, GE Healthcare) in Tromsø 7. The mean of the second and third measurements was used in the analysis. Non-fasting venous blood samples were obtained. Fresh serum was analysed at the University Hospital laboratories in Tromsø 7. In KYH, serum was processed, frozen within 2 hours, and shipped to Moscow for central analysis (2). In both studies, HDL cholesterol, LDL cholesterol, and HbA1c were determined using comparable standard methods (2).

Ultrasound examination

Both carotid arteries (common carotid artery (CCA), bifurcation, and internal carotid artery (ICA)) were scanned for carotid plaques using Vivid q (GE Health care) with 6~13 MHz linear transducers in KYH and Vivid 7 (GE Health care) with a linear 12 MHz transducer in Tromsø 7. The protocols of the examination procedure were aligned between the two studies. Carotid plaque was defined as a focal structure encroaching into the arterial lumen at least 0.5 mm or 50% of the surrounding intima-media thickness (IMT) value or IMT >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface based on the Mannheim Consensus (41).

In Tromsø 7, plaque detection was determined online by experienced sonographers and documented by digital still images of each plaque. In KYH, a similar procedure was used, supplemented by recording of digital cine loops in transverse and sagittal views. The off-line reading of recorded images was performed with EchoPAC software (GE VINGMED ULTRASOUND Horten Norway) by an experienced cardiologist (AR). In a previous survey of the Tromsø Study, acceptable levels of reproducibility of carotid plaque detection were demonstrated (167).

Assessment of the burden of plaque differed slightly between the two studies. In both studies, plaques were sought in each segment (common carotid artery (CCA), bifurcation, and internal carotid artery (ICA)) of both carotid arteries, a total of six carotid segments). The Tromsø 7 protocol explicitly further divided each segment into the far or near wall of the

artery, giving a total of twelve sites. However, only one plaque could be counted at each site because of concerns about differentiating between a single large plaque and two discrete plaques. In KYH however, the protocol did not involve recording the near or far wall location, and there was no restriction on how many plaques could be recorded for each of the six segments. This protocol difference could lead to an artefactually higher plaque burden in KYH compared to Tromsø 7. To reduce this risk, we created a total plaque score that was more comparable between the studies. This was done using the following rule: when three plaques (the maximum seen) were recorded for any one of the six carotid segments in KYH, this was converted to a score of two.

Because of the lack of information relating to wall location in KYH, we also conducted a sensitivity analysis by using a simpler plaque score which was independent of information about the wall in which the plaque was located. A score of one was assigned for the presence of one or more plaques in each of the six carotid segments (CCA, bifurcation, and ICA of each carotid artery). This resulted in a consistent approach for both studies, with a maximum possible score of six for each individual.

Statistical methods

Two outcome measures were used: the presence of plaques as a binary outcome and plaque score. As exposures, four anthropometric adiposity measures were used: BMI, WC, WHR, and WHtR. To enable direct comparison of the magnitude of the effects of these measures, adiposity z-scores standardised to Tromsø 7 participants were created by subtracting the mean and dividing by the SD of each measure in Tromsø 7.

The association between the presence of plaques and each adiposity measure was examined using a series of logistic regression models. The association between plaque score and each adiposity measure was estimated using an analogous series of linear regression models.

First, separately for each combination of study and sex, we fitted a sequence of three models for each outcome and adiposity measure. Model 1 adjusted for age (in five-year age bands). Model 2, our main model to elucidate the association between adiposity and plaque burden, further adjusted for potential confounders: smoking and education. Model 3 further adjusted for potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, HbA1c, medical history of DM) to see to what extent the association is mediated by these factors.

Second, we fitted sex-specific models to the pooled data from both studies. Our simplest models compared outcomes (presence of plaques and plaque score) between the two studies using an indicator for study and adjusting for age (in five-year age bands) alone. We then separately added each adiposity measure to these models, to obtain pooled estimates of the effect of each adiposity measure, adjusting for age (Pooled Model 1). We next fitted models comparing outcomes between the two studies adjusting for age, smoking, and education. To these models we then separately added each adiposity measure (Pooled Model 2), assessing to what extent between-study differences were altered by adjusting for adiposity, and to allow pooled estimates of the confounder-adjusted effect of each adiposity measure to be obtained. Evidence for a difference in these associations between the two studies was examined by adding an interaction between study and adiposity to each Pooled Model 2: testing for statistical significance using likelihood ratio tests for logistic regression and Wald tests for linear regression. These tests of interaction were not statistically significant other than for the association between BMI and the presence of plaques in women (Online table 1). Given this, we omitted the interaction terms. Finally, we extended Pooled Model 2 by further adjusting for the potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, HbA1c, diabetes) (Pooled Model 3).

STATA version 15 (Stata Corp) was used for all the analyses.

Results

Baseline characteristics of participants

Table 1 shows the participants' baseline characteristics. Median age was higher in Tromsø 7 (61 years (range 52-65)) than KYH (56 years (range 48-63)). The age-adjusted prevalence of current smoking in men was much higher in KYH than Tromsø 7, but only half in women in KYH compared to Tromsø 7. Higher education was more prevalent in Tromsø 7 than in KYH for both sexes. SBP was considerably higher in KYH than in Tromsø 7. In general, cardio-metabolic risk factor levels were less favourable in KYH.

Adiposity

Table 2 shows adiposity z-scores for the KYH participants standardised to the Tromsø 7 population adjusted for age. All mean adiposity z-scores for women in KYH were

consistently higher than those for women in Tromsø 7 but did not differ significantly between men, except for WC which was lower in KYH.

Prevalence of carotid plaques

Table 3 compares the prevalence of carotid plaques and the mean plaque scores in the two studies. The prevalence of carotid plaques and the mean plaque score increased with age in both women and men. The burden of plaques was consistently higher in KYH than Tromsø 7 in both sexes.

The association between carotid plaque burden and adiposity: a pooled analysis of the two studies

Table 4 shows the odds ratios for having at least one carotid plaque per 1SD increase in each adiposity measure by sex from the pooled analysis. After adjustment for confounders (Pooled Model 2), there was evidence of association between all adiposity measures and the presence of plaques except for BMI in women. WHR showed the largest odds ratios (women 1.15 95%CI: 1.06, 1.25, men 1.18 95%CI: 1.06, 1.31) while BMI had the smallest. After further adjustment for cardio-metabolic mediators (Pooled Model 3), all odds ratios decreased substantially.

Table 5 shows the difference in plaque score per 1SD increase in each adiposity measure. In women, adiposity was associated with an increase in plaque score, with WHR showing the largest effect size (increase per 1SD change 0.134 95%CI: 0.087, 0.180) and BMI the smallest. Additional adjustments for cardio-metabolic mediators reduced effect sizes substantially. For men, there was no evidence of an association between adiposity and plaque score.

In the study-specific analysis, there was some suggestion that these associations were weaker in KYH than in Tromsø 7 (Figure 1, Online table 2, 3). However, tests for interaction were not statistically significant except for the association between BMI and the presence of plaques in women, suggesting that there is little evidence that the association between adiposity and plaque burden differs between the two studies.

The effect of adiposity on the between-study differences in carotid plaque burden

Figure 2 compares the carotid plaque burden between the two studies with and without adjustment for adiposity. Without adjustment for adiposity measures, the odds ratio for having at least one carotid plaque in KYH compared to Tromsø 7 was 1.97 (95%CI: 1.62, 2.38) in women and 2.78 (95%CI: 2.21, 3.49) in men (Figure 2a, online table 4a). Further adjustment for each adiposity measure separately had only a small effect on this odds ratio for both men and women. The between-study difference remained large and statistically significant after further adjustment for cardio-metabolic mediators (Online table 4a). Similarly, without adjustment for adiposity, participants in KYH had a higher mean plaque score than those in Tromsø 7 by 0.51 (95%CI: 0.40, 0.62) for women and 0.90 (95%CI: 0.75, 1.05) for men; these estimates decreased slightly for women and hardly changed at all for men with further adjustment for adiposity (Figure 2b, online table 4b). The difference remained significant after further adjustment for cardio-metabolic mediators (Online table 4b).

Sensitivity analysis

To investigate whether the higher plaque burden might have been caused by a systematic overestimation of the plaque score in KYH due to lack of information about near or far wall location (see Methods), we repeated the analyses using a simpler plaque score (maximum score 6) which is not affected by the location of plaques. The results are presented in Online tables 5 and 6. Overall, using a simpler plaque score had little impact on the findings.

Discussion

Our analysis had four major findings. First, the adiposity level in women was higher in KYH than in Tromsø 7, whereas it was similar in men. Second, the burden of carotid plaque was higher in KYH than in Tromsø 7 regardless of age and sex. Thirdly, there was no significant difference between the two studies in terms of the association between adiposity and plaque: there was evidence of an association between plaque burden and adiposity, which was largely mediated by cardio-metabolic risk factors. Finally, adiposity failed to explain the difference in the burden of plaque between the two studies.

General and abdominal adiposity

The higher level of general adiposity in KYH women compared to Tromsø 7 women, and the similar level of adiposity in men in the two studies, agrees with previous reports. A recent pooled analysis showed that mean BMI in Russian women was higher than among Western European women, whereas mean BMI in Russian men was similar to or lower than their Western European counterparts (30). Although BMI is a convenient screening tool for obesity, it is a crude measure that provides limited information on fat distribution which is strongly associated with CVD. Additional information regarding body shape helps capture obesity-associated risks more accurately (83). In women, the differences in abdominal adiposity between KYH and Tromsø 7 were greater than those for general obesity assessed by BMI.

Prevalence of plaques and plaque score

Carotid plaques were more prevalent in KYH than in Tromsø 7 regardless of age and sex. Carotid atherosclerosis assessed by ultrasound has been used to predict CVD risk, which is intuitively understandable, considering atherosclerosis is the major cause of CVD. IMT predicts future CVD events (42). However, carotid plaque, representing a later stage of atherosclerosis, outperforms IMT as a predictor of CVD events (14).

Compared to a binary measure of the presence/absence of plaques, a more continuous measure such as plaque score may be expected to be more informative about plaque burden. Several continuous plaque variables such as total plaque area and plaque height have been proposed and have consistently predicted future CVD risk (50, 198, 199). Although plaque score is a simple quantitative variable based on the number of plaques, previous studies showed that it had reasonable predictive ability for future CVD events (24, 48, 200). Higher prevalence of plaques and increased plaque score in the KYH population reflect enhanced CVD risk in this population, consistent with the high CVD mortality rate in Russia compared to Western Europe.

Ultrasound imaging of carotid plaque has several advantages in large epidemiological studies. Compared to more sophisticated imaging such as coronary artery calcium and MRI, ultrasound assessment is more widely available and less expensive. Compared to other continuous plaque variables measured by ultrasound, plaque score is simpler, less operator dependent, less time-consuming and has similar prognostic value (24). Since atherosclerotic

changes occur far in advance of CVD events, imaging of carotid plaque enables us to compare CVD burden between apparently healthy populations with different CVD risks.

However, caution is needed when comparing carotid plaque burden assessed by ultrasound across different studies. The prevalence of carotid plaques in previous reports ranges between 25.1% and 91% (133, 200-206), reflecting various factors including different participant backgrounds such as age and risk profiles, different measurement site and method to assess plaque, and different definition of plaque, which makes a direct comparison of plaque burden difficult (45). Standardised protocols for plaque measurement help to strengthen comparisons. In our study, we used comparable methods of plaque assessment in the two studies and a harmonised approach to define a measure of plaque score with participants of similar age structure which enabled us to make a reasonable comparison of plaque burden.

The association between carotid plaque burden and adiposity

We found that the association between adiposity and carotid plaque burden was not significantly different between the two studies. A few reports have suggested regional differences in the association between adiposity and CVD events. For example, the INTERSTROKE study suggested that the association between WHR and stroke is weaker in Eastern and Central Europe than in high-income Western countries (116). However, in the present analysis, confidence intervals for WHR from the two studies largely overlapped, especially for women. This is reassuring because a similar benefit might be expected from an intervention to reduce adiposity levels in the two countries.

In the pooled data, there was some evidence of associations between adiposity and increased plaque burden, which was mediated by cardio-metabolic CVD risk factors, especially among women, and abdominal adiposity seemed more strongly associated with carotid plaque than general adiposity. This result agrees with a previous study using the fifth Tromsø Study survey (178). Whether abdominal adiposity is a stronger CVD risk factor than BMI is controversial. A pooled analysis with 221,934 individuals concluded that BMI, WC, and WHR have similar associations with CVD events (114). On the other hand, the INTERHEART and INTERSTROKE studies suggested that WHR was more strongly associated with CVD events than BMI (115, 116). A causal role for WHR adjusted for BMI on CVD was recently supported by two Mendelian randomisation studies (124, 125).

Adiposity does not explain the difference in plaque burden between studies

Our main finding is that none of the adiposity measures explained the higher burden of carotid plaque in KYH compared to Tromsø 7, and this difference remained even after further adjustment for potential mediators. This suggests that other CVD risk factors may play a more important role in the development of carotid atherosclerosis in Russia than in Norway. For example, a model with physical activity and diet might have explained the study difference better. However, physical activity and diet seem to be located in the upper stream of the association between adiposity and carotid burden, and they cannot be simply considered as confounders in this association.

Limitations

Our study has several limitations. First, WC was assessed differently in the two studies. However, the conversion equation of WC we used was derived from a relatively large sample size, mainly consisting of Caucasians with a similar age structure (161). The use of this equation seems reasonable for our population. It should be noted that WC in Tromsø 7 in this paper was converted, which may result in a discrepancy in the reported levels of abdominal adiposity between this and other publications using Tromsø 7 data. Second, there was a minor difference between the studies in the assessment of plaque number and in the information on plaque location which could have resulted in overestimation of plaque burden in KYH relative to Tromsø 7. However, to avoid this, the main analyses used a more comparable plaque score as described in the Methods. In addition, the results of a sensitivity analysis used a further simplified plaque score that produced results that were consistent with those from the main analysis. Third, we did not include alcohol in our regression models, although it is known to play a role in CVD mortality in Russia (168-170, 207). This was because there is a lack of clarity as to whether either volume of ethanol or pattern of drinking is related to atherosclerosis per se. Other confounders which may be important are inflammation markers such as high-sensitivity C-reactive protein: the level of systemic inflammation plays an important role in both atherosclerosis and obesity, thus including it in the model might be informative. Further research is needed to understand the association between atherosclerosis and potential risk factors such as alcohol and inflammation in Russia to facilitate effective strategies to reduce its exceptionally high CVD burden. Fourth, the cross-sectional study design did not allow us to draw any strong causal conclusions. Finally, as always, caution must be exercised in generalising the results we have obtained from the two cross-sectional

studies of inevitably selected groups in specific cities to the national populations of Russia and Norway.

The study has several strengths. First, the two studies were designed to be comparable in terms of the main examination, which enabled us to compare quantitative carotid plaque data between studies. Second, this study provides the prevalence of abdominal obesity as well as general obesity in the general population in Russia.

In summary, our findings suggest two directions from which cardiac health in Russia may be improved. First, although adiposity does not explain the excess carotid plaque burden in Russia, adiposity, especially abdominal adiposity, seems to be an important risk factor for carotid atherosclerosis in Russia as well as in Norway and an area for intervention. The especially high burden of adiposity in Russian women is a concern. The prevention and treatment of obesity are important for the reduction of the carotid plaque burden and its consequent CVD in Russia. In particular, control of cardio-metabolic risk factors will mitigate the negative effect of adiposity. Second, the determinants of excess carotid plaque burden explaining the high CVD burden in Russia should be explored. Further research is needed to investigate the role of potential determinants including alcohol intake, inflammation and other cardio-metabolic factors.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Informed consent

Informed consent was obtained from all participants included in the two studies.

Table 1: Participant characteristics in KYH and Tromsø 7

	KYH men (n=1389)	Tromsø 7 men (n=811)	Age-adjusted Comparison: KYH vs T7 (95%CI)	KYH women (n=1873)	Tromsø 7 women (n=989)	Age-adjusted comparison: KYH vs T7 (95%CI)
Age (year) median (IQR)	56 (48-63)	61 (52-66)		55 (48-63)	60 (52-65)	
Anthropometric measure			Difference (95%CI)			Difference (95%CI)
Height (cm)	174.7 (6.6)	177.9 (6.7)	-3.9 (-4.4, -3.3)	161.1 (6.3)	164.7 (6.3)	-4.1 (-4.6, -3.6)
Weight (kg)	84.5 (15.5)	87.5 (12.9)	-3.6 (-4.9, -2.3)	74.5 (16.1)	71.4 (12.9)	3.4 (2.3, 4.6)
BMI (kg/m^2)	27.6 (4.6)	27.7 (3.7)	0.0 (-0.3, 0.4)	28.8 (6.2)	26.4 (4.7)	2.7 (2.3, 3.1)
WC[a](cm)	96.8 (12.2)	98.7 (9.8)	-1.3 (-2.3, -0.3)	90.1 (14.2)	81.8 (11.2)	9.4 (8.4, 10.4)
WHR[a]	0.95 (0.07)	0.95 (0.07)	-0.00 (-0.00, 0.01)	0.84 (0.08)	0.79 (0.07)	0.06 (0.05, 0.06)
WHtR[a]	0.55 (0.07)	0.56 (0.06)	-0.00 (-0.00, 0.01)	0.56 (0.09)	0.50 (0.07)	0.07 (0.07, 0.08)
Potential confounder (%)			Odds Ratio (95%CI)			Odds Ratio (95%CI)
Non-smoker	351 (25.3)	316 (39.0)	(ref)	1304 (69.6)	383 (38.7)	(ref)
Ex-smoker	518 (37.3)	377 (46.5)	1.3 (1.1, 1.7)	285 (15.2)	451 (45.6)	0.2 (0.1, 0.2)
Current smoker	520 (37.4)	118 (14.6)	4.0 (3.1, 5.1)	284 (15.2)	155 (15.7)	0.5 (0.4, 0.6)
Higher education	478 (34.3)	388 (47.8)	0.6 (0.5, 0.7)	701 (37.4)	495 (50.1)	0.5 (0.4, 0.6)
Potential mediators			Difference (95%CI)			Difference (95%CI)
Systolic blood pressure (mmHg)	138.6 (19.9)	132.8 (17.9)	7.3 (5.6, 8.9)	129.8(19.6)	126.4(19.4)	5.7 (4.3, 7.2)
Total cholesterol (mmol/l)	5.38 (1.13)	5.42 (1.05)	-0.06 (-0.16, 0.04)	5.68 (1.17)	5.62 (1.04)	0.13 (0.04, 0.21)
Triglycerides (mmol/l) median (IQR)	1.35 (0.95, -1.92)	1.50 (1.00, -2.10)	-0.05 (-0.15, 0.06)	1.23 (0.89,-1.77)	1.10 (0.80, -1.60)	0.18 (0.11, 0.26)
HDL cholesterol (mmol/l)	1.32 (0.33)	1.41 (0.39)	-0.09 (-0.12, -0.06)	1.55 (0.36)	1.78 (0.49)	-0.24 (-0.27, -0.20)
LDL cholesterol (mmol/l)	3.66 (0.90)	3.63 (0.99)	0.01 (-0.07, 0.09)	3.80 (0.95)	3.60 (0.96)	0.27 (0.19, 0.34)
HbA1c (%)	5.60 (0.84)	5.74 (0.57)	-0.08 (-0.14, -0.01)	5.57 (7.69)	5.67 (0.51)	-0.04 (-0.09, 0.01)
			Odds Ratio (95%CI)			Odds Ratio (95%CI)
Existing	93 (6.7)	47 (5.8)	1.4 (1.0, 2.1)	182 (9.7)	52 (5.3)	2.5 (1.8, 3.5)

diabetes mellitus (%)	

Participants were restricted to those aged between 40 and 69 years with all covariates. Data are mainly presented in mean values with standard deviation for continuous variables and percentages for binary variables. Age, triglycerides are presented in the median with inter-quintile range.
BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio.
[a]WC in T7 assessed at the level of the umbilicus was converted to the narrowest WC so that it can be comparable with WC in KYH. WHR and WHtR in T7 are calculated using converted WC.

Table 2 Adiposity z-scores for KYH participants standardised to the Tromsø 7 population

	KYH men (n=1389)	KYH women (n=1873)
	Mean z-score (95%CI)	Mean z-score (95%CI)
BMI z-score	0.01 (-0.09, 0.11)	0.58 (0.49, 0.68)
WC z-score	-0.13 (-0.24, -0.03)	0.85 (0.76, 0.94)
WHR z-score	0.04 (-0.04, 0.13)	0.84 (0.76, 0.92)
WHtR z-score	0.08 (-0.02, 0.18)	1.01 (0.92, 1.10)

Results are adjusted for categorical age (5-year interval)
BMI: body mass index, WC: waist circumference, WHR: waist-to-hip ratio, WHtR: waist-to-height ratio

Table 3. The prevalence of carotid plaques and plaque score according to study and sex

	KYH men	Tromsø 7 men	Age-adjusted comparison KYH vs T7 [a]	KYH women	Tromsø 7 women	Age-adjusted comparison KYH vs T7 [a]
N	1389	811		1873	989	
Prevalence n (%)			Odds Ratio (95%CI)			Odds Ratio (95%CI)
All age	1050 (75.6)	499 (61.5)	3.2 (2.6, 4.0)	1043 (55.7)	478 (48.3)	1.9 (1.6, 2.3)
40-49	212/394 (53.8)	38/148 (25.7)	3.5 (2.3, 5.4)	174/570 (30.5)	40/189 (21.2)	1.7 (1.1, 2.5)
50-59	340/450 (75.6)	104/191 (54.5)	2.7 (1.9, 4.0)	328/605 (54.2)	112/273 (41.0)	1.7 (1.3, 2.3)
60-69	498/545 (91.4)	357/472 (75.6)	3.5 (2.4, 5.1)	541/698 (77.5)	326/527 (61.9)	2.1 (1.7, 2.7)
Plaque score [b] mean(SD)			Difference (95%CI)			Difference (95%CI)
All age	2.3 (2.0)	1.5 (1.6)	1.1 (1.0, 1.3)	1.2 (1.5)	0.9 (1.2)	0.5 (0.4, 0.6)
40-49	1.1 (1.4)	0.4 (0.8)	0.7 (0.5, 0.9)	0.5 (0.9)	0.3 (0.7)	0.2 (0.05, 0.3)
50-59	2.1 (1.9)	1.1 (1.3)	1.0 (0.8, 1.3)	1.1 (1.4)	0.7 (1.0)	0.4 (0.2, 0.6)
60-69	3.3 (2.0)	2.0 (1.7)	1.3 (1.1, 1.5)	2.0 (1.7)	1.3 (1.4)	0.7 (0.5, 0.8)

[a]Adjusted for categorical age (5-year interval)

[b] In Tromsø 7, when one or more plaques exit in each carotid segment (far/near wall of common carotid artery, bifurcation, internal carotid artery from each carotid artery), a score of one was assigned. Each individual can have a maximum score of 12. Comparable plaque score was created in KYH.

Table 4: Odds ratios for having at least one carotid plaque per 1 SD increase in each adiposity measure: pooled results from the two studies

	Pooled Model 1 OR (95%CI)	p-value	Pooled Model 2 OR (95%CI)	p-value	Pooled Model 3 OR (95%CI)	p-value
Men (n=2200)						
st BMI	1.12 (1.02, 1.23)	0.02	1.13 (1.03, 1.24)	0.01	1.01 (0.91, 1.12)	0.83
st WC	1.14 (1.04, 1.25)	0.005	1.13 (1.03, 1.24)	0.009	1.02 (0.92, 1.13)	0.76
st WHR	1.21 (1.08, 1.34)	0.001	1.18 (1.06, 1.31)	0.003	1.05 (0.93, 1.18)	0.46
st WHtR	1.18 (1.07, 1.30)	0.001	1.17 (1.06, 1.29)	0.002	1.04 (0.93, 1.16)	0.47
Women (n=2862)						
st BMI	1.06 (0.99, 1.14)	0.08	1.06 (0.99, 1.13)	0.09	0.94 (0.87, 1.01)	0.08
st WC	1.12 (1.04, 1.20)	0.002	1.11 (1.03, 1.19)	0.006	0.96 (0.88, 1.04)	0.30
st WHR	1.20 (1.11, 1.30)	<0.001	1.15 (1.06, 1.25)	0.001	1.00 (0.91, 1.09)	0.94
st WHtR	1.12 (1.04, 1.20)	0.001	1.11 (1.03, 1.19)	0.006	0.95 (0.88, 1.03)	0.23

St BMI: body mass index z-score, st WC: waist circumference z-score, st WHR:waist-to-hip ratio z-score, st WHtR: waist-to-height ratio z-score, OR: odds ratio, 95%CI: 95% confidence interval

Model 1: adjusted for categorical age (5-year) and country, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Table 5: Difference in plaque score per 1 SD increase in each adiposity measure: pooled results from the two studies

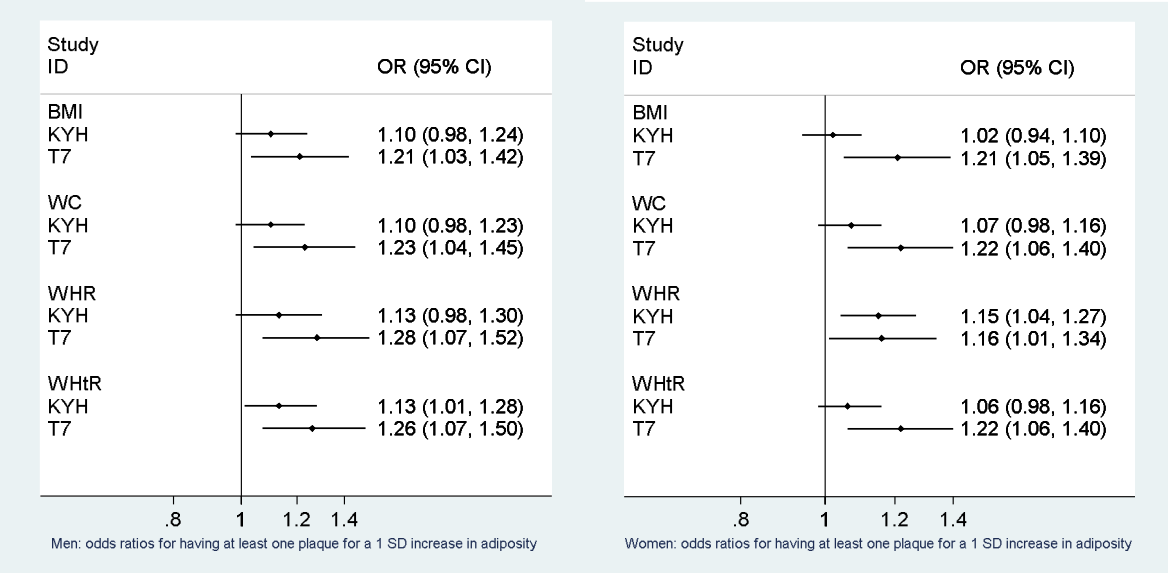
	Pooled Model 1 slope (95%CI)	p-value	Pooled Model 2 slope (95%CI)	p-value	Pooled Model 3 slope (95%CI)	p-value
Men (n=2200)						
st BMI	-0.024 (-0.085, 0.037)	0.44	-0.002 (-0.062, 0.058)	0.94	-0.102 (-0.167, -0.037)	0.002
st WC	-0.010 (-0.072, 0.052)	0.75	-0.006 (-0.067, 0.054)	0.84	-0.108 (-0.174, -0.042)	0.001
st WHR	0.057 (-0.017, 0.130)	0.13	0.032 (-0.040, 0.103)	0.39	-0.075 (-0.151, 0.002)	0.06
st WHtR	0.023 (-0.041, 0.087)	0.48	0.021 (-0.042, 0.083)	0.52	-0.084 (-0.152, -0.016)	0.02
Women (n=2862)						
st BMI	0.028 (-0.012, 0.067)	0.17	0.027 (-0.012, 0.066)	0.17	-0.072 (-0.114, -0.030)	0.001
st WC	0.076 (0.035, 0.118)	<0.001	0.067 (0.026, 0.108)	0.001	-0.045 (-0.091, 0.001)	0.05
st WHR	0.162 (0.116, 0.208)	<0.001	0.134 (0.087, 0.180)	<0.001	0.029 (-0.021, 0.080)	0.26
st WHtR	0.083 (0.042, 0.125)	<0.001	0.073 (0.032, 0.114)	0.001	-0.043 (-0.089, 0.004)	0.07

St BMI: body mass index z-score, st WC: waist circumference z-score, st WHR:waist-to-hip ratio z-score, st WHtR: waist-to-height ratio z-score, OR: odds ratio, 95%CI: 95% confidence interval

Model 1: adjusted for categorical age (5-year) and country, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Figure 1: Study-specific associations between carotid plaque and adiposity

a) Study-specific odds ratio for having at least one plaque for a 1 SD increase in each adiposity measure after the adjustment for age, smoking, and education (Model 2)



b) Study-specific difference in plaque score per 1 SD increase in each adiposity measure after the adjustment for age, smoking, and education (Model 2)

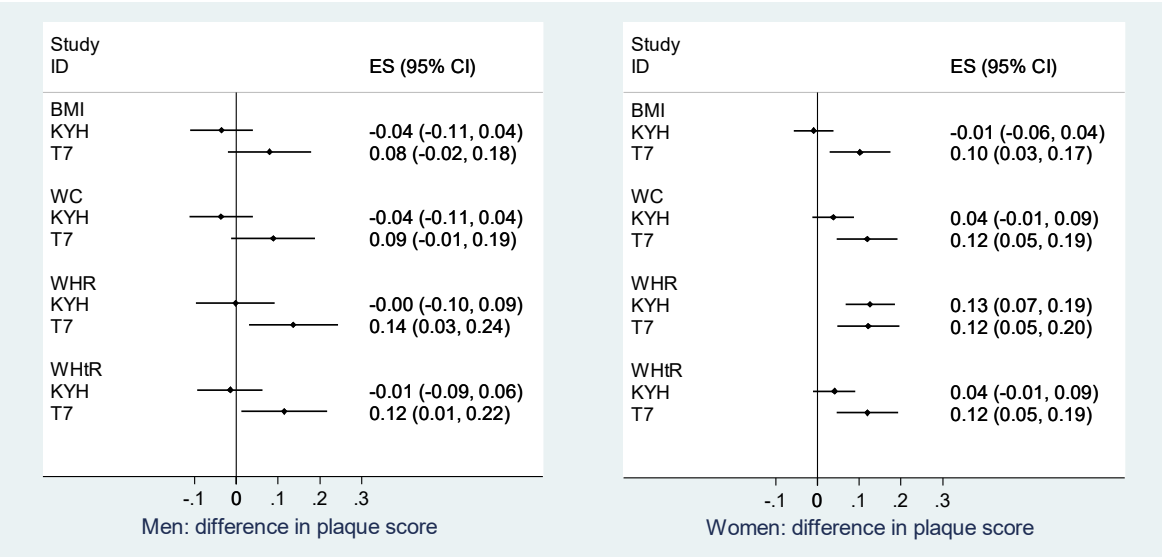
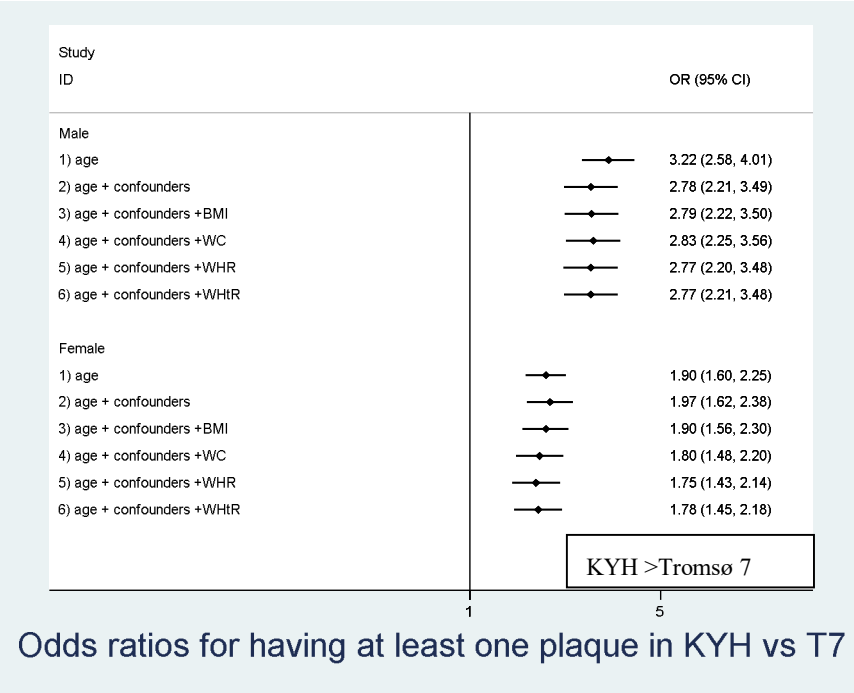
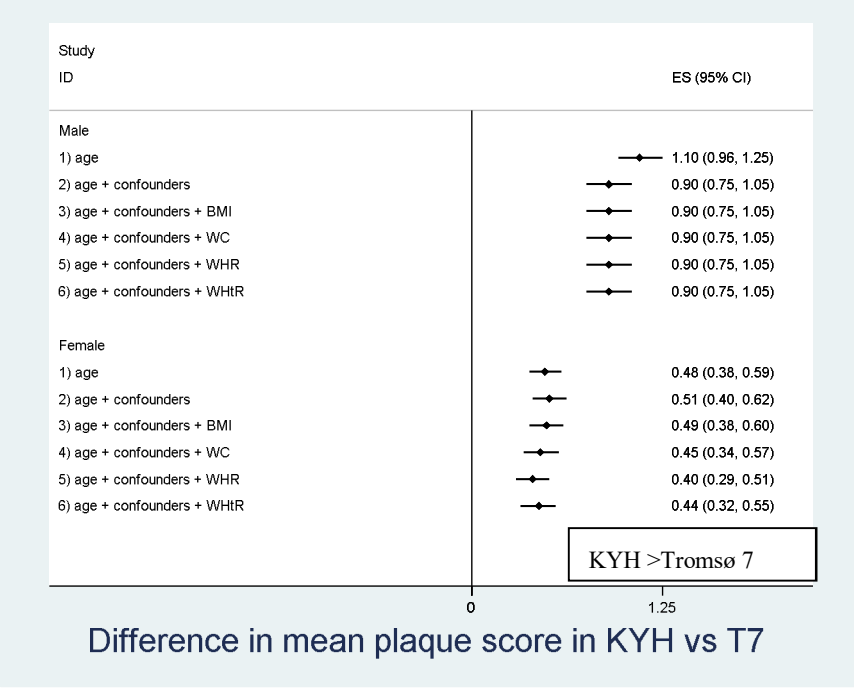


Figure 2: Comparison of carotid plaque burden in KYH compared to Tromsø 7 with and without adjustment for various adiposity measure

a) Odds ratios for having at least one plaque in KYH vs Tromsø 7 with and without adjustment for adiposity



b) Differences (95%CI) in the mean plaque score in KYH compared to Tromsø 7 with and without adjustment for adiposity



1) Adjusted for categorical age, 2) adjusted for variables in 1) plus potential confounders (smoking and education), 3)-6) adjusted for variables in 2) plus each adiposity measure separately

Online table 1: Interaction

Interaction between study and adiposity

Interaction: odds ratio for having at least one carotid plaque per 1 SD increase in each adiposity measure (adiposity#country)

Men	Pooled Model 1	Pooled Model 2	Pooled Model 3
BMI	0.22	0.35	0.39
WC	0.15	0.30	0.33
WHR	0.24	0.42	0.29
WHtR	0.21	0.40	0.37
Women			
BMI	0.11	**0.044**	0.21
WC	0.33	0.20	0.41
WHR	0.72	0.79	0.73
WHtR	0.29	0.21	0.47

Model 1: adjusted for categorical age (5-year) and country, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Interaction: difference in plaque score per 1 SD increase in each adiposity measure: pooled results from two studies (adiposity#country)

Men	Pooled Model 1	Pooled Model 2	Pooled Model 3
BMI	**0.02**	0.33	0.13
WC	0.05	0.23	0.33
WHR	0.24	0.72	0.55
WHtR	0.08	0.38	0.42
Women			
BMI	0.14	0.07	0.46
WC	0.78	0.60	0.91
WHR	0.10	0.09	0.08
WHtR	0.86	0.80	0.64

Model 1: adjusted for categorical age (5-year) and country, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Online table 2: Study-specific odds ratio for having at least one carotid plaque per 1 SD increase in each adiposity measure

a) Men (n=2200)

	Model 1 OR (95%CI)	p-value	Model 2 OR (95%CI)	p-value	Model 3 OR (95%CI)	p-value
KYH Men (n=1389)						
st BMI	1.08 (0.97, 1.20)	0.18	1.10 (0.98, 1.24)	0.10	0.95 (0.84, 1.09)	0.47
st WC	1.09 (0.98, 1.22)	0.13	1.10 (0.98, 1.23)	0.11	0.95 (0.83, 1.08)	0.46
st WHR	1.14 (1.00, 1.31)	0.06	1.13 (0.98, 1.30)	0.09	0.95 (0.81, 1.12)	0.53
st WHtR	1.13 (1.01, 1.27)	0.04	1.13 (1.01, 1.28)	0.04	0.98 (0.84, 1.11)	0.63
T7 Men (n=811)						
st BMI	1.22 (1.04, 1.43)	0.02	1.21 (1.03, 1.42)	0.02	1.10 (0.92, 1.32)	0.32
st WC	1.26 (1.07, 1.48)	0.006	1.23 (1.04, 1.45)	0.02	1.11 (0.93, 1.34)	0.25
st WHR	1.31 (1.11, 1.56)	0.002	1.28 (1.07, 1.52)	0.006	1.16 (0.96, 1.41)	0.13
st WHtR	1.29 (1.09, 1.52)	0.003	1.26 (1.07, 1.50)	0.007	1.15 (0.95, 1.39)	0.15

b) Women (n=2862)

	Model 1 OR (95%CI)	p-value	Model 2 OR (95%CI)	p-value	Model 3 OR (95%CI)	p-value
KYH Women (n=1873)						
st BMI	1.03 (0.95, 1.11)	0.50	1.02 (0.94, 1.10)	0.69	0.92 (0.84, 1.00)	0.045
st WC	1.09 (1.00, 1.18)	0.04	1.07 (0.98, 1.16)	0.13	0.94 (0.86, 1.04)	0.23
st WHR	1.20 (1.09, 1.32)	<0.001	1.15 (1.04, 1.27)	0.007	1.01 (0.90, 1.13)	0.87
st WHtR	1.09 (1.00, 1.18)	0.045	1.06 (0.98, 1.16)	0.15	0.93 (0.85, 1.03)	0.17
T7 Women (n=989)						
st BMI	1.18 (1.03, 1.35)	0.02	1.21 (1.05, 1.39)	0.008	1.01 (0.86, 1.19)	0.86
st WC	1.20 (1.05, 1.38)	0.008	1.22 (1.06, 1.40)	0.006	1.02 (0.87, 1.20)	0.81
st WHR	1.20 (1.04, 1.37)	0.01	1.16 (1.01, 1.34)	0.04	0.98 (0.83, 1.14)	0.76
st WHtR	1.21 (1.06, 1.39)	0.006	1.22 (1.06, 1.40)	0.006	1.01 (0.85, 1.19)	0.94

St BMI: body mass index z-score, st WC: waist circumference z-score, st WHR:waist-to-hip ratio z-score, st WHtR: waist-to-height ratio z-score, OR: odds ratio, 95%CI: 95% confidence interval. Model 1: adjusted for categorical age (5-year) and country, Model 2: adjust for variables in Model 1 plus potential confounders (smoking and education), Model 3: adjusted for variables in Model 2 plus potential mediators (systolic blood pressure, HDL cholesterol, LDL cholesterol, glycated haemoglobin, diabetes)

Online table 3: Study-specific difference in plaque score per 1 SD increase in each adiposity measure: pooled results from two studies

a) Men (n=2200)

	Model 1 Difference in plaque score (95%CI)	p-value	Model 2 Difference in plaque score(95%CI)	p-value	Model 3 Difference in plaque score (95%CI)	p-value
KYH Men (n=1389)						
st BMI	-0.071 (-0.147, 0.005)	0.07	-0.036 (-0.111, 0.039)	0.35	-0.149 (-0.231, -0.068)	<0.001
st WC	-0.057 (-0.134, 0.021)	0.15	-0.037 (-0.113, 0.039)	0.34	-0.153 (-0.235, -0.070)	<0.001
st WHR	0.003 (-0.094, 0.101)	0.95	-0.002 (-0.097, 0.092)	0.96	-0.132 (-0.234, -0.031)	0.01
st WHtR	-0.029 (-0.110, 0.051)	0.48	-0.015 (-0.094, 0.063)	0.70	-0.139 (-0.225, -0.053)	0.002
T7 Men(n=811)						
st BMI	0.088 (-0.012, 0.187)	0.09	0.079 (-0.021, 0.179)	0.12	-0.015 (-0.125, 0.094)	0.79
st WC	0.115 (0.014, 0.216)	0.03	0.088 (-0.013, 0.188)	0.09	-0.008 (-0.119, 0.103)	0.88
st WHR	0.179 (0.073, 0.285)	0.001	0.136 (0.030, 0.243)	0.01	0.049 (-0.067, 0.165)	0.41
st WHtR	0.146 (0.044, 0.249)	0.005	0.115 (0.012, 0.218)	0.03	0.021 (-0.093, 0.135)	0.71

b) Women (n=2862)

	Model 1 Difference in plaque score(95%CI)	p-value	Model 2 Difference in plaque score(95%CI)	p-value	Model 3 Difference in plaque score (95%CI)	p-value
KYH women (n=1873)						
st BMI	0.001 (-0.047, 0.048)	0.98	-0.009 (-0.056, 0.038)	0.70	-0.107 (-0.157, -0.057)	<0.001
st WC	0.057 (0.007, 0.108)	0.03	0.038 (-0.012, 0.088)	0.14	-0.080 (-0.136, -0.025)	0.005
st WHR	0.165 (0.107, 0.224)	<0.001	0.126 (0.068, 0.185)	<0.001	0.014 (-0.050, 0.079)	0.67
st WHtR	0.062 (0.011, 0.112)	0.02	0.041 (-0.010, 0.091)	0.11	-0.081 (-0.137, -0.025)	0.004
T7 Women (n=989)						
st BMI	0.089 (0.016, 0.162)	0.02	0.102 (0.030, 0.175)	0.006	0.009 (-0.073, 0.091)	0.83
st WC	0.113 (0.039, 0.187)	0.003	0.119 (0.046, 0.192)	0.001	0.027 (-0.056, 0.110)	0.52
st WHR	0.139 (0.064, 0.214)	<0.001	0.122 (0.048, 0.197)	0.001	0.042 (-0.040, 0.124)	0.31
st WHtR	0.118 (0.044, 0.192)	0.002	0.120 (0.046, 0.193)	0.001	0.025 (-0.059, 0.109)	0.56

St BMI: body mass index z-score, st WC: waist circumference z-score, st WHR:waist-to-hip ratio z-score, st WHtR: waist-to-height ratio z-score, OR: odds ratio, 95%CI: 95% confidence interval

Online table 4: Comparison of carotid plaque burden in KYH compared to Tromsø 7 with and without adjustment for various adiposity measure (data of figure 2)

a) Odds ratios for having at least one carotid plaque in KYH vs. Tromsø 7 with and without adjustment for adiposity

	Men (n=2200)	Women (n=2862)
	OR (95%CI)	OR (95%CI)
Model 1-adiposity	3.22 (2.58, 4.01)	1.90 (1.60, 2.25)
Model 2-adiposity	2.78 (2.21, 3.49)	1.97 (1.62, 2.38)
Model 2 BMI	2.79 (2.22, 3.50)	1.90 (1.56, 2.30)
Model 2 WC	2.83 (2.25, 3.56)	1.80 (1.48, 2.20)
Model 2 WHR	2.77 (2.20, 3.48)	1.75 (1.43, 2.14)
Model 2 WHtR	2.77 (2.21, 3.48)	1.78 (1.45, 2.18)
Model 3 - adiposity	2.51 (1.98, 3.18)	1.63 (1.33, 2.00)
Model 3 BMI	2.51 (1.98, 3.18)	1.63 (1.36, 2.04)
Model 3 WC	2.52 (1.98, 3.19)	1.67 (1.36, 2.06)
Model 3 WHR	2.51 (1.98, 3.18)	1.64 (1.33, 2.01)
Model 3 WHtR	2.51 (1.98, 3.19)	1.69 (1.37, 2.09)

b) Differences (95%CI) in the mean plaque score in KYH compared to Tromsø 7 with and without adjustment for adiposity

	Men (n=2200)	Women (n=2862)
	Difference in plaque score (95%CI)	Difference in plaque score (95%CI)
Model 1-adiposity	1.10 (0.96, 1.25)	0.48 (0.38, 0.59)
Model 2-adiposity	0.90 (0.75, 1.05)	0.51 (0.40, 0.62)
Model 2 BMI	0.90 (0.75, 1.05)	0.49 (0.38, 0.60)
Model 2 WC	0.90 (0.75, 1.05)	0.45 (0.34, 0.57)
Model 2 WHR	0.90 (0.75, 1.05)	0.40 (0.29, 0.51)
Model 2 WHtR	0.90 (0.75, 1.05)	0.44 (0.32, 0.55)
Model 3 - adiposity	0.76 (0.61, 0.92)	0.37 (0.26, 0.48)
Model 3 BMI	0.76 (0.61, 0.91)	0.39 (0.28, 0.50)
Model 3 WC	0.74 (0.59, 0.89)	0.39 (0.28, 0.51)
Model 3 WHR	0.76 (0.61, 0.91)	0.35 (0.24, 0.47)
Model 3 WHtR	0.76 (0.61, 0.91)	0.40 (0.28, 0.51)

Online table 5 Sensitivity analysis 1: mean plaque score using original plaque score and simplified plaque score

	KYH men	Tromsø 7 men	Age-adjusted comparison KYH vs T7 *	KYH women	Tromsø 7 women	Age-adjusted comparison KYH vs T7*
Original Plaque scoremean(SD)**	1389	811	Difference (95%CI)			Difference (95%CI)
All age	2.3 (2.0)	1.5 (1.6)	1.1 (1.0, 1.3)	1.2 (1.5)	0.9 (1.2)	0.5 (0.4, 0.6)
40-49	1.1 (1.4)	0.4 (0.8)	0.7 (0.5, 0.9)	0.5 (0.9)	0.3 (0.7)	0.2 (0.05, 0.3)
50-59	2.1 (1.9)	1.1 (1.3)	1.0 (0.8, 1.3)	1.1 (1.4)	0.7 (1.0)	0.4 (0.2, 0.6)
60-69	3.3 (2.0)	2.0 (1.7)	1.3 (1.1, 1.5)	2.0 (1.7)	1.3 (1.4)	0.7 (0.5, 0.8)
Simplified plaque score mean (SD)						
All age	1.9 (1.6)	1.2 (1.1)	1.0 (0.9, 1.2)	1.1 (1.3)	0.8 (0.9)	0.5 (0.4, 0.6)
40-49	1.0 (1.2)	0.3 (0.6)	0.6 (0.5, 0.8)	0.5 (0.8)	0.3 (0.6)	0.2 (0.1, 0.3)
50-59	1.8 (1.5)	0.9 (1.0)	0.9 (0.7, 1.2)	1.0 (1.2)	0.6 (0.8)	0.4 (0.3, 0.6)
60-69	2.7 (1.6)	1.5 (1.2)	1.2 (1.1, 1.4)	1.7 (1.4)	1.1 (1.0)	0.7 (0.5, 0.8)

Online table 6 Differences (95%CI) in the mean plaque score in KYH compared to Tromsø 7 with and without adjustment for adiposity

Men

Men (n=2200)	Original score	Simplified score
	Difference in plaque score (95%CI)	Difference in plaque score (95%CI)
Model 1-adiposity	1.10 (0.96, 1.25)	1.03 (0.92, 1.15)
Model 2-adiposity	0.90 (0.75, 1.05)	0.89 (0.77, 1.00)
Model 2 BMI	0.90 (0.75, 1.05)	0.89 (0.77, 1.00)
Model 2 WC	0.90 (0.75, 1.05)	0.88 (0.77, 1.00)
Model 2 WHR	0.90 (0.75, 1.05)	0.89 (0.77, 1.00)
Model 2 WHtR	0.90 (0.75, 1.05)	0.89 (0.77, 1.00)
Model 3-adiposity	0.76 (0.61, 0.92)	0.79 (0.67, 0.90)
Model 3 BMI	0.76 (0.61, 0.91)	0.78 (0.66, 0.90)
Model 3 WC	0.74 (0.59, 0.89)	0.76 (0.64, 0.88)
Model 3 WHR	0.76 (0.61, 0.91)	0.78 (0.66, 0.90)
Model 3 WHtR	0.76 (0.61, 0.91)	0.78 (0.66, 0.90)

Women

Women (n=2862)	Original score	Simplified score
	Difference in number of plaques (95%CI)	Difference in number of plaques (95%CI)
Model 1-adiposity	0.48 (0.38, 0.59)	0.49 (0.40, 0.57)
Model 2-adiposity	0.51 (0.40, 0.62)	0.51 (0.42, 0.60)
Model 2 BMI	0.49 (0.38, 0.60)	0.49 (0.40, 0.59)
Model 2 WC	0.45 (0.34, 0.57)	0.46 (0.37, 0.55)
Model 2 WHR	0.40 (0.29, 0.51)	0.42 (0.32, 0.51)
Model 2 WHtR	0.44 (0.32, 0.55)	0.45 (0.35, 0.54)
Model 3 - adiposity	0.37 (0.26, 0.48)	0.40 (0.31, 0.49)
Model 3 BMI	0.39 (0.28, 0.50)	0.41 (0.32, 0.50)
Model 3 WC	0.39 (0.28, 0.51)	0.42 (0.32, 0.51)
Model 3 WHR	0.35 (0.24, 0.47)	0.38 (0.29, 0.48)
Model 3 WHtR	0.40 (0.28, 0.51)	0.42 (0.32, 0.51)

9.3 The association between WHR and carotid plaque burden: Effect of age

In research paper 3, I showed that adiposity, especially WHR, was associated with increased carotid plaque burden. In this section, I additionally looked at the effect of age on carotid plaque burden and compared it with that of WHR because age is clearly a strong determinant of carotid plaque burden. Furthermore, the effect of WHR within the same age group was examined.

Methods

First, the separate effect of age and WHR on carotid plaque burden was investigated using the pooled dataset from KYH and Tromsø 7. Sex-specific tertiles of WHR z-score were generated. Odds ratios for having at least one plaque as age group (10-year interval) or the tertiles of WHR z-score increases were estimated using logistic regression model including both the tertiles of WHR z-score and age group, which were adjusted for study, smoking, and education. The lowest age group and the lowest tertile of WHR z-score were used as reference categories. Similarly, differences in the simplified plaque score as age group or the tertiles of WHR z-score increases were investigated using the linear regression adjusted for study, smoking, and education.

Then, the effect of WHR within each age group was examined. I divided participants into nine groups. Participants were divided into the tertiles of WHR z-score within each age group (10-year interval). Odds ratios for having at least one plaque were investigated using logistic regression adjusted for study, smoking, and education. The differences in the simplified plaque score were estimated using linear regression adjusted for study, smoking, and education.

Results

Separate effect of age and WHR

Table 9-1 shows ORs for having at least one plaque as age group or tertiles of WHR increases. Increase in age was strongly associated with an increase in the presence of carotid plaques. Although the associations between the increase in WHR and the presence of plaques were weaker than that with age, most of the associations were significant and positive, suggesting that WHR is also a risk factor for the presence of carotid plaques.

Table 9-2 shows differences in the simplified plaque score as age group or tertiles of WHR increases. Again, age was a strong determinant of the simplified plaque score, whereas WHR showed positive but weaker associations than age.

The effect of WHR within each age group

Table 9-3 shows the distribution of the tertiles of WHR within each age group. As age increased, WHR considerably increased for both men and women.

Table 9-4 shows ORs for having at least one plaque according to age group and WHR tertiles. ORs considerably increased as age group increased. Within each age group, ORs tended to increase as the tertiles of WHR increased although there were large overlaps of 95% CIs.

Table 9-5 shows the difference in the simplified plaque score according to age group and standardised WHR tertiles. Again, age was a strong determinant of increase in the simplified plaque score. Within each age group, the association between WHR tertile and simplified plaque score was not clear among men, while the increase in the tertile of WHR seemed associated with an increase in simplified plaque score in women.

Comments

This analysis showed that age was a very strong determinant of carotid plaque burden as expected. Although the effect of WHR on carotid plaque burden was weaker than that of age, an increase in the tertiles of WHR seemed associated with an increase in carotid plaque burden within each age group, especially among women. This finding has an important clinical implication. Regardless of age, the increase in WHR may contribute to carotid atherosclerosis. The control of abdominal adiposity is important.

Table 9-1 Odds ratios for having at least one carotid plaque: the effect of age and WHR

	Men (N=2200)		Women (N=2862)	
	N	OR (model 2)	N	OR (model 2)
Age				
40-49 years	542	1.00 [ref]	759	1.00 [ref]
50-59 years	641	2.83 (2.19, 3.64)	878	2.63 (2.11, 3.26)
60-69 years	1017	7.99 (6.11, 10.46)	1225	6.93 (5.53, 8.67)
WHR tertiles				
1st	734	1.00 [ref]	954	1.00 [ref]
2nd	734	1.29 (1.01, 1.65)	954	1.13 (0.93, 1.38)
3rd	732	1.42 (1.10, 1.85)	954	1.46 (1.18, 1.80)

Table 9-2 Differences in simplified plaque score: the effect of age and WHR

	Men (N=2200)		Women (N=2862)	
	N	Difference (model 2)	N	Difference (model 2)
Age				
40-49 years	542	0.00 [ref]	759	0.00 [ref]
50-59 years	641	0.75 (0.60, 0.91)	878	0.45 (0.35, 0.56)
60-69 years	1017	1.54 (1.40, 1.69)	1225	1.05 (0.94, 1.15)
WHR tertiles				
1st	734	0.00 [ref]	954	0.00 [ref]
2nd	734	0.06 (-0.08, 0.19)	954	0.05 (-0.05, 0.15)
3rd	732	0.04 (-0.10, 0.18)	954	0.29 (0.18, 0.39)

Table 9-3 Distribution of the tertiles of WHR in each age group

	WHR 1st	WHR 2nd	WHR 3rd	Total
Men	N (%)	N (%)	N (%)	
40-49 years	268 (49.4)	173 (31.9)	101 (18.6)	542
50-59 years	224 (34.9)	224 (34.9)	193 (30.1)	641
60-69 years	242 (23.8)	337 (33.1)	438 (43.1)	1017
Women				
40-49 years	383 (50.5)	220 (29.0)	156 (20.6)	759
50-59 years	282 (32.1)	313 (35.6)	283 (32.2)	878
60-69 years	289 (23.6)	421 (34.4)	515 (42.0)	1225

Table 9-4 Odds ratios for having at least one carotid plaque: the joint effect of age and WHR

Age	Thirds of WHR		
	1st	2nd	3rd
Men	OR (model 2)	OR (model 2)	OR (model 2)
40-49 years	1.00 [ref]	1.37 (0.92, 2.05)	1.46 (0.90, 2.36)
50-59 years	3.02 (2.05, 4.44)	3.73 (2.51, 5.52)	3.93 (2.58, 5.99)
60-69 years	8.11 (5.31, 12.39)	10.27 (6.87, 15.36)	12.07 (8.17, 17.83)
Women			
40-49 years	1.00 [ref]	0.96 (0.65, 1.41)	1.28 (0.85, 1.94)
50-59 years	2.19 (1.56, 3.08)	2.92 (2.11, 4.05)	3.66 (2.60, 5.14)
60-69 years	6.54 (4.60, 9.30)	7.16 (5.19, 9.87)	9.31 (6.78, 12.78)

Table 9-5 Differences in simplified plaque score: the joint effect of age and WHR

Age	Thirds of WHR		
	1st	2nd	3rd
Men	Difference (model 2)	Difference (model 2)	Difference (model 2)
40-49 years	0.00 [ref]	1.13 (-0.12, 0.38)	0.11 (-0.19, 0.41)
50-59 years	0.77 (0.54, 1.01)	0.77 (0.54, 1.00)	0.95 (0.70, 1.19)
60-69 years	1.64 (1.41, 1.87)	1.67 (1.46, 1.88)	1.56 (1.36, 1.76)
Women			
40-49 years	0.00 [ref]	-0.06 (-0.23, 0.12)	0.01 (-0.19, 0.21)
50-59 years	0.30 (0.13, 0.46)	0.45 (0.29, 0.61)	0.68 (0.51, 0.84)
60-69 years	0.90 (0.73, 1.07)	1.00 (0.85, 1.15)	1.29 (1.14, 1.43)

9.4 Comparison of strength of WHR with carotid plaques compared to that of conventional CVD risk factors

In research paper 3, I showed that the associations between adiposity and carotid plaque burden were to a large extent mediated by cardio-metabolic mediators (SBP, LDL cholesterol, HDL cholesterol, HbA1c). Although this finding shows that these cardio-metabolic mediators, combined together, are strongly associated with increased carotid plaque burden, paper 3 did not make direct comparisons of the strength of associations of these cardio-metabolic mediators with plaque burden. This final section investigates the association between carotid plaque burden and a 1 SD change in each cardio-metabolic mediator. This enables a direct comparison with the effect of a 1 SD unit increase in the adiposity measures of BMI and WHR, and in this way provides an important context for judging the importance of the adiposity associations presented in paper 3.

Methods

Z-scores for SBP, LDL-c, HDL-c, and HbA1c were generated in the same way as was used to create the adiposity z-scores (see paper 3 method section). Odds ratios for having at least one plaque were estimated per 1 SD unit increase in each cardio-metabolic mediator or adiposity measure using logistic regression adjusted for categorical age and study. The associations of the simplified plaque score with each cardio-metabolic mediator or adiposity measure changes were estimated using linear regression adjusted for categorical age and study. All analyses were done separately for men and women.

Results

Table 9-6 shows the odds ratios for having at least one plaque per 1 SD increase in each cardio-metabolic mediator or adiposity measure. SBP was the most strongly associated variable, notably so for men. However, the ORs for a 1 SD increase in LDL-cholesterol, and HbA1c were of the same magnitude as for WHR. The weakest association was shown for BMI.

Table 9-7 shows the differences in the simplified plaque score per 1 SD increase in each cardio-metabolic mediator or adiposity measure. Again, SBP showed the largest effect estimates, although, for women, it was only slightly bigger than seen for WHR. Again, BMI showed the smallest associations for both sexes.

Comments

In this analysis, 1 SD increase in WHR was as strongly associated with the presence of carotid plaques as 1 SD increase in traditional cardio-metabolic risk factors such as cholesterol and HbA1c, suggesting that WHR is an equally important risk factor for carotid atherosclerosis. Especially, in women, the strength of association was comparable with that of SBP. On the other hand, the weakest association of BMI suggests that BMI may not be appropriate adiposity measure to predict risk for carotid atherosclerosis.

The associations between WHR and the simplified plaque score seemed relatively weak compared to traditional cardio-metabolic risk factors among men. However, this association was the second strongest, followed by SBP among women.

Table 9-6 Odds ratios for having at least one plaque per 1 SD increase in BMI, WHR, SBP, LDL cholesterol, HDL cholesterol and HbA1c adjusted for age and study: pooled results from the two studies

	Men OR(95%CI) (n=2200)	Women OR(95%CI) (n=2862)
BMI (1 SD increase)	1.12 (1.02, 1.23)	1.06 (0.99, 1.14)
WHR (1 SD increase)	1.21 (1.08, 1.34)	1.20 (1.11, 1.30)
SBP (1 SD increase)	1.43 (1.28, 1.59)	1.29 (1.18, 1.41)
LDL-cholesterol (1 SD increase)	1.17 (1.05, 1.30)	1.22 (1.12, 1.32)
HDL-cholesterol (1 SD increase)	0.91 (0.81, 1.01)	0.78 (0.71, 0.86)
HbA1c (1 SD increase)	1.18 (1.06, 1.32)	1.16 (1.08, 1.25)

Table 9-7 Difference in simplified plaque score per 1 SD increase in BMI, WHR, SBP, LDL cholesterol, HDL cholesterol and HbA1c adjusted for age and study: pooled results from the two studies

	Men difference (95%CI) (n=2200)	Women difference(95%CI) (n=2862)
BMI (1 SD increase)	-0.021 (-0.069, 0.026)	0.023 (-0.009, 0.056)
WHR (1 SD increase)	0.033 (-0.025, 0.090)	0.131 (0.093, 0.169)
SBP (1 SD increase)	0.221 (0.168, 0.273)	0.159 (0.118, 0.201)
LDL-cholesterol (1 SD increase)	0.078 (0.020, 0.137)	0.102 (0.062, 0.143)
HDL-cholesterol (1 SD increase)	-0.087 (-0.148, -0.025)	-0.141 (-0.188, -0.094)
HbA1c (1 SD increase)	0.073 (0.031, 0.116)	0.094 (0.064, 0.124)

9.5 Summary

- The adiposity level was substantially higher in KYH women than in Tromsø 7 women while men showed a similar adiposity level in both studies.
- The burden of carotid plaque was consistently higher in KYH than in Tromsø 7 for both sexes regardless of age group.
- The test for interaction suggested that the association between adiposity and carotid plaque burden is not significantly different between KYH and Tromsø 7.
- Adiposity failed to explain the difference in the burden of carotid plaque between KYH and Tromsø 7.

10 Discussion

The aim of this thesis was to examine the contribution of adiposity to atherosclerotic changes in the carotid artery in general population samples from Norway and Russia, which have very different CVD mortality rates. The two main objectives of this thesis were to: 1) investigate the role of general/abdominal adiposity on measures of carotid plaque assessed by ultrasound in population-based studies from Norway (Tromsø 7) and Russia (Know Your Heart); and 2) determine whether differences in carotid plaque burden (the presence of plaques, number of plaques) between the two populations, if any, can be statistically accounted for by various measures of adiposity.

This concluding chapter is subdivided into five sections. In the first, I briefly summarise the main findings. This is followed by a discussion of the extent to which these results provide new insights into the links between adiposity and carotid atherosclerosis in Norway and Russia and how far differences in adiposity may explain some of the differences in cardiovascular disease mortality between these countries. In doing this, I will pay attention to the strengths and limitations of the data and analyses employed in this thesis. Finally, I consider the implications of my conclusions for future areas of research.

10.1 Summary of findings

10.1.1 Objective 1: How is adiposity associated with carotid plaque burden?

My preliminary analysis using the Tromsø Study fourth and fifth survey (Chapters 6 and 7) had three main findings: First, there was some evidence of a cross-sectional association between adiposity and carotid plaque burden (presence of plaques, TPA) after adjustment for potential confounders (age, sex, smoking, education). Secondly, abdominal adiposity, especially WHR, seemed more strongly associated with plaque burden than general adiposity assessed by BMI. These associations were, to a large extent, mediated by cardio-metabolic CVD risk factors (SBP, LDL cholesterol, HDL cholesterol, glycosylated haemoglobin). These associations were confirmed in my prospective analysis (Chapter 7). Overall, the observed association was similar to the known association between adiposity and CVD events, reflecting that sub-clinical atherosclerosis is an intermediate outcome of CVD.

In my main analysis (Chapter 9), the formal test for interaction showed little evidence of a difference in the association between carotid plaque burden and adiposity between KYH and

Tromsø 7, implying that the impact of adiposity on carotid atherosclerosis is not significantly different between the two studies. In spite of little evidence of interaction, however, the association between carotid plaque and adiposity seemed slightly weaker in KYH than in Tromsø 7, which agrees with previous studies suggesting a weaker association between adiposity and CVD events in Russia compared to Western Europe (4, 116). Nevertheless, the 95% confidence intervals from the two studies overlapped substantially.

Results using the pooled data (KYH and Tromsø 7) were generally consistent with results of my preliminary analysis described above, despite using the plaque score based on the number of plaques instead of TPA. There was some evidence of an association between adiposity and carotid plaque burden (the presence of plaques, the plaque score), especially abdominal adiposity, which was mediated by cardio-metabolic CVD risk factors. This is expected because TPA and plaque score are correlated and behave similarly in terms of the association with CVD events (24).

10.1.2 Objective 2: Can adiposity explain the inter-study difference in carotid plaque burden?

Regarding the adiposity level in the two studies, the general adiposity level and the prevalence of general obesity in KYH women were much higher than in Tromsø 7 women whereas they were similar in KYH men and Tromsø 7 men (Chapters 8 and 9), which agreed with a previous study (30). Abdominal adiposity level and prevalence of abdominal obesity showed a similar pattern with considerably higher adiposity levels in KYH women than Tromsø 7 women and similar levels in men. However, the difference in abdominal adiposity level in women was even larger than in the general adiposity level. The prevalence of abdominal obesity was much higher than that of general obesity among KYH women, suggesting that using BMI alone to screen obesity might underestimate the actual obesity burden and obesity-related health risks in the population.

Regarding carotid plaque, use of a comparable protocol allowed us to make a reasonable comparison of plaque burden between the two studies. Consistent with the substantially higher burden of CVD in Russia compared to Norway, the prevalence and number of carotid plaques were consistently higher in KYH than in Tromsø 7, regardless of age group and sex (Chapter 9).

To the best of my knowledge, this is the first study to examine the contribution of adiposity to excess CVD burden in Russia. In my analysis, adiposity failed to explain the higher carotid plaque burden in KYH not only in men (who had similar adiposity levels to Tromsø 7 men), but also in women who had a considerably higher adiposity level in KYH than in Tromsø 7 (Chapter 9). The between-study difference remained large after further adjustment for cardio-metabolic CVD risk factors, strongly suggesting that there are factors other than adiposity that explain the differences in carotid atherosclerosis between the two studies, despite the fact that adiposity in both studies is a risk factor for carotid plaque.

10.2 Clinical/Public health implications of these findings: adiposity and atherosclerosis in Russia

In my analysis, KYH women had a higher level of adiposity than Tromsø 7 women, regardless of the adiposity measure used. Abdominal obesity was much more prevalent than general obesity in KYH women with almost half the participants being affected despite the fact WC was assessed at the narrowest part of the trunk which would make underestimation of the prevalence of abdominal obesity more likely. This suggests that monitoring of adiposity using BMI alone may underestimate the burden of obesity and obesity-related risks in this population. Regular monitoring/screening of both general and abdominal adiposity such as WC will be beneficial for a more accurate assessment of adiposity-related risks.

Our findings showed a higher plaque burden in the KYH population than the Tromsø 7 population, which is accords with the fact that the CVD mortality rate in working-age people in Russia is higher than in high-income European countries. There was some evidence of an association between adiposity and carotid plaque burden, which was not statistically different between KYH and Tromsø 7. This is reassuring because this result implies that through prevention and treatment of obesity, a positive effect on cardiac health might be expected in Russia, as has been shown in extensive evidence from high-income European countries, mainly through improvement of cardio-metabolic mediators or directly. An effective strategy to reduce the adiposity burden will contribute to reducing the high CVD mortality in Russia, and is likely to further reduce levels in Norway. Improved control of cardio-metabolic mediators such as hypertension and hyperlipidemia will also mitigate the negative impact of obesity in both countries.

A key conclusion of my thesis is that adiposity did not explain the difference in carotid plaque burden between the two studies after adjustment for confounders (age, education, smoking), especially in men. Furthermore, even after further adjustment for cardio-metabolic CVD risk factors, such as high blood pressure in KYH, the difference in plaque burden between the two studies remained large and statistically significant. This agrees with previous studies which showed an excess CVD mortality rate in Russia beyond traditional CVD risk factors (4, 5). An implication of this is that other factors play an important role in excess CVD burden in Russia. In addition to interventions to reduce adiposity level and other traditional CVD risk factors, the identification of risk factors explaining the high plaque burden will be essential to improve cardiac health in Russia. Alcohol, specifically harmful drinking patterns, are considered to be involved in CVD development in Russia and could be an important candidate (170, 207, 208). Other potential risk markers include psychosocial risk factors and diet.

10.3 Strengths and limitations

Two population-based studies with similar study periods and study protocols enabled me to directly compare adiposity burden, carotid plaque burden, and its association with adiposity in the studies with a contrasting CVD mortality rate. Although carotid plaque is receiving more attention as an imaging biomarker to predict CVD risk, not many population studies have quantitative carotid plaque variables. Even among the limited studies with carotid plaque variables, the comparison of plaque burden between studies can be challenging due to the different methods of assessing carotid plaque. Comparison of quantitative carotid plaque in this study provided insights into different atherosclerotic changes and their association with adiposity between the two studies.

However, the methods of assessing carotid plaque in KYH and Tromsø 7 are not fully comparable. As mentioned in detail in Chapter 5, a difference between the two studies in assessing plaque score may result in overestimation of plaque burden in KYH compared to Tromsø 7. However, as reported in Chapter 9, the results of a sensitivity analysis using a simplified plaque score, which is not affected by the information on the wall location of the plaque, showed results consistent with those using the original plaque score. Further, the prevalence of plaques (my other outcome, measured consistently in the two studies) was also substantially higher in KYH than in Tromsø 7.

Turning to the adiposity variable, the difference in WC measurement site between the two studies is another potential limitation. My calibration of WC in Tromsø 7 seems acceptable based on a conversion equation derived from relatively large Caucasian samples (161). However, the conversion using the equation does not allow for individual variability, which can introduce considerable imprecision. WC is a crude measure of visceral adiposity, and I am not aware of any previous study that shows either measurement site of WC is better than another in terms of the estimation of visceral adiposity and its consequences, including CVD events. Because neither WC is considered as a "gold standard" for assessing visceral adiposity, any correction of errors is not straightforward. Therefore, I chose a relatively simple approach and did not consider random variability when converting WC. A more advanced method is needed in terms of taking into account that neither of the WC measures is perfectly appropriate. Considering my simple approach to make two WC measurements comparable it is possible that differences in abdominal adiposity level between the two studies are under or overestimated. Although the importance of WC measurement has been well recognised, other studies, in addition to the current study, have used different measurement sites to assess WC. Standardisation of WC measurement protocols would be beneficial for interpretation and comparison of individual studies, which will assist the accumulation/synthesis of evidence.

Furthermore, I did not correct measurement error in adiposity measures because ICCs I obtained might not be suitable for this purpose due to true changes in adiposity measures which occurred between two measurements. Heterogeneity in true changes will result in ICCs being reduced and correction factors being overestimated. Besides, recent research shows excellent reproducibility of adiposity measures, including abdominal adiposity measures (161, 165). Nevertheless, the associations between adiposity and carotid plaque burden in this study may be underestimated due to regression dilution bias.

Another limitation regarding adiposity is the use of anthropometric measures. The three abdominal adiposity measures used in this study can provide information on fat distribution and compensate for the limitations of BMI. However, these measures cannot distinguish visceral adipose tissue from subcutaneous adipose tissue. Visceral adipose tissue is the most important player in obesity-related risk as it has a stronger association with CVD risk factors than subcutaneous adipose tissue (209). Although abdominal anthropometric adiposity measures correlated with visceral adipose tissue better than BMI, they still do not reliably

reflect visceral adipose tissue (83). Thus, ideally, the use of visceral adipose tissue as exposure will provide a more accurate picture of the association between adiposity and carotid plaque burden. However, it will require the use of MRI, DXA or CT scan, which, being more expensive and time-consuming, will probably not be suitable for large epidemiological studies consisting of healthy participants.

Regarding the adiposity measures, a further limitation lies in the lack of body composition data. As was described in Chapter 2, the use of body composition data, especially in terms of lean mass, can be informative for understanding the health conditions, particularly in the elderly population. Several studies found that decreased lean mass was associated with an increase in CVD events, coronary calcium score, and arterial stiffness (210-212). Considering that the participants in both KYH and Tromsø 7 are relatively old, information on lean mass and sarcopenic obesity may be valuable, and an investigation of the association between lean mass and carotid plaque will undoubtedly be interesting. However, body composition was estimated using BIA in KYH, and DXA in Tromsø 7, and making two body composition data comparable was challenging. Further work is needed to investigate the role of lean mass in CVD burden among the Russian population.

Finally, the statistical model in this thesis might have included other CVD risk factors such as physical activity and diet. In particular, physical activity is a crucial CVD risk protective factor. However, it was challenging to make physical activity variables comparable between the two studies. Further, as I mentioned in Chapter 5, physical activity is not simply considered as a pure confounder or a mediator regarding the association between adiposity and carotid atherosclerosis; physical activity is likely to be located in the upper stream of the causal pathway between adiposity and carotid atherosclerosis. Nevertheless, it might be interesting to conduct a sensitivity analysis to investigate the potential effect of physical activity on this association. A small sample of participants in both studies underwent an objective assessment of physical activity using heart rate and movement sensors. Sensitivity analysis using this objective measure of physical activity might be informative in the future. This objective measure could also be used to calibrate the two reported physical activity measures in the two studies.

Alcohol is another potential confounder which I did not include, although it may play a significant role in the CVD burden in Russia. As discussed in detail in Chapters 5 and 9, alcohol was not included in the model because comparable alcohol variables in the two

studies were not available when I obtained the dataset. In addition, it is questionable whether alcohol is considered as a confounder in the association between adiposity and carotid atherosclerosis: in spite of suggestive evidence between alcohol consumption and CVD events, it is still controversial whether alcohol is associated with atherosclerotic changes. Furthermore, one study showed the inclusion of hazardous drinking did not improve the SCORE performance in Russia (213), so the effect of alcohol on CVD might also be limited. Nevertheless, further research regarding the impact of alcohol is needed.

Height may prove to be an interesting variable for inclusion in the model. Observational studies show that short adult height is associated with a higher risk for CVD events (214). This finding is supported by recent Mendelian Randomisation studies (215, 216). It seems that not only are environmental factors such as poor nutrition and socioeconomic status associated with higher CVD risk but also the genetic disposition to short height. One possible explanation here relates to the diameter of the coronary artery. In simple terms, taller people have a coronary artery with a larger diameter, which makes them less susceptible to the blockage of the artery (217). Because the participants in KYH were significantly shorter than those in Tromsø 7 (for both sexes), it is perhaps worth exploring the contribution of height to the association between adiposity and atherosclerotic changes in the carotid artery.

10.4 Future areas of research

The findings from this thesis suggest two strategies to improve cardiac health in Russia: 1) to monitor, prevent, and treat obesity in Russia to reduce the carotid plaque burden 2) to explore the critical risk factors explaining the excess carotid plaque burden in Russia. This identifies four domains of research questions to explore in the future.

First, regular monitoring of adiposity in a general population in Russia will be necessary to estimate the accurate burden of obesity and trends in Russia. Since Russia is such a massive country, the population in KYH cannot be considered as being fully representative of the whole nation. Besides, KYH just provides a screenshot of the adiposity level instead of following its prospective change. Accurate and prospective epidemiological data from more extensive areas will be a foundation for implementing the effective intervention. The use of BMI alone may result in underestimation of the obesity burden and obesity-related risks. The assessment of abdominal adiposity will compensate for the limitation of BMI, and help to capture the burden of obesity more precisely.

However, it should be noted that the standardised protocol of waist measurement is important. Although two recent population studies provide the prevalence of abdominal obesity in Russia (65, 191), the use of different adiposity measures (WC vs WHR) and different measurement sites (the mid-point between the top of iliac crest and the lower rib vs umbilicus site) makes the interpretation/comparison of the abdominal adiposity level difficult. In particular, the prevalence of abdominal obesity is considerably affected by the difference in measurement site of WC because the cut-off point is fixed (men: >102 cm, women>88 cm) regardless of the measurement site, which can lead to misleading outcomes (161). Monitoring abdominal obesity using a standardised WC assessment protocol, combined with proper staff training to reduce measurement error, is vital to providing comparable data among different sites.

Second, to prevent and treat obesity in Russia, understanding of regional determinants of obesity will be crucial because they may be different from those in Western Europe. In Western Europe, generally, a low adiposity level is associated with health-conscious behaviours such as high educational attainment, increased physical activity, and healthy diet. However, this pattern might not be applicable to the Russian population. For example, a previous study suggests that high educational attainment is not significantly associated with a decrease in obesity level in Russian men, unlike Western European men (190). A relatively weak association between adiposity and plaque burden observed in KYH might imply that Russian participants with low adiposity levels have different characteristics from Norwegians; for example, being more likely to engage in unhealthy behaviour.

A few studies have investigated the determinants of obesity in Russia (189, 218). However, these data are relatively old and may not be relevant today. Investigation of the determinants using contemporary data will be needed. Since the KYH Study has data on most of the potential determinants of obesity, such as detailed physical activity variables and educational attainment, it will be a useful dataset for further exploration. However, the limitation of the KYH Study for this purpose is that information on diet and nutrition is limited to a simple diet quality score. A previous study pointed out that diet might be an important factor to determine obesity in Russia (219). Russian diet seems different from Western European diet, being characterised by high animal food and fat and low fruit consumption (220). An investigation into the impact of diet and nutrition on obesity based on a detailed diet questionnaire would be informative.

Third, although this cross-sectional study can serve as a baseline study to explore the role of adiposity on carotid atherosclerosis in Russia, further research with better study design will be needed to confirm our findings. The prospective association between adiposity and carotid plaque burden will be important to estimate the longitudinal impact of adiposity on carotid atherosclerosis. In particular, the impact of the change in adiposity level on the progression of atherosclerotic change will be clinically informative. A study using visceral adipose tissue assessed by MRI, CT, and DXA instead of anthropometric measures will provide a more accurate estimation of the effect of adiposity. However, a study regarding adiposity, no matter how well designed, will be susceptible to residual confounders. Mendelian Randomisation studies may help to overcome this problem and elucidate this association more clearly. For example, investigation of the association of genetic score of BMI and WHR with carotid plaque variables might provide better information on a causal pathway between carotid atherosclerosis and adiposity, and reduce the effect of residual or unmeasured confounders.

Finally, since adiposity failed to explain the higher carotid plaque burden in Russia, determinants for excess carotid plaque need to be explored. Potential risk factors such as alcohol consumption, diet, and psychological factors should be explored. Quantitative carotid plaque variables could be useful outcomes in future studies.

10.5 Concluding remarks

The disproportionally high CVD burden in Russia compared to high-income Western European countries must be addressed. The rise in obesity prevalence is an increasing concern for both high-income Western European countries and Russia and may lead to further CVD burden in Russia. The current study provides insights into the impact of general/abdominal adiposity on carotid atherosclerosis in Russia compared to Norway, and the contribution of general/abdominal adiposity to the excess carotid plaque burden in Russia. The abdominal adiposity level seemed more problematic than the general adiposity level in Russian women. The burden of carotid plaque was consistently higher in both sexes in Russia, which agreed with the high burden of CVD in Russia. Although no adiposity measure was a determinant of excess carotid plaque burden in Russia, adiposity, especially abdominal adiposity, seemed to have a similar negative impact on carotid atherosclerosis in the two populations, suggesting that prevention and treatment of obesity will be as beneficial to reducing the CVD burden in Russia as it has been in high-income European countries.

Therefore, even if adiposity makes little contribution to explaining inter-country differences in CVD risk, the level of adiposity in both countries needs to be reduced to lessen the burden of CVD. In Russia, epidemiological data on obesity are limited compared to Norway and other CVD low-risk countries. Therefore, in addition to a recent attempt to provide the prevalence of abdominal obesity, regular and prospective monitoring of both general and abdominal obesity will be beneficial in Russia. Moreover, an investigation of the determinants of obesity in Russia will help to implement effective interventions to reduce the obesity burden in Russia. Further research on the prospective association between adiposity and carotid plaque burden will be needed to confirm these findings.

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Appendix A

A.1 Systematic literature review search term

Search term of systematic literature review

1. Obesity-related terms: Obesity OR Overweight OR Body Mass Index OR "Body Weights and Measures" OR obes* OR obesity OR BMI OR Body mass index OR Body-mass index OR Weight for height OR Weight-for height OR Body fat OR Body fat percent* or Percent* body fat OR Fat mass OR Adiposity OR Waist circumference OR Waist measurement OR Waist-Hip ratio OR A Body Shape Index

AND

2. Carotid ultrasound related terms: Carotid Intima-Media Thickness OR Intima*media thickness OR Carotid intima*media thickness OR Arterial thickness OR Arterial wall thickness OR carotid plaque OR plaque size OR plaque echogenicity

AND

3. Adult

AND

3. human

A.2 Systematic literature review: Data extraction sheet

*Reference (first author/year):

*country:

*Study design:

*year:

*characteristic of population

Sample size total	
N male	
N female	
Age lower range	
Age upper range	
Age mean	
ethnicity	
Source of sample	
Study population	
other	

Source of sample: 1. random sample 2. Non-random volunteer 3. Non-random other 4. Not clear

Study population: 1. community 2. health check-up 3. Specific occupation 4. other

*measure of adiposity (only one used in analysis)

adiposity	BMI	WC	WHR	WHtR	other	other
Mentioned?						

Objective measurement?						

DEXA	bioimpedance	CT	MRI	

*IMT measurement:

1.R 2.L 3.Both	
1.CCA 2.Bulb 3.ICA	
1.far 2.near wall 3.both	
Angle(single/multiple)	
Cardiac cycle (1. Diast 2.other 9.?)	
Max/average/both	
IMT calculation	
Use of software (0.1)	
definition	
other	
Assessor blinding	

*Plaque measurement:

1.R 2.L 3.Both	

1.CCA 2.Bulb 3.ICA	
1.far 2.near wall 3	
definition	
Mannheim Consensus	
Use of software	
Quantitative? (0.1)	
Quantitative measure	
Assessor blinding	

*Result

Total population:

Obesity/overweight prevalence:

Plaque prevalence:

Population IMT:

Prevalence of thick IMT:

History of CVD:

Used adiposity var: binary/categorical/con	
Used IMT var: binary/con	
Association mentioned 1 (adiposity and IMT/plaque)	
association	

Adjusted for	
(Statistical method)	
Association mentioned 2	
association	
Adjusted for	
(Statistical method)	
Association mentioned 3	
association	
Adjusted for	
(Statistical method)	

Male:

Obesity/overweight prevalence:

Plaque prevalence:

Population IMT:

Prevalence of thick IMT:

Used adiposity var: binary/categorical/con	
Used IMT var: binary/con	
Association mentioned 1 (adiposity and IMT/plaque)	
association	
Adjusted for	
(Statistical method)	
Association mentioned 2	

association	
Adjusted for	
(Statistical method)	
Association mentioned 3	
association	
Adjusted for	
(Statistical method)	

Female:

Obesity/overweight prevalence:

Plaque prevalence:

Population IMT:

Prevalence of thick IMT:

Used adiposity var: binary/categorical/con	
Used IMT var: binary/con	
Association mentioned 1 (adiposity and IMT/plaque)	
association	
Adjusted for	
(Statistical method)	
Association mentioned 2	
association	
Adjusted for	
(Statistical method)	

Association mentioned 3	
association	
Adjusted for	
(Statistical method)	

Conclusion:

*Note:

This article is..

Reference to check

A.3 Systematic literature review: quality assessment sheet

Quality assessment criteria	Acceptable(*)	Yang	Yeboah	Youn	Yun				
Selection		1	1	2	1				
Representativeness of the sample	Truly representative of the average in the target population. Somewhat representative	-	*	*	-				
Sample size	Justified and satisfactory	-	-	-	-				
Non-respondents	Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory	-	-	-	-				
Ascertainment of the exposure	Validated measurement tool	*	-	*	*				
Comparability		2	0	2	2				
The study controls for the most important factor(age/sex?)	Yes	*	-	*	*				
The study controls for any additional factor		*		*	*				
Outcome		3	2	2	2				

Assessment of the outdcome	Independent blind assessment** Validated measurement*	**	*	*	*				
Statistical test	The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including CI and p-value	*	*	*	*				
Overall quality score		6	3	6	5				

A.4 The measurement of anthropometric measures (extracts from Know Your Heart protocol)

10.0 Waist and Hip Circumference

10.1 Preparation

7.1.1 Equipment needed

- Seca measuring tape

10.2 Procedure

10.2.1 Explain to participant you are now going to measure their waist and hip circumference.

10.2.2 Ask participant to remove bulky clothing and bulky objects from pocket. Participant should be wearing only 1 layer of thin clothing. Give them plastic tray for any objects they may have in their pockets.

10.2.3 Participants should lift up the top half of their clothing (i.e. so participant naked at the waist and measurement is done on bare skin, not over clothes) to make it easier to assess position of the waist. Ask participant to stand with feet one foot apart and with arms folded across the chest. This is to keep arms out of the way during measurement. The participant should continue to hold their clothes up during measurement.

10.2.4 Standing to the back of the participant, identify the waist as the smallest part of the trunk (i.e. natural indent). If it is not possible to locate the natural indent of the trunk, identify the umbilicus ("belly button") and measure the circumference at this level.

10.2.5 Working side on to participant hold the seca measuring tape in your left hand and pass the tape measure around the participant's body with your right hand. Check tape measure is horizontal and not twisted. Insert the white plastic end of the tape into the groove of the housing, and ask participant to relax and breathe in and out slowly. The button on the measuring tape should be facing upwards.

10.2.6 While participant is breathing out, tighten or loosen measuring tape using button catch of the spring coil. Tape should fit comfortably around the participant without feeling either loose or tight. As participant slowly breathes out, but before they breathe in again record waist circumference on the proforma. **Measure in centimetres accurate to the nearest millimetre - if it falls between two millimetres round upwards.**

10.2.7 With participant remaining in the same position slide the seca measuring tape to the widest part of the hips, checking it is horizontal and not twisted. Record hip circumference on the proforma. **Measure in centimetres, accurate to the nearest millimetre – if it falls between two millimetres round upwards).**

10.2.8 Repeat measurement of both waist and hip circumference and record on the proforma. Two measurements of both waist and hip circumference should be recorded in total.

11.0 Height

11.1 Preparation

11.1.1 Equipment

- Seca Portable Height measure

Assemble height measure before starting.

11.2 Procedure

11.2.1 Explain to participant you are going to measure their height.

11.2.2 Participant should be barefoot.

11.2.3 Ask participant to stand with their back against the vertical scale, feet parallel to each other, toes pointing forward and soles flat on the floor. Check participant is standing unsupported, with legs straight and with buttocks and shoulder blades touching the vertical scale. Their shoulders should be relaxed, with arms by sides and they should not be slouching or leaning to one side

11.2.4 Ensure participant has no neck problems or recent neck surgery prior to positioning head then ask participant if it is ok to position their head. If so, gently position participants head so they are looking straight forward (not nose in the air), with their ear holes in the same horizontal plane as the lower border of their eye sockets (Frankfurt Plane).

11.2.5 Ask participant to stand as tall as possible and take a deep breath in. While they are doing this bring the horizontal measure down

on top of their head. Record measurement on proforma in centimetres, **accurate to the nearest millimetre (if falls between two millimetres round upwards).**

11.2.6 Repeat height measurement and record in the proforma (2 height measurements in total). Ask participant to walk around room in between height measurements.

12.0 Weight, body composition analysis

12.1 Preparation

12.1.1 Equipment

- Tanita body composition analyser
- Laptop with the software “Tanita”

The platform for the Tanita body composition analyser should be wiped with disinfectant wipe between participants. Metal handles of the Tanita body composition analyser should be wiped with disinfectant wipe between participants.

12.2 Procedure

12.2.1 Ask participant if they have a pacemaker or if female if they are pregnant.

If answer is "yes" explain to the participant you are going to measure their weight and switch body composition analyser to weight only mode (go to section 12.4).

If answer is "no" explain to the participant you are going to measure their weight and assess body composition. Explain analyser measures body

composition by passing a small electric current through body. If participant wishes to undergo this procedure then use body composition mode (section 12.3). If participant does not wish to undergo this procedure then use weight only mode instead (section 12.4)

12.3 Measurement using Tanita body composition analyser (Standard)

12.3.1 Ask the participant to remove their shoes and socks/stockings/tights. Participants should be barefoot. Bulky clothing and bulky item should have been removed from pockets. Participant should be wearing only 1 layer of thin clothing. (see section 10 on measurement of waist circumference). Take measurement in body composition mode

12.3.2 Enter information on clothing weight (1 kg). In the "name" field, fill in the participant ID (no not fill in the actual name).

12.3.3 Ask participant to place their bare feet on analyser platform's markings and not to move their feet. Ask the participants to use their hands to grip the two metal handles firmly with arms hanging loosely by sides. Participant's arms should not touch their sides and inner thighs should not be touching.

12.3.4 Enter information on:

- Body type (designate as standard)
- Gender
- Date of birth (15th of the month/ month /year not actual date of birth)
- Height (cm)

12.3.5 The analyser measures weight first. After measuring weight ask the participant to firmly hold on to the two metal handles, their hands need to hang freely at their side. The analyser emits a "beep" each time it measures body impedance (make sure beep setting is on). It emits a "beep beep" noise to indicate it has finished measuring both weight and body impedance.

12.3.6 Ask participant to step off device.

12.3.7 Save results of body composition analysis directly to laptop (no paper record). If there is a problem with saving to the laptop a paper printout should be obtained directly from the Tanita device.

12.4 Measurement of weight only (alternative method)

Note: Use this method if participant has a pacemaker, is pregnant or refuses body composition measurement.

12.4.1 Explain to the participant you are going to measure their weight. Participant should remove footwear heavy jewellery, bulky clothing and bulky item should have been removed from pockets. Participant must be barefoot. Participant should be wearing only 1 layer of thin clothing (see section 10 on measurement of waist circumference).

12.4.2 Participants should stand with weight equally distributed on legs, looking straight ahead, arms hanging loosely by their side not holding on to anything.

12.4.3 Make sure the body composition analyser is switched to weight only mode, perform and record measurement of weight.

12.4.4 Record weight measurement in the proforma **accurate to the nearest 0.1 kg**

12.4.5 Ask participant to step off scale.

A.5 The mean value of waist circumference before and after the conversion in Tromsø 7 visit 1 participants

	Men		Women	
Age category (year)	Original umbilicus WC Mean (SD)(cm)	Converted Minimal WC Mean (SD)(cm)	Original umbilicus WC Mean (SD)(cm)	Converted Minimal WC Mean (SD)(cm)
40-44	98.58 (11.79)	95.96 (11.14)	88.40 (12.89)	80.05 (11.63)
45-49	99.87 (11.80)	97.64 (11.14)	90.03 (13.69)	81.67 (12.35)
50-54	99.64 (11.25)	97.90 (10.63)	89.42 (12.99)	81.28 (11.72)
55-59	100.29 (10.65)	98.99 (10.06)	91.31 (12.42)	83.15 (11.20)
60-64	100.34 (11.08)	99.52 (10.48)	91.22 (12.42)	83.23 (11.20)
65-69	101.09 (11.37)	100.69 (10.75)	92.16 (12.59)	84.23 (11.36)

WC: waist circumference

Umbilicus WC: measured at the level of the umbilicus, minimal WC: measured at the smallest part of the trunk

Original umbilicus waist circumference was converted using the following conversion equations taken from Mason et al.

Women: -1.02517 + 0.03207*age + 0.90184*original waist

Men: -1.19141 + 0.09503*age + 0.94491*original waist

A.6 Protocol for ultrasound examination

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Study of Cardiovascular Disease in Russia

Title: Standard Operating Procedures for Ultrasound Image Acquisition			
Document number: SOP9		Version number:1.6	
Creation Date: 08/11/2013		Number of pages: 24	
Comment: This SOP is based on draft imaging and instrumental protocol produced in Novosibirsk 23/5/12 Name:Andrey Ryabikov, Sofia Malyutina			
Content originators	Name: Andrey Ryabikov, Sofia Malyutina	Signed:	Date:
SOP Production	Name: Sarah Cook	Signed:	Date:
Content Approval	Name:	Signed:	Date:
Contact: sarah.cook@lshtm.ac.uk			

Amendment Record				
Version number	Date	Changes Made	Reasons for Changes	Changes made by
1.1	20/11/13	Incorporation of comments from Darryl Leong and Alun Hughes	Further development of protocol	Sarah Cook
1.2	24/11/13 20/01/14	Incorporation of comments from Andrey Ryabikov, Sofia Malyutina	Further development of protocol	Andrey Ryabikov, Sofia Malyutina
1.3	12/02/14	Incorporation of comments from project meeting Meeting points from Henrik Schrimer, Elisiv Mathiesen, Darryl Leong,Alun Hughes, Andrey Ryabikov, Sofia Malyutina	Further development of protocol	Andrey Ryabikov, Sofia Malyutina
1.4 (1)	24/04/14	Incorporated comments from GE London (7.1; 9.1-9.3)	Further development of protocol	Natan Simcox
1.4 (2)	26/06/14	Incorporated comments from Nov training session	Further development of protocol	Andrey Ryabikov, Sofia

		Points form Nov team (Tab 1, Tab 4; 7.1; 7.2;7.4 7.5;9.0);		Malyutina
1.4	28/09/14	Incorporated comments from GE Moscow (9.2-9.3)	Further development of protocol	Denis Rusakov, Ilya Botvin
1.4	24/10/14	Comments from Tromsoe	Further development	H. Schirmer
1.5	06/03/15 04/08/15 10/09/15	After Tromsoe comments & after Pilot	Further development	Andrey Ryabikov, Sofia Malyutina
1.6	12/01/16	Patient's initials format; AUS update (NOV optional US study); Minimal clarification Echo (Frame rate, PW AoV); VUS preferable position	Further development	Dave Leon comments Andrey Ryabikov Sofia Malyutina

1.0 Purpose

The purpose of this SOP is to provide the correct procedure for ultrasound examinations.

2.0 Responsibilities

3.0 Definitions and abbreviations

Terminology	Definition
SOP	Standard Operating Procedure
ECHO	Echocardiography
VUS	Vascular Ultrasound
AUS	Abdominal Ultrasound
QC	Quality Control
CRL	Central Reading Laboratory

LAN	Local Area Network
IQC	Internal Quality Control
EQC	External Quality Control
IMT	Intima Media Thickness
CCA	Common carotid artery
ICA	Internal carotid artery
ECA	External carotid artery
IVC	Inferior Vena Cava
Nov	Novosibirsk
Arkh	Arkhangelsk
SW	Software
AF	Atrial Fibrillation

4.0 Staff, training, quality control

4.1 The staff for ultrasound team should satisfy formal criteria: to be certified physicians in Ultrasound with experience in ECHO and vascular ultrasound; optionally in Novosibirsk - should also have experience in abdominal ultrasound. The number of specialists should be at least 4 per centre to ensure examination 2 terms a day and potential substitution. 1 medical nurse is needed to assist ultrasound exam at each site/district within participating centre.

4.2 The team should read SOP and organize at least one local “hands-on” training for standardized ECHO and vascular examination under supervision of local senior specialist (AR –Nov, AK-Arkh). Each observer should prepare 1 test record for review in Central Reading Lab (CRL, Nov).
Test record should correspond to the following criteria:
- fulfilled on digital ultrasound machine (GE’s Vivid family preferable). Otherwise the scanner selection among available is to be advised by CRL supervisor (AR);
- include still images and cine loops for Echo/VUS/AUS(Nov) depending on observer’s responsibility in the project;
- correspond to SOP according to image acquisition (required views and modalities), system setting and image quality;
- recorded in original raw format for Vivid or in DICOM (other machines), otherwise recorded as sequence of files containing still images (*.jpg) and cine loops (*.avi; *.mpeg);

- records from each observer should be collected in one folder and followed with inventory file containing Centre number, observer ID number, type of Exam, date of record, list of image files;
- the folder(s) are to be saved on DVD disks and sent to CRL for qualification review; DVD should be marked with permanent marker: indicate number of disk, centre name, personal ID's of observers included, and mark as "Test Records" project into cover box from physical damage;
- CRL should fulfil review of test records and provide "Quantification ECHO/VUS QC scan review";

4.3 The team will attend centralised training sessions and read SOP. Training for all ECHO, VUS (and AUS) observers and readers should take place in a central location (e.g. headquarters of the GE company in Moscow). The sessions will include "hands-on" training for standardized acquisition/ standardized ECHO and vascular examination procedure/data storage.

4.4 Timelines:
- test records preparing and sending to CRL - April 2014, CRL review – May 2014);
- centralized training session – 1-2 October 2014;

4.5 Internal quality control (IQC) – senior specialist at site will monitor the technical quality of ultrasound performance from all specialists in pre-pilot period, in pre-survey period and once a month across survey period; encode study quality in IQC worksheet;

4.6 External QC (EQC) – the supervisor(s) from CRL (Novosibirsk) and from comparative centre (Tromso) will visit all centres in 3 months after field work started, then once a year during the course of study.

5.0 Related documents

5.1 SOP for vascular ultrasound
5.2 SOP for EchoCG (ECHO)
5.3 SOP for abdominal ultrasound
5.4 SOP for image analysis
5.5 Table with cine loops to be acquired and their sequence for use during examination

6.0 Health and Safety

7.0 Ultrasound Image Acquisition

7.1 **Preparation**

7.1.1 Equipment needed:

- Ultrasound machine (Vivid q)
- Correct probe for Ultrasound (phased array Sector probe M4S-RS for ECHO, Linear probe 12L-RS for VUS, Convex probe 4C-RS for AUS)
- Desktop/Laptop workstation running EchoPAC SW
- High capacity external HD/USB memory stick
- Examination couch
- Lubricating jelly, tissue towels, disposable linen (sheets)
- Electrocardiogram leads and disposable electrodes

7.1.2 Setting up machine.

- Linear probe should be attached to the scanner before plug in *(Vivid q can only have 1 probe attached at a time)*
- Turn on the power on main unit, wait till machine complete it's loading

7.1.3 Log on as USR (No password required), select "Archive" and "Create new patient".

- Upload patient's ID number with barcode scanner: place the blinking cursor in the {Patient ID} box & scan the barcode by reader (this should fulfil the Patient ID box with the details contained in the barcode), check this with paper copy).
- Add to Patient's ID suffix with a "V" for Vascular, "E" for Echo, "A" for Abdominal exam.
- Enter patient's Initials ; Birth date in the format *15/mm/19yy*; enter an operator's personal ID, enter appropriate {Echolab} as "Nov" or "Ark" and select examination type (start with "Vascular"); then push "Start exam" button on the screen.
- [Application] – select the {12L-RS / M4S-RS / 4C-RS} probe, then the {Project name} preset. Change to 'AF' preset if patient is in atrial fibrillation (AF).

- At the end of the Vascular procedure press [Patient], {Create New Patient/Exam} and repeat the procedure above to create a new patient ID for the Echo / Abdominal exams.

7.1.4 Linear barcode IDs should be used throughout. Study ID consists of 7 digits (centre-specific digit which allow identification of study centre by co-ordinators, check digits, order number and reporting unit number).

7.2 **Timing**

Diagram of the ECHO & US consequence and timing (totally 75 min per participant).

i. Patient preparations (5 min)	*ii. Vascular Ultrasound* (20 min)	*iii. BP measurement** (5 min)	*iv. EchoCG* (30 min)	*v. Abdominal Ultrasound* (10 min)	*vi. Data archiving* (5 min)

*For BP measurement use dominant arm (the same arm as during survey).

7.3 **Participant Preparation**

7.3.1 Explain to participant you are now going to carry out an ultrasound of their blood vessels, heart and abdomen. This should take approximately 1 hour.

7.3.2 Ask participant to remove top half of their clothing.

7.3.3 Position participant appropriately.

7.3.4 Place 3 electrocardiogram leads on the participant's chest wall in positions that will not interfere with positioning of the ultrasound probe during image acquisition. In general, leads should be placed as follows: 1 near the right shoulder, 1 near the left shoulder and 1 on the lower ribs near the *right* abdomen. The most important is that a clear QRS signal is seen *on the screen* with no noise in the signal.

7.4 **Vascular Ultrasound Procedure**

7.4.1 Vascular carotid ultrasound (VUS) should be performed first (before ECHO)

7.4.2 The VUS examination should take approximately 20 minutes

7.4.3 In addition to this SOP sonographers should be given a table 4 detailing the cine loops and still images to be recorded and their sequence which can be used during the VUS

7.4.4 Attach ECG leads with stable ECG curve on the screen, provide high voltage

7.4.5 Patient position: *supine*

- Thin (half empty) pillow could be used as under-neck support to achieve moderate extension in short-necked; it also helps on muscular compliance in stooped patients
- Light head rotation opposite to examined site is recommended to improve submandibular acoustic window (rotation angle is unspecified)

7.4.6 *Both sides will be examined (Left & Right)*

- always start from the left
- repeat for right side

7.4.7 Image acquisition:

-*B-mode* is a basic regimen for all obligatory images and videos.
-Harmonics and Colour flow Doppler could be used for better plaque detection and defining its surface.
-Preparing to measure intima-media thickness:
-10 consecutive cardiac cycles should be acquired for each image (in case of atrial fibrillation or otherwise)
-Use for visualization that probe position which is closer to the vessel and where intima/lumen interface is better discernible
-indicate in a commentary sheet probe position (A-anterior, L-anterolateral, P-posterolateral)
-probe should be perpendicular to the intima-media surface
-use highest probe frequency (not sacrificing clear visualisation)
-only 1 focal zone is required (to increase frame rate)
-frame rate (FPS) should be the highest (not less than 40/sec if "Vivid *q*" is used)
-don't use 2nd harmonics for IMT video clip.

-don't use "Virtual convex" (or "Wide view" or "Trapezoid view") option
-use magnified images with high frame rate (but not zoomed images)
-The following images will be acquired with the participant in supine position:

7.4.8 *Longitudinal view (B-mode)*:

- ECG-gated longitudinal view of at least **10-mm at the far wall of the distal CCA** for further measurement of IMT and interadventitial diameter, preferably in plaque-free zone (Video clip)
- Fast scanning of common carotid arteries (CCA) with branches whatever is visible to assess the anatomy and atherosclerotic lesions (video clip of CCA, bulbus, ICA, ECA).
- Plaque is defined as a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or demonstrates a thickness 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface *(Mannheim Consensus, 2006).*
- In case of suspected plaque and/or intima bulging freeze the image; still image and additional video should be stored to assess plaque length and morphology

7.4.9 Turn the probe 90 degrees clockwise for left side and counter clockwise for the right to acquire transverse views.

7.4.10 *Transverse view (B-mode)*:

- **Repeat careful scanning** of CCA with branches whatever is visible to assess the anatomy and atherosclerotic lesions (video clip of CCA, bulbus, ICA, ECA). In case of plaque and/or local thickening freeze the image; still image should be stored to assess plaque circumference at most prominent segment

7.4.11 **Complete the procedure by BP measurement on dominating arm (in clinostasis)**

7.5 **ECHO Procedure**

7.5.1 The ECHO examination should take approximately 30 minutes

7.5.2 In addition to this SOP sonographers should be given a table detailing the cine loops and still images (Table 1) to be recorded and their sequence which can be used during the ECHO.

7.5.3 Patient position: *left decubitus*

7.5.4 Image acquisition:

For all 2D images, optimisation of gain, depth and sector width settings should be performed to maximise endocardial and epicardial definition and to achieve a frame rate at least 50/sec. Frame rate above Only 1 focal zone is required (to increase frame rate). Keep 2D image frozen in M-mode and spectral Doppler recording (PW, TDI). Always use breath hold to optimize image quality for TDI measurements; for other images, only if necessary.
If the participant is in atrial fibrillation, 10 cardiac cycles should be acquired for each image; otherwise 5 *consecutive* cardiac cycles should be acquired.
The following images will be acquired

The manipulations are arranged by approach order and by technology.

Parasternal long-axis.

- 2D images of the left ventricle (LV), aortic root and left atrium (LA).
- B-guided M-mode of the basal left ventricle. The image acquisition plane should be as close as possible to perpendicular to the long-axis of the LV. M-cursor is positioned anatomically below the tips of mitral valve cooptation point, on mitral chordae.
- Parasternal long-axis M-mode of aorta (AO) and left atrium. The image acquisition plane should be as close as possible to perpendicular to the long-axis of the LA.
- Colour-flow Doppler recordings to search for **MR** and **AoR.**
- Parasternal long-axis 2D with depth adjusted to enlarge image of the aortic annulus *together with adjacent left ventricle outflow tract (do not use Zoom function; increase frame rate by reducing sector width).*

Parasternal short-axis.

- 2D level of aorta and left atrium. PW Doppler recording in pulmonary artery.
- Optionally: 2D of LV in appropriate levels if myocardial asynergy present.
- Repeat **Parasternal long-axis** B-guided M-mode of the basal left ventricle.

4-chamber apical view.

- 2D of LV and LA with maximal chamber dimensions, good LV endo-/epicardial definitions, and including right chambers.
- Colour-flow Doppler recordings of *mitral valve*.

- Pulsed-wave Doppler with the sample volume positioned in LV cavity at the mitral leaflet tips.
- Colour-flow Doppler recordings in *tricuspid valve* and CW Doppler recordings for detection of tricuspid regurgitation, if any (adjust CW velocity scale lower, start with 3-4 m/sec at least).

Off-plane 4-chamber apical 2D image of right ventricle (RV). View focusing on the RV between 4- and 5-chamber view (showing the maximum RV transversal diameter). The depth adjusted so that tricuspid annulus is at the bottom. *Imaging should be performed during breath hold.*

Off-plane 4-chamber apical

- M-mode with the imaging plane through the *lateral tricuspid annulus*. For this image, the position of the heart within the imaging sector should be such that the plane of movement of the lateral tricuspid annulus is as close to vertical as possible. Use the patient's breath hold to avoid excessive heart movement.

3-chamber apical view;

- continuous-wave Doppler (CW) with the cursor placed in mainstream of AO transvalvular flow.
- 3-chamber apical; pulsed-wave Doppler (PW) with the sample volume placed in the centre of the left ventricular outflow tract with just one of two leaflet closure signals present.

4-chamber apical 2D image of the left ventricle, with the depth setting adjusted so that the mitral annulus is at the bottom of the sector throughout the cardiac cycle, and with the sector width adjusted to encompass the entire thickness of the left ventricular myocardium *with the frame rate within 50-80 fps*.

2-chamber apical 2D image of the left ventricle, with the depth setting adjusted so that the mitral annulus is at the bottom of the sector throughout the cardiac cycle, and with the sector width adjusted to encompass the entire thickness of the left ventricular myocardium *with the frame rate within 50-80 fps and with the anterior wall optimally visualized, if necessary during breath hold*.

4-chamber apical 2D image of the left atrium, with the depth setting adjusted so that the left atrial roof is at the bottom of the sector throughout the cardiac cycle, and with the sector width *narrowed* to encompass *only* the entire left atrium. Change focus position accordingly. *Frame rate is to be within 50-80 fps*

2-chamber apical 2D image of the left atrium, with the depth setting adjusted so that the left atrial roof is at the bottom of the sector throughout the cardiac cycle, and with the sector width slightly *narrowed* to encompass the entire left atrium. *Frame rate is to be within 50-80 fps*

4 chamber apical; Mitral Annular Doppler Tissue Imaging: the DTI PW Doppler sample volume is placed at the medial (**septal**) corner (5 beats) and, subsequently, at the **lateral** corner of the mitral annulus (5 beats). 'Tilt' function could be used for better alignment between cursor and wall. (Optimally this is achieved by changing probe position). Always keep *breath hold* at expiration.

7.6 **Abdominal ultrasound***

** Abdominal sonography is to be performed in NOV only (optional for Arkhangelsk)*

8.0 Transducer Cleaning

8.1 All transducers must be cleaned after every use.

8.1.1 After every exam, ensure the acoustic coupling gel is completely wiped off the transducer. Transducers should not be left soaking in gel

8.1.2 Remove any transducer cover, biopsy guides, or protective devices from the transducer.

8.1.3 Use a moistened soft cloth or wipe to remove any remaining contaminants that remain on the transducer or cable. Do not re-use cloths or wipes. Soap, detergents or enzymatic cleaners should be used in accordance with the manufacturer's instructions. (Notes: Cleaning products should be as close to neutral PH as possible. Any gel, cleaning or disinfectant products containing concentrations surfactants, methanol, ethanol, benzyl or methyl alcohol, bleach, methyl or ethyl paraben, polyethylene glycol, mineral oil, lubricant oil, oil based lotions, acetone, ammonia, anhydrous ammonia, iodine, iodine compounds, acids with 5PH or greater may damage or discolour your transducer. The use of any type of brush is not recommended as bristles may damage lens materials. Ultrasonic cleaning should not be performed.

8.1.4 If rinsing is required, use caution not to expose the system connector to moisture or liquids.

9.0 Data storage and transfer

9.1 All exams (images/cine loops) should be stored in RAW format on machine's internal HD and copied to Workstation running EchoPac SW (capacity 0.5 Tb) located in ECHO room. Files should be backed up at regular basis (weekly).

9.2 **Coping of examinations from the scanner to EchoPAC PC done offline**

The procedure is to be done by dataflow manager or supervisor at regular basis (weekly). There are two options to copy data : using external HD /Memory stick OR by direct connect or network with EchoPac Workstation. When reconnected, copy the data (images, demographics, measurements and report) for the examination(s) done offline to USB HD and EchoPAC PC by the following steps:

1. Press **PATIENT** on the Front panel, then select **Patient List**.
The *Search/Create Patient window* is displayed
2. Select the source archive in the *Dataflow field*: **Local Archive-Int.HD**: exports data from the local archive.).
3. Press **Export** in the *Search/Create Patient window*.
4. Select the following destination from the *Destination drop-down menu*:
   - **USB HD/Memstick Archive** (to copy to USB HD/Memstick) or
   - **Remote Import/Export Archive** (to copy to EchoPAC PC by direct connect or network): exports raw and DICOM (if present) data.

5. To **FORMAT** USB HD/ Memstick as required.
Insert the USB external HD (or USB Memory Stick) and press {Config} οτ the panel, go to {Connectivity}, then to the {Tools}, select {USB Memstick} from the dropdown menu; insert the name into the "Label" box (if needed), press {Format}, press {ok}.

6. Search and Select the patient records/examinations to export in the *Patient list*.
The following selection methods can be used:
- Press and hold down **SHIFT** while selecting patient records/examinations to select several consecutive items at a time.
- Press and hold down **CTRL** while selecting patient records/examinations to select several discrete items.
- Press **Select all** in the *Export patient window* to export all patient records.
7. Adjust the following settings:

• Tick **Copy images**
• Caution: **NOT tick "Delete selected patient(s) after copy"!**
8. Press **Copy**.
If one or more patient examination is already present in the destination archive the *Export/Import conflict window* is displayed. For each conflicting item, select:
Keep: to keep the existing examination in the destination archive.
Replace: to replace the existing examination with the corresponding item in the source archive.
Press **OK** to resume export.
A progress indicator is displayed. When done a status window is displayed showing the number of patient records that have been successfully exported.
9. Press **OK**.
A check mark is displayed in the *Copied field* in the *Export patient window* for each item exported.
A status message is displayed for each item exported.
Make sure that the operation was successful for each item exported.
10. Press **Done** in the *Export patient window* to complete the process.
11. If copy to USB HD/Memstick - then press [Alt + E] to bring up the eject menu. Select the {USB HD} from the eject list & wait for the USB HD/Memstick to be ready to disconnect (message at bottom of screen) before removing.

12**. Copy the data from USB HD/Memstick to EchoPac Workstation**
(if direct connection or network is not used for data flow)
To copy these images to the EchoPac Workstation - log in to the EchoPac application as ADM. Insert the USB HD/Memstick & go to {Import}, select {USB HD/Memstick} from the dropdown menu. On this Import screen highlight the studies to be imported (use CTRL or SHIFT to select multiple studies), then press {copy}. Once copying to the EchoPac Workstation has completed select {Done}, then [Alt + E] to bring up the eject menu. Select the {USB HD} from the eject list & wait for the Memstick to be ready to disconnect (message at bottom of screen) before removing.
13. **Review on the EchoPAC PC Workstation**
- Select the **Local Archive-Int.HD** dataflow on the EchoPAC PC (can be configured as default dataflow).
- Press **Archive** and select the patient to be reviewed.

In case of copy via network - do NOT open *a study on the EchoPAC PC Workstation before the study is closed on the scanner.*

9.3 Acquired data (images/cine loops) should be later exported from EchoPac Workstation to external HD (high capacity USB memory stick) for reading and back-up copy in following steps:

-To copy exams on to USB Memory stick log on to the EchoPac application as ADM in the EchoPAC Workstation;
-Insert the USB Memory stick and go to {Import}, select {USB Memstick} from the dropdown menu;
-**SELECT** patients from EchoPac server to be exported; select ECHO, VUS (+AUS in NOV) for the same patient (notice if summary capacity of selected records is not higher that capacity of memory stick);
-On this IMPORT/EXPORT screen highlight the studies to be exported (use CTRL or SHIFT to select multiple studies), then press {**COPY**}.
-To **FORMAT USB Memory Stick** (if needed):
Insert the USB Memory stick and press {Config} от the panel, go to {Connectivity}, then to the {Tools}, select {USB Memstick} from the dropdown menu; insert the name into the "Label" box (if needed), press {Format}, press {ok}.
-Once copying to the Memory stick has completed select {**DONE**}, then [**Alt + E**] to bring up the eject menu. Select the {**USB HD/Memstick**} from the eject list and wait for the Memory stick to be ready to disconnect (message at bottom of screen) before removing;
-Repeat all steps for second copy (local storage);
-Sign/mark memory sticks with permanent marker: indicate number of stick and centre name. Each data carrier should be supplied by inventory form which include number of stick, centre name, IDs of records included, and mark as "CRL" or "Local"; place into cover box from physical damage.

9.4 There should be regular checks by one member of team collecting ECHO and ultrasound data that all data is being saved correctly.

9.5 Memory sticks with ECHO, VUS (+AUS in NOV) are to be periodically transferred to Central Reading Lab (CRL) for qualitative assessment, calculations and analysis by independent readers.

9.6 To ensure enough capacity of ultrasound scanner to save new RAW data – the data copied on *EchoPAC PC Workstation* should be deleted from scanner's internal HD when internal HD is loaded for 75-80%. Before erasing, local senior specialist should check the presence of relevant records on *EchoPAC PC Workstation* and register the batch of records deleted (ID's range or dates range) in IQC worksheet.

9.7 Data backup should be provided by local centre for local storage. Backup data might be used locally in order to provide clinical conclusion for examined patients

10.0 Image analysis (reading)

10.1 Independent off-line analysis will be carried out by Central Reading Lab (CRL) using EchoPAC workstations

- In CRL (NOV) two senior readers with established good inter-reader reproducibility and 2 trained readers will be involved.
- -Periodic joint reading sessions will train for parallel reading styles.
- -Repeated reading of same images will be done periodically to check temporal drift.
- -To establish intra-reader and inter-reader variability, a double blind reading of image samples (30 records) will be done within Novosibirsk CRL between all readers at the initial stage of reading period and annually.
- For compatibility purposes, also a double blind reading (30 records) per year will be done between Novosibirsk Reading Centre and Tromsø Reading Centre.

10.2 The reading for ECHO, VUS and AUS will include quantitative measurements/calculations and grading listed in Tables 2 and 3 (ECHO), Tables 5 and 6 (VUS), Tables 8 and 9 (AUS).

10.3 Reading strategy :

- Batch read when possible to minimize systemic temporal drifts.
- Repeat reading of same images periodically to check temporal drift.
- -Average measures for multiple beats (minimum 3 in regular rhythm, 5 in atrial fibrillation).

10.4 ECHO, VUS (+AUS in NOV) report which will be fed back to participants will be done independently of standardised reading for study within 1 month of ultrasound examination.

Table 1. Sequence and type of echocardiographic images to record (each ECHO site)

Echocardiographic window and view	Type of images (still / loop)
Parasternal long-axis:	
2D of LV: 5 beats	cine loop
M-mode cursor perpendicular through **LV** below the mitral leaflet: 5 beats	Still
M-mode cursor perpendicular through **AO and LA**: 5 beats	Still
Additional M-mode scan for LA if necessary (in case of enlarged Valsalva sinuses)	
- 2D of LVOT and Ao annulus: 5 beats.	cine loop
- **Colour-flow** Doppler recordings to search for **MR** and **AoR:** 5 beats	cine loop
Parasternal short-axis view with perpendicular approach from long axis kept:	
- **2D** at level of **AO** and **LA**: 5 beats.	cine loop
- **PW** Doppler recording in **PA**: 5 beats.	Still
- *Optional*: 2D of LV in appropriate level in case of a-/ hypokinesia: 5 beats.	cine loop
- **Repeated M-mode of LV Parasternal long-axis**: 5 beats	Still
Apical 4-chamber view:	
- **2D of LV and LA**: 5 beats with max chamber dimensions, good LV epi/endocardial definition and both right chambers visualised.	cine loop
- **PW** Doppler **transmitral** flow with sample volume at leaflet tips during diastole: 5 beats	Still
- **Colour-flow** Doppler of **mitral** valve: 5 beats	cine loop
- Colour-flow Doppler in tricuspidal valve: 5 beats	cine loop
- **CW** Doppler for **TR**: 5 beats	still
Off-plane RV between 4- and 5-chamber:	
- **2D of RV:** the depth adjusted so that tricuspid annulus is at the bottom; max RV transversal diameter and area: 5 beats	cine loop
Off-plane apical 4-chamber view for Tricuspid Annulus:	
- **M-mode:** cursor perpendicular through the lateral Tricuspid annulus: 5 beats	Still

Apical 3-chamber view (LV)	
- **CW** Doppler **Ao valve:** 5 beats	Still
- **PW** Doppler of **LV outflow tract** ~~*(in case of Ao stenosis and for SV calculation)*~~**:** 5 beats	Still
Apical 4-, 2-chamber view for LV (strain):	
- **A4C 2D of LV**: 5 beats, the depth adjusted so that mitral annulus is at the bottom of image sector	cine loop
- **A2C 2D of LV**: 5 beats, the depth adjusted so that mitral annulus is at the bottom of image sector	cine loop
Apical 4- & 2-chamber view for LA (strain, volume):	
- **A4C 2D of LA**: 5 beats with depth adjusted so that the LA roof is at the bottom	cine loop
- **A2C 2D of LA**: 5 beats with depth adjusted so that the LA roof is at the bottom	cine loop
Apical 4-chamber view for Mitral Annular DTI:	
- **Apical 4C. Mitral Annular DTI:** the DTI PW Doppler at the septal corner (5 beats) and, at the lateral corner (5 beats).	Still

Table 2. Quantitative echocardiographic measurements by ECHOPAC software (Central Reading Lab)

Echocardiographic window and view	*Measurements*	*Calculated variables*
Parasternal long-axis; M-mode (short axis might also be used)	LV internal dimension (LVID), interventricular septal (IVS) and posterior wall (PW) thicknesses measured at end-diastole (d) and end-systole (s)	LV structure - LV mass, relative wall thickness LV systolic function - systolic endocardial fractional shortening, ejection fraction
Parasternal long-axis; M-mode	Left atrial dimension (end-systole), aortic root dimension	
Parasternal long-axis; 2D 3-chamber apical; Pulsed Doppler of LVOT	LVOT diameter (systole) Velocity-time integral (VTI)	LV systolic function - Doppler stroke volume
4-chamber apical; 2D	LV cavity volume measurements at the end diastole and end systole (methods of discs), LV long axis.	LV systolic function – ejection fraction
4-chamber apical; Pulsed Doppler of transmitral flow	Peak early diastolic mitral flow (E peak) and late diastolic mitral flow (A peak, atrial filling fraction) wave velocities, deceleration times of E peak and duration of A peak	LV diastolic function – E/A ratio
4-chamber apical; M-mode of lateral site of tricuspid annulus	Tricuspid annulus.	RV systolic function – motion amplitude of tricuspid annulus
4-chamber apical; Tissue Doppler of septal and lateral corner of mitral annulus	Mitral annulus. Peak systolic myocardial (Sm), early diastolic myocardial (Em), and late diastolic myocardial (Am) velocities.	LV diastolic function: – Em/Am ratio, – E/Em ratio
4-chamber + 2-chamber apical; LV 2D strain Echo (2DSE) (Speckle Tracking)	AFI technology. Aortic Valve closure (AVC) time; LV endocardial border tracing - to calculate longitudinal peak systolic LV strain (LVPSS LAX, A4C, A2C) and Strain Rate (LVSR LAX, A4C, A2C).	LV systolic function: – global LV strain (LV GSS), – peak systolic & peak diastolic myocardial strain – Strain Rate (LV SR)
4-chamber and 2-chamber apical; LA 2DSE (Speckle Tracking);	*AFI* novel technology (LV program). P wave onset; LA endocardial border tracing to calculate peak positive, peak negative and total LA strain (A4C, A2C); LA Strain Rate (late negative, early negative, positive).	LA structure & function: – global LA strain (3 measures); – global LA Strain Rate (3 meas);
4-chamber and 2-chamber apical; LA 2D	LA cavity volume measurements at the end diastole and end systole (methods of discs, A4C, A2C), LA long axis	LV diastolic function: – LA volume index (LAVI)

- **Table 3. Grading of Valvular Regurgitation and Stenosis**

- Semi-quantitative evaluation	- Valvular disorder	- Echocardiographic window and images

- **1** – mild - **2** – mild to moderate - **3** – moderate - **4** – severe	- MR, MS	- PSLA – colour-flow - Apical 4-chamber: colour-flow, CW, PW
	- AR, AS	- PSLA – colour-flow - Apical 4-chamber and long-axis: colour-flow, CW, PW
	- TR	- Apical 4-chamber and long-axis: colour-flow, CW

Table 4. Sequence and type of vascular images to record (each VUS site)

Site, position and view	Type of images (still / cine loop)
Carotid arteries left	
2D longitudinal view; anterior (preferable) or anterolateral or posterior position (wherever IMT visualization is the best and closer to the probe), distal **CCA left,** 10 beats To register position of IMT visualization and to mark the image (**A, L, P**)	cine loop
2D longitudinal view**;** to document key regions where plaques could exist; **CCA, bulbus, ICA, ECA** (left), 10 beats	cine loop
2D transverse view**;** to document key regions where plaques could exist; **CCA, bulbus, ICA, ECA** (left), 10 beats	cine loop
*In case of **plaque or intima bulging**: longitudinal view of relevant segment in CCA/bulbus/ICA/ECA*	still
In case of plaque or intima bulging: transverse view of relevant segment in CCA/bulbus/ICA/ECA *Indicate the number of plaques in comments to records*	still
Carotid arteries right	
2D longitudinal view**;** anterior (preferable) or anterolateral or posterior position (wherever IMT visualization is the best and closer to the probe), distal **CCA left,** 10 beats To register position of IMT visualization and to mark the image **(A, AL, P**)	cine loop
2D longitudinal view**;** to document key regions where plaques could exist; **CCA, bulbus, ICA, ECA** (right), 10 beats	cine loop
2D transverse view**;** to document key regions where plaques could exist; **CCA, bulbus, ICA, ECA** (right), 10 beats	cine loop
*In case of **plaque or intima bulging**: longitudinal view of relevant segment in CCA/bulbus/ICA/ECA*	still
In case of plaque or intima bulging: transverse view of relevant segment in CCA/bulbus/ICA/ECA *Indicate the number of plaques in comments to records*	still

Table 5. Quantitative vascular measurements by ECHOPAC software (Central Reading Lab)

Vascular site and view	*Measurements*	*Calculated variables*
2D; Anterior position (preferable), longitudinal view of **CCA left;** ** anterolateral or posterior*	IMT (at end diastole) on ECG-gated longitudinal view at **10-mm** segment of the far wall of distal **CCA** in plaque-free zone (automatic; min, mean, max ; 3 cycles); CCA diameter To register position of IMT measurement	-
2D; Anterior position (preferable), longitudinal view of **CCA right;** **anterolateral or posterior*	IMT (at end diastole) in ECG-gated longitudinal view at **10-mm** posterior segment of the far wall of the distal **CCA** in plaque-free zone (automatic; min, mean, max ; 3 cycles); CCA diameter To register position of IMT measurement	-
In case of plague: **2D**; CCA/bulbus/ICA/ECA; longitudinal view; transverse view	Number of plaques; stenosis % (diameter, ECST); stenosis % (area) ;	-

Table 6. Grading of vascular abnormalities

Semi-quantitative evaluation	Vascular abnormalities: abbreviation/ definition	Vascular site and images
0 – no plaques **1** – single plaque **2** – multiple plaques (indicate number of plaques)	PL Plaque is a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or demonstrates a thickness 1 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface *(Mannhein Consensus, 2006)*.	CCA/bulbus/ICA/ECA: 2D; longitudinal view; transverse view
0 – no triple line pattern of carotid wall **1** – triple line carotid pattern	TLP Triple pattern is defined as divergence from the normal double line pattern of carotid wall, characterised by additional discernable reflection line in intima-media compartment of the far wall.	CCA/bulbus: 2D; longitudinal view; transverse view

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A.7 The original number of plaque in KYH and plaque score in Tromsø 7 according to carotid segment

		Number of plaque N (%)		Plaque score	
		KYH men	KYH women	T7 men	T7 women
Right CCA	0	1237 (89.1)	1805 (96.4)	778 (95.9)	973 (98.4)
	1	125 (9)	66 (3.5)	29 (3.6)	15 (1.5)
	2	26 (1.9)	2 (0.1)	4 (0.5)	1 (0.1)
	3	1 (0.1)	0		
Right bulb	0	639 (46.0)	1196 (63.9)	436 (53.8)	664 (67.1)
	1	602 (43.3)	576 (30.8)	238 (29.4)	243 (24.6)
	2	148 (10.7)	99 (5.3)	137 (16.9)	82 (8.3)
	3	0	2 (0.1)		
Right ICA	0	981 (70.6)	1620 (86.5)	751 (92.6)	950 (96.1)
	1	378 (27.2)	234 (12.5)	53 (6.5)	37 (3.7)
	2	30 (2.2)	19 (1.0)	7 (0.9)	2 (0.2)
	3	0	0		
Left CCA	0	1180 (85.0)	1765 (94.2)	764 (94.2)	966 (97.7)
	1	169 (12.2)	97 (5.2)	46 (5.7)	21 (2.1)
	2	35 (2.5)	10 (0.5)	1 (0.1)	2 (0.2)
	3	5 (0.4)	1 (0.1)		
Left bulb	0	604 (43.5)	1162 (62.0)	439 (54.1)	649 (65.6)
	1	606 (43.6)	607 (32.4)	251 (31.0)	255 (25.8)
	2	176 (12.7)	103 (5.5)	121 (14.9)	85 (8.6)
	3	3 (0.2)	1 (0.1)		
Left ICA	0	988 (71.1)	1618 (86.4)	765 (94.3)	966 (97.7)
	1	354 (25.5)	235 (12.6)	41 (5.1)	23 (2.3)
	2	46 (3.3)	19 (1.0)	5 (0.6)	
	3	1 (0.1)	1 (0.1)		

CCA: common carotid artery, bulb: bifurcation, ICA: internal carotid artery