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A biomarker approach to explain high cardiovascular disease burden in Russia: insights from population-based studies in Russia and Norway

Know Your Heart and The Tromsø Study

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Summary

Although the problem of cardiovascular disease (CVD) in Russia has been the subject of attention for decades, a definitive and comprehensive explanation of why the CVD burden there is so high and generally greater than in many other countries has still not been found. In this thesis, I have attempted to advance research on these issues by examining the role of blood lipids, heart damage biomarkers (high sensitivity cardiac Troponin T and NT-proBNP), alcohol use, and diabetes.

The methodological approach that I have chosen for Paper 1 and Paper 3 was to compare the biomarker levels in two population-based studies: Know Your Heart (Russia) and Tromsø 7 (Norway). There were no substantial differences in lipid profiles between Know Your Heart and Tromsø 7, however, higher mean high sensitivity C-reactive protein reflected higher pro-inflammatory status in Russian sample. Moreover there was evidence of higher levels of cardiac wall stretch (NT-proBNP) and heart damage (high sensitivity cardiac Troponin T) biomarkers in Know Your Heart compared to Tromsø 7. This work is the first time that levels of these heart damage biomarkers in two population-based studies in Russia and elsewhere have been undertaken.

In Paper 3, I compared diabetes prevalence defined as self-reported diabetes and/or medication use for diabetes and/or glycated haemoglobin (HbA1c) ≥ 6.5 % between Know Your Heart and Tromsø 7. Obesity (measured as BMI and waist circumference) explained a substantial proportion of differences in diabetes prevalence between KYH and Tromsø 7 in women but not in men.

The analysis in Paper 2 was based on data from Know Your Heart study only and was a comparison of biomarker levels in extremely heavy drinkers in Russian addiction treatment centers to those in the general population of Arkhangelsk (Russia). The levels of NT-proBNP,

high sensitivity cardiac Troponin T, and high sensitivity C-reactive protein were much higher in extremely heavy drinkers compared to non-problem drinkers.

This thesis implicates non-atherosclerotic pathways as a possible explanation for high cardiovascular disease burden in Russia. This conclusion is supported by higher levels of NT-proBNP and high sensitivity cardiac Troponin T in Know Your Heart compared to Tromsø 7, while atherogenic lipoproteins are at similar levels in both studies. The biomarker profile of extremely heavy drinkers in Russian addiction treatment centers supports the non-ischemic damage as an aetiological pathway leading to heart disease as a consequence of heavy alcohol use. High prevalence of diabetes mellitus in Russia, including a higher proportion of undiagnosed and untreated cases, contributes to cardiovascular disease burden of both atherosclerotic and non-atherosclerotic origin. Strategies to reduce the burden of high cardiovascular disease in Russia should include steps to reduce the prevalence of heavy drinking as well as tackling the high burden of diabetes.

Abbreviations

ATC – Anatomical Therapeutic Chemical

AUDIT – Alcohol Use Disorder Identification Test

BMI – body mass index

CAGE – cut-annoyed-guilty-eye-opener

CDT – carbohydrate deficient transferrin

CHD – coronary heart disease

CI – confidence interval

CVD – cardiovascular disease

DAG – directed acyclic graphs

DCM – dilated cardiomyopathy

ECG – electrocardiography

eGFR – estimated Glomerular Filtration Rate

GGT – gamma-glutamyl transferase

HbA1c – glycated haemoglobin

HDL – high-density lipoproteins

hsCRP – high sensitivity C-reactive protein

hs-cTnT – high sensitivity cardiac Troponin T

KYH – Know Your Heart

LDL – low-density lipoproteins

MI – myocardial infarction

NT-proBNP – N-terminal pro-B-type Natriuretic peptide

WC – waist circumference

WHR – waist-to-hip ratio

List of Papers

This thesis is based on the following papers:

1. Iakunchykova O, Averina M, Wilsgaard T, Watkins H, Malyutina S, Ragino Y, Keogh RH, Kudryavtsev AV, Govorun V, Cook S, Schirmer H. Why does Russia have such high cardiovascular mortality rates? Comparisons of blood-based biomarkers with Norway implicate non-ischaemic cardiac damage. J Epidemiol Community Health. 2020 Sep;74(9):698-704.
2. Iakunchykova O, Averina M, Kudryavtsev AV, Wilsgaard T, Soloviev A, Schirmer H, Cook S, Leon DA. Evidence for a direct harmful effect of alcohol on myocardial health: A large cross-sectional study of consumption patterns and cardiovascular disease risk biomarkers from northwest Russia, 2015 to 2017. Journal of the American Heart Association. 2020 Jan 7;9(1): e014491.
3. Iakunchykova O, Averina M, Wilsgaard T, Malyutina S, Kudryavtsev AV, Cook S, Wild S, Eggen AE, Hopstock LA, Leon DA. What factors explain the much higher diabetes prevalence in Russia compared to Norway? Major sex-differences in the contribution of adiposity. BMJ Open Diabetes Research & Care, submitted.

Chapter 1 Introduction

1.1 Trends in life expectancy and cardiovascular disease mortality in Russia and Norway

Life expectancy at birth in Russia has been lower compared to many countries in the developed world for many years and significant fluctuations were recorded since 1990. Life expectancy in men dropped to 57 years between 1990 and 1994, and 71 years in women. The gender gap during this period equalled 14 years in life expectancy. Life expectancy increased from 1994 until 1998, decreased between 1998 and 2003, and then started a long-lasting upward trend from 2003/2004 (Figure 1). By 2018 Russian life expectancy had increased to almost 68 years for men and 78 years for women with the gender gap narrowing to 10 years. This is still much lower than life expectancy in Norway which in 2018 was 81 years for men and 84.5 years for women (1).

The major contributors to high mortality in Russia especially among men of working age were cardiovascular diseases and external causes (2). The fluctuations in mortality followed trends in alcohol consumption (3, 4). However, more recent data suggest that there is now a weaker correlation of markers of the prevalence of harmful drinking with mortality trends, and other factors may be driving the positive mortality changes in Russia (5). A consistent decline in CVD mortality was observed starting 2005 (6), but Russia still has one of the highest rates of mortality from cardiovascular disease (CVD) in the world (7). In 2015 age-standardized CVD mortality in Russia was 512.5 per 100000 in men, and 273.7 per 100000 in women (6). It is much higher compared to counties in the geographical region and similar economic development ranking. Taking an example of Norway, age-standardized CVD mortality was 112.3 per 100000 in men, and 71.6 per 100000 in women in 2015 (8).

In the most developed countries, including Norway, life expectancy was increasing consistently in the late 20th century and beginning of the 21st century and CVD mortality has been declining (9). The decline in CVD mortality has been largely attributed to the successful primary and secondary prevention of CHD which led to the reduction of major risk factor prevalence at the population level (9, 10). The success in reduction of smoking levels was remarkable as well as pharmacological management of high blood pressure and high total cholesterol (10, 11). Also, the improvements in intensive care and treatment of acute cardiac events have contributed to a decline in mortality (12). However, CVD are still in the top three causes of death in Norway, along with cancer and diseases of respiratory system (13).

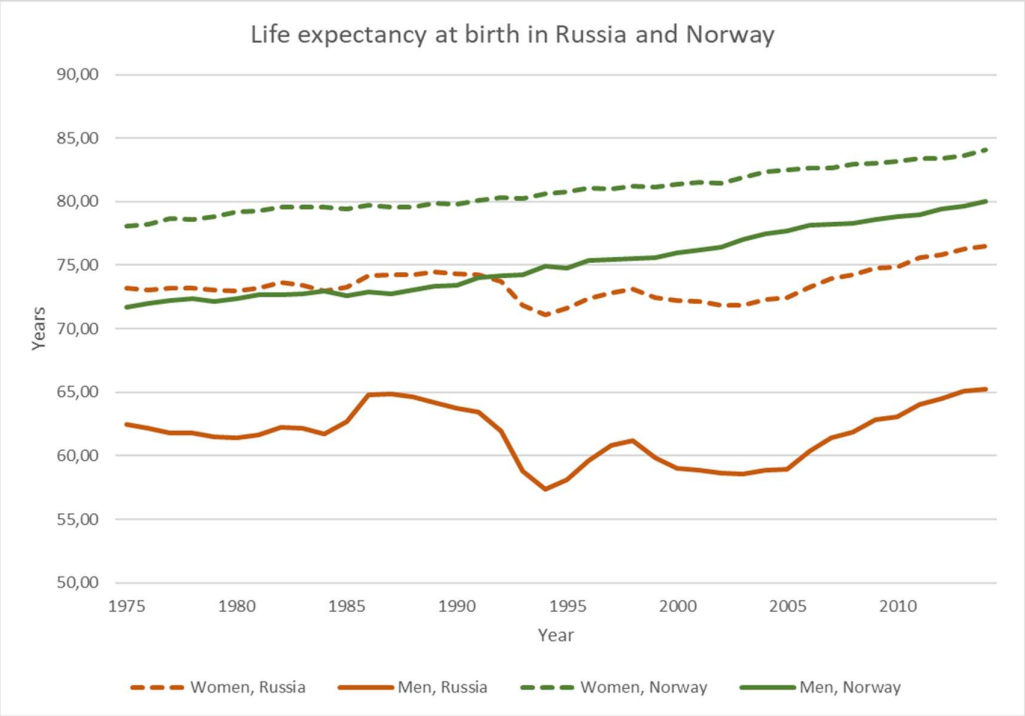

Figure 1. Life expectancy at birth in Russia and Norway (Source: Human Mortality Database (1))

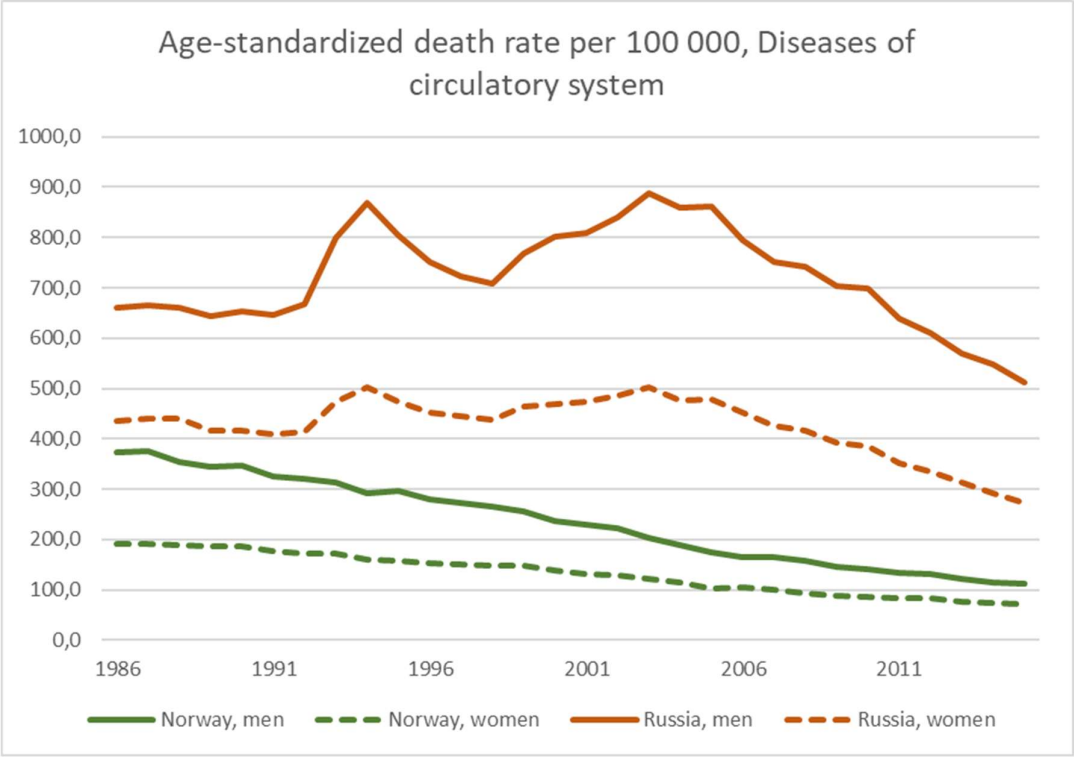

Figure 2. Age-standardized death rate per 100 000, Diseases of circulatory system. Standardized to the WHO world standard population (Source: WHO mortality database (8)).

Although the problem of high cardiovascular disease mortality in Russia has been the subject of attention for decades, a definitive and comprehensive explanation has still not been found. Among the potential explanations that have been suggested are differences in coding practices (for CVD mortality), access to medical care for acute cardiovascular events, differences in level and pattern of alcohol use, diet, levels of classic CVD risk factors (smoking, cholesterol, blood pressure, obesity, diabetes), socio-economic challenges and stress.

This thesis will attempt to advance research in three directions to further look at lipid levels, alcohol use, and diabetes as potential explanations for high CVD mortality in Russia. The completely novel aspect is the measurement of heart damage biomarkers (high sensitivity

cardiac Troponin T and NT-proBNP) to assess the underlying CVD morbidity in the population-based samples in Russia and Norway.

Previous studies did not find big differences in cholesterol levels between Russia and many other Western countries, which is paradoxical given the much higher CVD mortality in Russia. Although alcohol use was implicated in the fluctuations of life expectancy, the mechanisms by which alcohol could influence CVD mortality are less clear. Prevalence of obesity and diabetes has been increasing in most countries of the world over the last decades, which has led some researchers to suggest that the downward trend in CVD mortality may slow down or reverse. Recent population-based studies have suggested that the prevalence of diabetes is considerably higher in Russia than in most Western countries (14-16), although little is known about why this is the case.

1.2 Explaining the differences in CVD between populations

1.2.1. Methodological approach to comparison studies between populations in epidemiology

Diseases are distributed unevenly between countries resulting in health inequalities. Epidemiology has a key role in helping to provide evidence to inform strategies to reduce these differences in disease distribution. The hypotheses about the possible reasons behind the differences can be put into several groups: 1. Differences in the diagnosis and coding practices during the collection of surveillance data. 2. Differences in the distribution of risk factors that have been established to play a role in disease causation 3. Risk factors that are specific for the particular population with a high prevalence of the disease but are absent or unknown in other populations; 4. Differences in health care systems, including access to prevention, screening, and treatments.

This section will be focused on methodological approaches to comparison studies of diseases and their risk factors between populations. The basic aim of comparison studies is to evaluate the potential role of known disease determinants in generating the observed differences in disease incidence and prevalence and to quantify the extent to which these differences remain unexplained by measured factors. Although this thesis focuses on two populations defined by geographical region, similar approaches can be applied to studying changes in the population over time.

One of the first studies in CVD epidemiology that collected data on a number of risk factors using comparable protocols in several countries was the Seven Countries Study. This was a ten-year investigation of the epidemiology of coronary heart disease that recruited 12 763 men aged 40-59 in Yugoslavia, Finland, Italy, the Netherlands, Greece, the United States, and Japan in 1958-1964 (17). It was an ecological analysis that related the incidence of the disease to levels of risk factors. Investigators plotted the CHD rates against a proportion of hypertensive participants (above an arbitrary threshold of 160 mm or more) and found that the population with a lower frequency of hypertension tended to have lower CHD rates. Similarly, 64% percent of the variance in death from CHD in cohorts was explained by median cholesterol values. However, applying a similar approach they did not find that differences among the cohorts in the incidence rates of CHD can be explained by differences in smoking habits (17).

The foundation of standardized research on determinants of CVD in different countries was the World Health Organization's MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) Project (18). Twenty-one countries participated in the project which started in the 1980s with the objective to measure trends in cardiovascular mortality and coronary heart disease and cerebrovascular disease morbidity and to assess the extent to which these trends were related to changes in known risk factors and health care measured at the same time in defined populations in different countries (18). MONICA showed, for the first time,

comparable and standardized estimates of risk factor levels in populations from a large number of countries (19, 20).

Studies to compare CVD risk factor levels in different countries without trying to relate them to morbidity or mortality are today relatively common. Ideally nationwide representative and comparable data on prevalence or mean levels of risk factors would be collected. However, this is difficult to achieve due to financial, logistical constraints, selection, and measurement bias inherent in epidemiological studies. Nevertheless, the WHO STEPwise approach to Surveillance (STEPS) project conducts regular collection of core data on the established risk factors using the comparable data collection procedures to be able to compare levels between countries and monitor trends (21). The WHO Study on global AGEing and adult health (SAGE) is a longitudinal study on aging conducted in several low- and middle-income countries, including Russia, which in addition to standard behavioural risk factors like alcohol and tobacco consumption, diet and physical activity collected data on a number of more objective risk factors, including waist and hip circumferences, weight, height, and blood pressure (22).

Large meta-analytic studies (Non-communicable disease risk factor collaboration (NCD-Risc) or Global Burden of Disease use data from many available national studies to produce local and regional estimates of risk factor levels for non-communicable disease (cholesterol, obesity, diabetes, blood pressure), as well as describe the trends over time.

1.2.2. Innovations in analytic approaches

One of the analytic approaches that is referred sometimes as a between-country comparison is essentially a comparison of hazards and population attributable fraction (PAF) of individual risk factors in different populations. Within a counterfactual approach, PAF is defined as the

proportional reduction in disease or death that would occur if exposure to the risk factor were reduced to zero, ceteris paribus.

Recently more sophisticated approaches have been developed that use individual-level data to quantify the contribution of variation in distribution of the risk factors to the observed difference in the prevalence of the disease. Conceptually, it requires a quantification of the degree of confounding for the 'population effect' induced by different factors, which are known to be determinants of the disease. Scarce methodological work is available on this problem, and comparison of 'unadjusted' and 'adjusted' regression-based estimates are mostly used in practice. The disadvantage of this approach is limited interpretability of obtained regression estimates due to confounding, non-collapsibility, and interactions (23, 24). More recently the step was taken to formalize the problem using directed acyclic graphs and the potential outcomes framework (23). Under a set of clearly stated assumptions, the change in the morbidity difference induced by compositional variations in measured risk factors relative to a reference population can be estimated. The defined set of estimands (the change in the prevalence or incidence difference induced by compositional variations in measured risk factors, all at once and individually, relative to a reference population; and the proportion of the crude difference that remains unexplained by measured factors) can be obtained using standardization (g-computation), inverse probability weighted (IPW) and doubly robust IPW estimation (23). This approach still depends upon the availability of good and comparable measurements of risk factors and outcomes for each country. It has not yet been widely applied in practice of comparison studies in epidemiology, and in CVD research in particular.

Morbidity and mortality due to a particular disease are often affected by multiple distal and proximal risk factors that act jointly in the disease causation process (25). Counterfactual causal attribution of disease and injury to individual risk factors does not normally allow additive decomposition and the sum of attributable fractions or burdens for a single disease due to

multiple risk factors is therefore theoretically unbounded (26). Methodological approaches to comparison studies described in this section do not allow one to capture this complexity. Finally, the accumulated effects of sustained exposure and the temporal profile of exposure on disease are also not accounted for in comparison studies.

In the next section, I will make an overview of the comparison studies of CVD that included Russia or were conducted to explain the high prevalence of CVD in Russia relative to most other countries of the world.

1.2.3. Conventional risk factors of cardiovascular disease can only partially explain high CVD rates in Russia

It could be expected that a high cardiovascular mortality rate in Russia is explained by high levels of these conventional risk factors: smoking, blood pressure, high LDL-cholesterol, obesity, diabetes (27, 28). MONICA was the first project to assess the contribution of the classical risk factors to CVD in different countries and their trends over time using purposefully collected individual and ecological data for selected populations. In an ecological analysis of trends in CHD over 10 years across the WHO MONICA Project populations, classic risk factors made a moderate contribution with around 15% in women and 40% in men of the variability of trends in coronary-event rates being "explained" by trends in these risk factors (29). Population-level trends in systolic blood pressure showed a strong association with stroke event trends in women (38%), but there was no association in men (30). In addition, risk factor gradients in blood pressure, total cholesterol, and smoking across MONICA project countries had poor ability to explain international variations in CVD death rates and explained 25% in men and 33% in women (31).

Some studies have attempted to quantify CVD risks in different populations using individual-level data but were not focussed on how far the risk factors could explain between-country differences. In the large multi-country case-control study INTERHEART, Yusuf et al. (2004) quantified the contribution of risk factors to acute (non-fatal) myocardial infarction within each country (27). The study reported a consistently high Population Attributable Risk percent associated with smoking, lipids, hypertension, diabetes, abdominal obesity, and the combined psychosocial index in most regions of the world and in every ethnic group (27). In the context of this thesis, notably, it was lower for Eastern Europe compared to other regions (27). However, for alcohol consumption, exercise, or diet greater variability was noted across regions. In all regions, the nine risk factors accounted for between three-quarters and virtually all the PAR for acute myocardial infarction while the relative importance of every risk factor was related to its prevalence (27). However, the case-control design of INTERHEART does not allow an analysis of risk factors explaining CVD differences between countries because it cannot account for absolute differences in the multi-dimensional baseline CVD rates in each population.

The problem of differences in baseline CVD rates has recently been recognised in a major revision of the WHO SCORE tool (32). In fact, recalibration of SCORE equations for Russia (based on cohort data from Moscow and Saint Petersburg) found that the original SCORE-High model tends to substantially underestimate 10-year cardiovascular mortality risk for women at all ages and men at younger ages (33).

Averina et al. (2003) took an approach of joint comparison of several conventional CVD risk factors by calculating CVD risk scores in Russian and Norwegian cross-sectional samples. The study concluded that high cardiovascular mortality in Russia seemed to be only partially explained by conventional risk factors like smoking, blood pressure, obesity, and high total

cholesterol (34), however, the study was likely to be biased due to recruitment of the healthier working part of the population.

In a later study by Sergi Trias-Limos et al., 2020 authors used the new WHO CVD risk prediction algorithms to directly quantify the contribution of conventional CVD risk factors (blood pressure, total cholesterol, smoking, diabetes) to CVD mortality differences between Russia and Norway (35). This study concluded that conventional CVD risk factors account for a third of the male and a fifth of the female CVD mortality gap between Russia and Norway on the counterfactual that the Russian risk factor profile was altered to become the same as in Norway (35).

It is worth noting that most comparison studies that looked at the explanatory power of blood pressure, LDL-cholesterol, smoking, obesity, diabetes were focused on coronary heart disease and myocardial infarction as endpoints, while total CVD mortality includes other classes of CVD including those of non-atherosclerotic origin. CVDs of non-atherosclerotic origin have different aetiological pathways and may be explained by these established risk factors to a lesser extent.

1.3 Types of cardiovascular disease

Cardiovascular diseases are a heterogeneous group of diseases which affect primarily cardiovascular system. Here I will briefly describe CVD that contribute to mortality the most.

1.3.1 Coronary heart disease and myocardial infarction

Coronary heart disease has been the major component of cardiovascular morbidity and mortality in much of the western industrialized world and globally (36). Atherosclerosis is the primary underlying mechanism of CHD. It is a pathological condition that occurs in medium

and large arteries throughout the body (37). Its clinical manifestations appear especially in the heart, brain, lower extremities, and aorta. The atherosclerotic plaque, whether fully matured or in the intermediate stages of development, is regarded as the key to precipitating blood clot formation (thrombosis) with sudden interruption of blood flow (38). A variety of outcomes may follow, depending on the location, severity, and duration of the interruption. It is the underlying condition in the occurrence of myocardial infarction (MI), ischemic stroke, peripheral arterial disease of the lower extremities, and aortic aneurysm. Coronary heart disease is characterized by occlusion of arteries in the heart due to the development of atherosclerotic plaque (39). Atherosclerotic plaque develops as a protrusion on the arterial wall which can then become vulnerable to damage with consequent thrombus formation. Such a thrombus can stop or severely restrict the blood supply to the heart muscle leading to a myocardial infarction MI. MI caused by plaque rupture/erosion is classified as Type I MI according to the Fourth Universal Definition of myocardial infarction (40). In case of a mismatch between oxygen supply and demand, but without acute atherothrombotic plaque disruption, in patients with stable known or presumed CHD, the diagnosis of type 2 MI is made.

1.3.2 Stroke

Stroke, or cerebrovascular accident (CVA), is a second major class of cardiovascular diseases, that is characterised by disturbance of blood flow in the cerebral circulation (41). It constitutes a large proportion of overall cardiovascular morbidity and mortality globally (42). There are two major types of stroke: ischemic and haemorrhagic. Ischemic stroke accounts for 80-85% of stroke cases, the remainder being intracerebral haemorrhage (10-15%), and subarachnoid haemorrhage (3-5%) (41). In low-income countries and historically in high-income countries haemorrhagic stroke is/was of greater importance (43). The most common mechanism of

haemorrhagic stroke is a hypertensive small-vessel disease, where small lipohyalinotic aneurysms are formed and subsequently rupture (41). Ischemic stroke (IS) is defined as neurological dysfunction after focal cerebral infarction due to occlusion of cerebral arteries (41). Mechanisms include atherothrombosis (extra- or intracranial), embolism (cardiogenic due to atrial fibrillation or artery-to-artery embolism), primary occlusive disease of the small penetrating arteries, and non-atherosclerotic abnormalities (dissections, vasculitis, and coagulopathies) (44). Hence, some stroke subtypes do not have an atherosclerotic component in their pathophysiology, although the majority of ischemic stroke cases occur as a consequence of atherosclerotic disease.

1.3.3. Cardiomyopathies, heart failure, and cardiac arrhythmias

Chronic heart failure is an impairment of the fundamental function of the heart as a pump and prime mover of the circulatory system. Heart failure is classified into subtypes with preserved (HFpEF), mildly reduced (HFmrEF), and reduced ejection fraction (HFrEF) (45). Heart failure with reduced ejection fraction occurs as a result of systolic dysfunction when the left ventricle ejects a reduced amount of blood with each contraction. Heart failure with preserved or mildly reduced ejection fraction has diastolic dysfunction as a major component in its pathology. Diastolic dysfunction involves incomplete relaxation of the left ventricle and therefore a reduced volume of blood entering the left ventricle to be ejected with the next contraction (45). Heart failure has a complex and diverse aetiology. It may be consequent of an acute coronary event of atherosclerotic origin, for example, MI, which causes significant localized damage to the ventricular wall. Other underlying processes leading to heart failure are longstanding high blood pressure; cardiomyopathies; or valvular heart disease, abnormalities of heart rhythm (45). Dilated cardiomyopathy (DCM) is defined as left ventricular (LV) dilation and systolic

dysfunction in the absence of coronary artery disease or abnormal loading conditions proportionate to the degree of LV impairment (46). The main causes of dilated cardiomyopathy are alcohol use, toxins, illicit drug use, metabolic/endocrine disturbances, inflammatory/autoimmune disorders, and genetic causes (46).

Cardiac arrhythmias, or disturbances of heart rhythm, reflect dysfunction of electrophysiological control of the rate and rhythm of the cardiac cycle (47). One very serious consequence is a disturbance of blood flow through the left atrium of the heart due to atrial fibrillation, promoting formation and dislodging of thrombi that can be carried through the circulation to the brain and result in a thromboembolic/occlusive stroke (47). Another is an increased rate of ventricular contraction (ventricular tachycardia or fibrillation), with loss of effective pumping action of the heart, potentially leading to cardiac arrest and sudden cardiac death.

According to the Fourth universal definition of myocardial infarction, Type 2 MI can occur in the absence of significant atherosclerotic disease, but as a result of mismatch between oxygen supply and demand that can be caused by vasospasm or coronary microvascular dysfunction, non-atherosclerotic coronary dissection, coronary embolism, or other mechanisms that reduce oxygen supply such as severe bradyarrhythmia, respiratory failure with severe hypoxemia, severe anemia, and hypotension/shock; or to increased myocardial oxygen demand due to sustained tachyarrhythmia or severe hypertension with or without left ventricular hypertrophy (40).

1.4 Risk factors and biomarkers of cardiovascular disease

In this section, I will give a short description of the risk factors and biomarkers of cardiovascular disease that this thesis has as its main focus. Special attention is given to diabetes (Paper 3) and alcohol use (Paper 2).

1.4.1 Conventional CVD risk factors

The established conventional modifiable CVD risk factors are high blood pressure, high total and LDL-cholesterol, tobacco smoking, and obesity (27, 28). They are cited as intervention targets in guidelines for primary and secondary prevention of CVD with the highest grading for evidence (48). It is suggested that these risk factors can explain approximately 75% of the occurrence of CHD within populations (49, 50). A decline in population levels of mean cholesterol, blood pressure, and frequency of smoking contributed greatly to the declining trends in CHD within many developed countries including Norway (10, 11).

1.4.2 Diabetes

Independent of other major vascular risk factors, diabetes substantially increases the risk of deaths that are attributed to occlusive vascular disease among both men and women. The risk is doubled among men aged 35–89, but tripled among similarly aged women, even after controlling for total cholesterol, blood pressure, body mass index (BMI), and smoking (51). Apart from increasing the risk of occlusive vascular disease, diabetes also leads to diabetic cardiomyopathy that is characterised in its early stages by diastolic relaxation abnormalities and at a later stage by clinical heart failure in the absence of dyslipidaemia, hypertension, and coronary artery disease (52). A retrospective cohort study showed a 2.5-fold increase in heart failure risk in those with type 2 diabetes (53). Furthermore, an observational study involving

25,958 men and 22,900 women with type 2 diabetes indicated that a 1% increase in glycated haemoglobin (HbA1c) was associated with an 8% increase in the risk of heart failure, independent of blood pressure, obesity, age, and the presence of CHD (54).

Although diabetes is considered a heterogeneous group of diseases, three types are distinguished in most clinical and epidemiological research: Type 1 diabetes, Type 2 diabetes, and gestational diabetes. Type 1 diabetes and Type 2 diabetes have different aetiology but both significantly increase the risk of CVD. The onset of Type 1 diabetes predominantly happens at a young age (less than 18 years old) although adult-onset Type 1 diabetes can constitute about 50% of all Type 1 diabetes cases. Both Type I and Type 2 have a large genetic component, although different genetic loci are involved as part of the pathophysiologic pathway. More than 250 loci significant at the genome-wide level have been identified for Type 2 diabetes (55). Among environmental and behavioural factors, obesity, physical inactivity, and poor nutrition increase the risk of Type 2 diabetes (56) (57-59). Visceral adipose tissue which is part of abdominal fat deposit is most strongly related to the risk of Type 2 diabetes (60). It is associated generally with a more pro-inflammatory state and insulin resistance. Dietary patterns that are characterized by high consumption of fruit and vegetables, whole grains, fish, and poultry, and by decreased consumption of red meat, processed foods, sugar-sweetened beverages, and starchy foods are protective of diabetes (56). Both observational and intervention studies demonstrated evidence for an inverse association between physical activity and risk of type 2 diabetes, which may partly be mediated by reduced adiposity (20-30%) (57-59). All subtypes of physical activity appear to be beneficial (58), and a sedentary life style adds additional risk (61). Beyond these factors, others have also been found to be associated with an increased risk of type 2 diabetes including smoking and certain environmental pollutants (62, 63).

1.4.3 Alcohol use and CVD

The role of alcohol in CVD morbidity and mortality is still debated in the scientific literature (64). The beneficial effects of light to moderate alcohol consumption for ischemic diseases were consistently demonstrated over many years of research leading to claims about "safe" or "beneficial" alcohol drinking (65, 66). However, these studies have been criticized for methodological flaws like week adjustment for confounders and using biased comparison group (current abstainers) that could lead to reverse causality (66). A large meta-analysis that looked separately at different cardiovascular outcomes in relation to alcohol use still found some protective effect of moderate alcohol use on myocardial infarction (67). However, the risk of all other CVD outcomes, including stroke, CHD, heart failure, fatal hypertensive disease, and fatal aortic aneurism was increased among drinkers generally in a dose-response fashion (67). Chronic heavy drinking particularly increases the risk of hypertension, coronary heart disease, cardiomyopathy, atrial fibrillation and flutter, and all types of stroke (64, 68, 69). A recent meta-analysis of alcohol and blood pressure confirmed the consistent increased risk of hypertension with higher total alcohol consumption (70).

Apart from the quantity and frequency of alcohol consumption, the pattern of alcohol use is suggested to be important when considering it as a risk factor for cardiovascular disease (66). Episodic heavy drinking does not provide any beneficial effect on CHD, therefore average alcohol consumption inadequately captures the relationship between alcohol consumption and CHD (66). Episodic heavy drinking, sometimes also called binge drinking, is usually defined in the study settings as six or more standard drinks in one sitting (approximately 60 g of pure alcohol during 2 hour time period), although other, slightly different definitions are sometimes used (71). The debate about the role of alcohol in CVD has had been of particular interest in the Russian context because drinking patterns in Russia can go far beyond this definition (72).

The traditional drinking culture in Russia can be characterized by consumption of large quantities of spirits, non-daily drinking, irregular heavy drinking episodes, and the acceptance of public drunkenness (73). Nordic countries, including Norway, and some other Eastern European countries historically have a similar drinking culture (73). In Russia and several other former Soviet countries drinking of non-beverage alcohol and 'zapoi' are found. 'Zapoi' is a term with origins in colloquial Russian language and means a period of consecutive drunkenness lasting two or more days and significantly impairing the social life of the individual (74).

Trends in alcohol use have corresponded to the trends in mortality in Russia over the years. According to the different authors between 34% and 59% of death among men in Russia have been attributable to alcohol use (75-77). Alcoholic cardiomyopathy is among conditions that occur at high levels of alcohol use and is characterised by dilation and impairment of the left ventricle (78). Although the causal relationship between heavy alcohol use and cardiomyopathy is well established (79), it is less clear what proportion of alcohol drinkers develop alcoholic cardiomyopathy and what amount of alcohol is required to produce the condition (80). By definition the diagnosis of alcoholic cardiomyopathy requires confirmation of heavy alcohol use in the patient, therefore there is less clarity on prevalence of any intermediate phenotypes which occur before the clinical diagnosis or at lower levels of alcohol use.

1.4.4 Novel biomarkers of cardiovascular disease

The classical CVD risk prediction models include blood lipids (total cholesterol, LDL-cholesterol, or non-HDL-cholesterol) (32). A range of further blood-based biomarkers has been proposed to improve the prediction of CVD in clinical practice in primary prevention (81). Although there have been many candidates that were shown to be associated with the CVD risk,

most of them did not improve the performance of the prediction models or improved it only slightly, and therefore were not introduced clinically in primary prevention (81-84). However, these biomarkers may nevertheless give some insight into the pathophysiology of cardiovascular disease and characterize cardiovascular health in populations (85).

Some of the conventional biomarkers, like LDL-cholesterol, are causally related to cardiovascular diseases through the atherosclerotic process. However, certain other biomarkers are instead markers of end-organ damage and indicate the presence of cardiovascular pathologies even if asymptomatic (85). The three biomarkers of this type that were most strongly associated with CVDs outcomes in population-based studies are hsCRP, NT-proBNP, high sensitivity cardiac Troponin T (hs-cTnT)/Troponin I (81, 86). High sensitivity cardiac Troponin T (hs-cTnT) and NT-pro-BNP have an established role in clinical cardiology diagnostics, although until recently they have not tended to be measured in population-based samples (86). The measurement of hsCRP has been strongly advocated by some researchers (87) and was recommended in the AHA/CDC Scientific Statement for clinical use (88). However, the 2016 European Guidelines on cardiovascular disease prevention in clinical practice do not recommend routine measurement of this biomarker for risk prediction (48).

In the following paragraphs, I will summarize the potential contribution of hs-cTnT, NT-proBNP, and hsCRP to our understanding of the pathophysiology of CVD.

1.4.4.1 Troponin T and Troponin I

Troponin T and Troponin I is an intracellular protein of myocytes and is expressed almost exclusively in the heart (89). When cardiac cells are injured, both types of troponins are released into the bloodstream and can be detected with by laboratory tests (89). The abrupt elevation of Troponin T and Troponin I relative to the usual values in a particular individual usually

indicates acute cardiac damage, therefore it is used in clinical practice for diagnostics of MI (40). Chronic cardiac damage can lead to persistent elevation of Troponin T, which occurs in non-ischemic conditions like heart failure, cardiomyopathy, atrial fibrillation, myocarditis, sepsis, chronic kidney disease, use of chemotherapeutic agents, etc. (90). Currently, high sensitivity cardiac Troponin T tests which can detect concentrations as low as 3 ng/L are recommended for use in clinical practice (89). Furthermore, the development of high-sensitivity assays allowed measurement of hs-cTnT concentrations in individuals without previous CVD recruited from the general population. In population-based studies, elevations in circulating cardiac troponin were associated with a higher risk of a first-ever CVD event (86).

Chronic elevation of troponin levels was associated with indices of heart failure (such as higher left ventricular mass, lower left ventricular ejection fraction, increased NT-proBNP levels) but not with indices of atherosclerosis or ischemia (91-93). Therefore, hs-cTnT is potentially useful for risk prediction of heart failure at the population level (85).

1.4.4.2 N-terminal pro-B-type natriuretic peptide: NT-proBNP

N-terminal proBNP (NTproBNP) is created as a result of proteolytic cleavage of the prohormone pro-B-type natriuretic peptide (proBNP) in the LV myocardium in response to end-diastolic wall stress through volume and pressure overload (94). NT-proBNP does not have biological functions while BNP is s a vasoactive hormone involved in volume homeostasis, vasodilation, and cardiovascular remodeling (94). NT-proBNP is used for diagnosis and prognosis in the setting of heart failure (45). Evidence from measuring of NT-proBNP in population-based samples indicate that it may be useful for prediction of heart failure in people without a diagnosis of CVD (85). In a meta-analysis the concentration of NT-proBNP strongly predicted first-onset heart failure and improved prediction of coronary heart disease and stroke

in people without known cardiovascular disease (95). Also, NT-proBNP concentration predicted stroke as strongly as coronary heart disease. This could partly be explained by associations between NT-proBNP concentration and stroke risk factors: left ventricular hypertrophy and atrial fibrillation (95-97). In a community sample of older adults change in NT-proBNP concentration conferred greater risk for systolic dysfunction, incident HF and cardiovascular death (98).

1.4.4.3 C-reactive protein: CRP

Increased levels of C-reactive protein (CRP) are associated with CVD (84), although evidence accumulated over the last two decades indicates that the association is probably not causal, with CRP being instead a downstream marker of atherosclerotic disease (99). While a 1000-fold elevation of CRP is an established clinical indicator of acute inflammation (100), mild and persistent elevation of CRP can be measured using the high sensitivity test (hsCRP) which has a lower detection limit of approximately 0.03 mg/L that is much lower than used in a routine clinical setting of diagnosing infection (5-8 mg/L). Elevated hsCRP levels have been interpreted as a marker of low-grade systemic inflammation that characterizes atherosclerosis (101). CRP was found within atherosclerotic plaque (102) and associated with coronary plaque burden (103). The high levels of hsCRP are strongly associated with future cardiovascular events even after adjustment for other CVD risk factors (104, 105). Risk ratios (RRs) for coronary heart disease per 1-SD higher log-transformed CRP concentration were 1.63 when initially adjusted and 1.37 when adjusted further for conventional risk factors. However, risks of a similar magnitude were observed for death from several cancers and lung disease (104). Thus, the elevation of hsCRP in individuals with no overt disease is non-specific and may reflect exposure to adverse inner and outer environment: chemical pollutants, diet, smoking, alcohol

consumption, medications, periodontal disease, obesity, diabetes, metabolic syndrome, and hypertension (101).

1.5. Trends and differences in CVD risk factors and biomarkers in Norway and Russia

In the next few paragraphs, I will provide a short summary of the levels and trends of conventional CVD risk factors (total cholesterol, blood pressure, smoking, obesity, diabetes), alcohol use, and novel biomarkers in Russia and Norway, with a more detailed description of studies conducted in Russia.

1.5.1. Lipid levels and lipid-lowering medication use

Previous research on lipid levels in Russia has failed to find any substantial differences in mean total cholesterol compared to other countries. Nationwide representative studies on lipid levels in Russia do not exist with only a few population-based studies conducted at different locations providing mean levels for total cholesterol and LDL-cholesterol among men and women. They are named here in the chronological order of when they were conducted: Lipid Research Clinics (1975-1977) (106), MONICA (1992-1995) (107), Arkhangelsk study (2000) (34), Pitkaranta study (1992, 1997, 2002, 2007), Izhevsk Family Study 2 (2008-2009) (108), Stress Aging and Health in Russia (SAHR) (2007-2010) (15), Epidemiology of cardiovascular diseases in the regions of Russian Federation (ESSE-RF) (2012-2014) (14), HAPIEE (2003-2005, 2006-2009, 2015-2017) (109), Know Your Heart study (2015-2017) (110). The WHO MONICA study (Novosibirsk and Moscow) reported the lower age-standardized hyperlipidemia (8 – 20%) compared to many other countries (107). Besides, favorable mean values of the ratio of Apo B to Apo A1 (111) and HDL to total cholesterol (34) were observed in Arkhangelsk study compared to Norway (Tromsø and Finnmark studies) (34). The HAPIEE (Novosibirsk)

reported high mean levels of total cholesterol (6.3 mmol/L) among participants aged 45–69 years, and the mean levels of LDL- and HDL-cholesterol were 4.1 mmol/l and 1.5 mmol/l, respectively, similar to countries with lower CVD mortality rates such as Poland and the Check republic (109). I could not identify any studies in Russia that reported trends over time in total cholesterol.

In contrast, more is known about the situation in Norway where data on longitudinal trends in total cholesterol are available. There has been a definite downward trend in mean total cholesterol observed in Norway since 1979 (11). In the Tromsø study mean total cholesterol decreased during 1979–2016 in both women and men and all age groups (11). Norway experienced the largest decline in total cholesterol among Western countries, of 0.4 mmol/L per decade (112), driven by both declines in HDL and non-HDL cholesterol (112).

The success of CVD mortality reduction in many developed countries is partially attributed to control of cholesterol levels that can be achieved with lipid-lowering medications and diet modifications (11). The Norwegian Prescription Database (NoPD) gives an overview over all prescription drugs dispensed from pharmacies in Norway since 2004 (113). According to NoPD use of lipid-modifying agents in Norway increased from 66.7 per 1000 (6.7%) in 2004 to 111.4 per 1000 (11.1%) in 2019, all age groups (113). The prescriptions increased with age, for example in 2019, 333.9 per 1000 (33.7%) were prescribed lipid-modifying agents in the age group 65-69 years (113). According to the Tromsø Study, the use of lipid-lowering drugs in Tromsø municipality was very rare in 1994 but increased steadily between 1994 and 2016 (11). Among women and men younger than 50 years, the use of lipid-lowering drugs was less than 5% in Tromsø 5-7 surveys. In persons older than 50 years old, lipid-lowering drugs use was higher in men than in women, and reached 20-25% by the seventh decade of life (11).

Unfortunately, there is no national prescription database in Russia that can provide data on lipid-lowering medication use in representative samples of the national population. Few

population-based studies that are limited to certain regions report a low prevalence of lipid-lowering medication use. The Izhevsk family study (2008-2009) reported that fewer than 2% of those with hyperlipidemia were taking lipid-lowering medications (108). A multi-centre population survey ESSE-RF (25-64 years old, 13 regions, 2012-2014) found that only 7 % of persons with high or very high CVD risk (including patients with previously diagnosed CVD) took lipid-lowering medications, and the target levels were reached in 14.4% of men and 4.8% of women in these groups (114). In EUROASPIRE IV (2012-2013), a study on management of CVD risk factors following hospitalisation with coronary heart disease in 24 European countries, three centres in Russia (Moscow region) had lower use of lipid-lowering medications and fewer patients reaching targets for cholesterol reduction compared to other countries (115). However, the health care system in Russia has gone through substantial change after the introduction of the "dispansarisation" program in 2013 which is essentially a screening program for CVD risk factors and chronic diseases (116). The Know Your Heart (KYH) study (2015-2018) reported that 40% of participants with previous MI or stroke were taking statins or other lipid-lowering medications, but the proportion meeting treatment targets for LDL-cholesterol was low (MI: 5%, stroke: 11.6%) (117). In total, data on lipid-lowering medications prescriptions in Russia are scarce and point at suboptimal use and low success of hypocholesteraemia control in Russia.

1.5.2 Blood pressure and blood pressure control

According to WHO Study on Global Aging and Adult Health (2010), 50% of Russians have hypertension, which is one of the highest among middle-income countries (118, 119). The study of 25–64-year-olds in nine regions of Russia, ESSE-RF (2012-2013), reported an age-standardized prevalence of hypertension of 44% (48.2% men and 40.8% women) (120). Mean

SBP and DBP were 130.7 mmHg and 81.6 mmHg respectively (120). Russia has also one of the highest prevalence of hypertension globally according to the analysis of WHO Global Health Observatory (121). The age-standardized prevalence of elevated blood pressure in Russia was 30% in 2010, and 28.7% in 2014, while the prevalence of hypertension in Norway was estimated to be at 20.7% (2010) and 18.1% (2014) (121).

Untreated hypertension remains a major problem in Russia. In the Know Your Heart (KYH) study (2015-2018), control of blood pressure was achieved in 22% of men and 43% of women with hypertension (122). The findings for KYH are consistent with ESSE-RF results, which reported that 14.4% of men and 30.9% of women had their hypertension controlled (120). In 2010, 83% of persons with hypertension in WHO SAGE study (Russia) had uncontrolled hypertension (118).

Mean systolic and mean diastolic blood pressure decreased substantially during the last four decades in high-income western countries including Norway, being among those with the highest blood pressure in 1975 they moved to the lowest in 2015 (123). Mean systolic and diastolic blood pressure in the Tromsø study decreased from 1994 to 2008 in both genders (124). For example in the age group 30 - 59 years, the age-adjusted drop in SBP per decade was 5.9 mm Hg in women and 3.7 mm Hg in men (124). The use of antihypertensive medication increased in all age groups in both genders from 1979 to 2008, and with age in all birth cohorts (124). The proportion of drug-treated hypertension increased from 8% to 19% in 1994 – 2008 (10).

1.5.3 Smoking

Smoking is the CVD risk factor that can be considered to be a definite driver of CVD morbidity and mortality among Russian men because of its sustained high prevalence over many decades.

For example, in 2000 smoking in Russian men was among the highest in the world according to the WHO Global Adult Tobacco Survey (GATS) (125). However, smoking among women in Russia has been historically low compared to Western countries and therefore it is unlikely to explain excess CVD mortality in Russian women compared to women in other countries. The studies of smoking prevalence in Russia over the last 30 years were reviewed by Shkolnikov et al. (126) in a meta-analysis that synthesised evidence from many population-based studies in Russia. The reductions in smoking started for men in 2008 with a simultaneous decline in all age and educational groups (126). One of the reasons cited for that decline was the implementation of a series of policy initiatives over the past 10 years, which started with the ratification of the Framework Convention on Tobacco Control in 2008 (127). Recent trends in smoking among women differ by age and educational group: smoking prevalence is declining at younger ages, but an upward trend remains at older ages; those with the highest levels of education showing small declines, whereas those women with minimal educational attainment have shown a persistent steady increase (126).

The comparison with data from other countries still shows higher contemporary levels of smoking in men (age-standardized prevalence of current smoking 48.1 % (RLMS in 2016) vs 24.2% in NHANES (2015–2016) and 23.4% in HSE (2012). Age-standardized smoking prevalence in Russian women was 17.5 % (RLMS in 2016) which is similar to 15.4% in NHANES (2015–2016) and 19.2% in HSE (2012), with prevalence among younger women in Russia (up to 45–50 years old) being higher than in older age groups (126).

Smoking prevalence has dropped steadily in Norway since 1998, and it is very low among young people currently. According to Statistics Norway, 51 percent of men and 32% of women (aged 16-74) smoked daily in the early 1970s (128). In 2019, 9% of people aged 16–74 smoked daily, with no difference in prevalence between men and women (128). The prevalence of young daily smokers was reduced from 17% to 2% in the last ten years (128). There are

significant differences in smoking status by educational status in Norway, and low-educated residents smoke more (129). In the Tromsø study age-adjusted daily smoking decreased from 34% in 1994-1995 to 22% in 2007-2008 (10).

1.5.4 Obesity

There has been an increase in the prevalence of overweight and obesity in both Russia and Norway over the past decades (130). Among men, however, it is striking that today the prevalence in Russia is if anything lower than in Norway. Among Russian men over 20 years of age the prevalence of obesity was around 9 % in 1980-1990, and increased to 12.8% in 2000, and to 15.3% in 2013; in Norway the prevalence of obesity in men was 14% in 1980, and increased to 16.6% in 2000, and to 19.1% in 2013. The prevalence of obesity in women in Russia was around 22.3 % in 1980-1990, and increased to 29.2% in 2000, and to 28.5% in 2013; in Norway prevalence of obesity in women was 13.5% in 1980, and increased to 16.4% in 2000, and to 18.0% in 2013. In 2013, 54.3% of Russian men and 58.9% of Russian women over 20 years old were considered overweight or obese, the corresponding numbers for Norway were also very high (58.4% men and 47.3% women) (130).

In the Tromsø Study, the age-adjusted (ages 30–84) the prevalence of obesity increased from 9.8% in men and 11.8% in women in 1994–1995 to 20.9% and 18.5%, respectively, in 2007–2008 (131). Abdominal obesity also increased from 1994–1995 (20% men and 35% women) to 2007–2008 (37% men and 55% women). In the longitudinal analyses over 13 years the mean waist circumference increased in all examined birth cohorts in both men (mean change 6.1 cm) and women (mean change 8.4 cm), but the increase was more remarkable in younger participants (132).

1.5.5 Diabetes.

Due to aging populations and lifestyle changes diabetes has become a global epidemic (133). Nevertheless, its prevalence varies widely in different countries being remarkably high in Russia. Data on Type 2 diabetes prevalence in Russia has been reported in a few population-based studies based either on glycated haemoglobin (14-16) or fasting glucose (134-136), although not all of them were published in the peer-review literature. These studies report a relatively high prevalence of Type 2 diabetes in different age groups in Russia ranging from 7% or 16% with the burden being higher in women compared to men at older ages which is not the case in most other countries (133, 137). The Russian multi-regional NATION study (2013-2015) estimated Type 2 diabetes prevalence based on both HbA1c and self-report: 7.0% in women versus 7.9% in men aged 45–59 years old, and 14.1% in women versus 9.9% in men aged 60–79 years (16). The ESSE-RF study (10 regions of Russian Federation, 2012-2014) estimated prevalence of diabetes mellitus based on self-report and fasting glucose: 9.4% in men and 7.4% in women aged 45-54 years old, and 13.6% in men and 16.5% in women aged 55-64 years old (134).

Diabetes can develop with no or minimal symptoms. As a consequence a substantial proportion of diabetes remains undiagnosed and not managed (138, 139). A high proportion of undiagnosed diabetes has been reported in Russia: 54% in NATION study (16), 43% in HAPIEE (135), 27% in UEMS (136). Time trends of diagnosed and undiagnosed diabetes prevalence in Russia are difficult to estimate because of a diversity of locations, sampling frames, and diagnostic tests used in the studies.

Estimates of the prevalence of diabetes in Norway are available from national registries with prospectively collected data on prescriptions of antidiabetic drugs and diabetes diagnoses from hospitals and primary care visits for all residents in Norway aged from 30 to 89 years. The crude

prevalence of Type 2 diabetes increased from 4.9% to 6.1% from 2009 to 2014; and diabetes prevalence was higher in men than women (6.8% vs 5.3% in 2014) (140). However, these estimates do not include undiagnosed diabetes cases that would be detected by screening for biomarkers.

1.5.6 Alcohol

Currently, alcohol use in Russia shows a declining trend, particularly among younger age groups, and there has been a shift in the type of beverage consumed from spirits to beer (141). Acute alcohol poisoning mortality which indicates the prevalence of harmful drinking has declined substantially since the mid-2000s (5). Fluctuations in total mortality/ life expectancy are no longer well predicted by mortality from acute alcohol poisonings (5). Improvements in life expectancy during the last decade are larger than would be expected based on changes in mortality rates from acute alcohol poisoning (5).

In the 1990s and the beginning of the 2000s, Russia was one of the heaviest drinking countries in Europe characterized by extremely high levels of alcohol consumption and hazardous drinking (142). Unrecorded alcohol made up a third of total alcohol consumed in the Russian Federation, including illegal production of homemade and surrogate / non-beverage alcohol. After the peak of 20.4 litres of alcohol per capita in 2003 alcohol consumption declined, by 43% by 2016, with a substantial decline in spirits drinking (67%) and consumption of unrecorded alcohol (48%). Within the same period, consumption of lighter alcoholic beverages decreased slightly; wine drinking declined by about 8% and beer drinking by about 4%. Despite this important success, in 2016 total per capita alcohol consumption for the Russian Federation was estimated at 11.7 litres of pure ethanol (for the population 15+), which is still among the

highest levels of consumption worldwide and higher than the WHO European average (9.8 litres) (142).

In Russia heavy episodic drinking (at least 60 g of alcohol on at least one occasion in the past 30 days) declined in men from about 75% of the adult population (aged 15 and older) in 2004 to 48% in 2016. A similar proportional drop was observed in women, from 52% in 2004 to 24% in 2016. Alcohol per capita consumption in drinkers only was 30.5 litres in Russian men and 10.5 litres in women in 2016.

In Norway, alcohol per capita consumption (in litres of pure alcohol) declined from 9.0 in 2010 to 7.5 in 2016 (143). Among drinkers only, men consumed 13.2 litres of pure alcohol in 2016, and women 4.6 litres. The prevalence of heavy episodic drinking (at least 60 g of alcohol on at least one occasion in the past 30 days) in men (aged 15 and older) was 48.6% in 2016, and 15.3% in women. Prevalence of alcohol use disorders and alcohol dependence was 10.6% in men and 3.8 % in women in 2016 (143).

It is important to mention that most indices of alcohol use mentioned above are based on the population surveys which are known to suffer from selection bias as well as social desirability bias, and are likely to underestimate alcohol consumption. Also, specific patterns of alcohol use recorded in Russia make indices of alcohol use less comparable with other countries. Selection bias and measurement error in studies of the relationship between alcohol use and CVD are considered in more depth in the Discussion section of this thesis.

1.5.7. Differences in levels of novel biomarkers: hs-cTnT, NT-proBNP, hsCRP

These novel biomarkers are still very rarely used in epidemiological assessments of CVD at the population level. I was unable to find any published papers that compared levels of these biomarkers between populations or across time. Comparing levels of these biomarkers on the

population level is particularly challenging because of the variety of commercial laboratory assays used many of which are not comparable. Several previous studies have investigated predictors of increased hsCRP levels in Russian populations but did not report mean levels or systematically compare them with studies from other countries (144, 145).

1.6 Aims of the thesis

The overall aim of the thesis is to contribute to understanding why the CVD burden is much higher in Russia compared to other countries.

Specific aims:

1. To compare the differences in CVD biomarker profiles in Norway and Russia;
2. To investigate the association between heavy alcohol use in Russia and biomarkers of heart damage and general inflammation;
3. To compare the prevalence of diabetes mellitus in Russia and Norway and explore what factors can contribute to these differences.

Chapter 2 Materials and methods

2.1 Study design and population

2.1.1. The Tromsø Study (Norway)

The Tromsø Study is a large population-based longitudinal study conducted in the Tromsø municipality. The study was initiated by the University of Tromsø in 1974, and its focus was on the extremely high cardiovascular mortality in Northern Norwegian men which later on expanded to other chronic diseases and included both genders (146). So far seven consecutive surveys of the Tromsø Study have been conducted. This thesis only uses data from the seventh wave (Tromsø 7).

Tromsø 7 was conducted in 2015-2016, and all inhabitants of the municipality of Tromsø aged 40 and above were invited and 21083 participated (65%), age 40-99. Potential participants received an invitation to visit the study site along with a first questionnaire which collected information on medical history, physical activity, smoking, and ethnicity. This could be completed digitally with a personal password or returned in paper during a visit to the study site. The second, more extensive, questionnaire had to be completed digitally at home or the study site where assistance was provided if needed. This questionnaire collected information on dietary habits, alcohol consumption, lifestyle, medication use, symptoms, and medical history. During the visit to the study site, participants also went through a physical examination and provided blood and urine sample. Physical examination included blood pressure measurements and measurements of weight, height, waist and hip circumference. Jenix® height & weight scale DS-103 (Jenix Co, Ltd) was used for weight measurement, systolic and diastolic blood pressure measurement was performed with automated oscillometric upper arm blood pressure monitors Dinamap (ProCare 300, GE Healthcare). A subsample of 8346 participants, aged 40-99, (mostly a random sample with some participants who were specifically invited

because of their participation in previous Tromsø surveys) attended a second visit, which included more extensive clinical examinations (12-lead electrocardiogram (ECG), carotid ultrasound, echocardiogram, and many other tests) and additional collection of blood. Schiller AT-104 PC (Schiller, USA) was used for 12-lead ECG. Blood samples (non-fasting) at both visits were processed immediately after collection and laboratory assays of the biomarkers were performed the same day at the Department of Laboratory Medicine, University Hospital of Northern Norway (ISO certification NS-EN ISO 15189:2012).

Paper 1 and Paper 3 utilised data from the Tromsø 7 study on 17649 men and women aged 40-69 years (Figure 3).

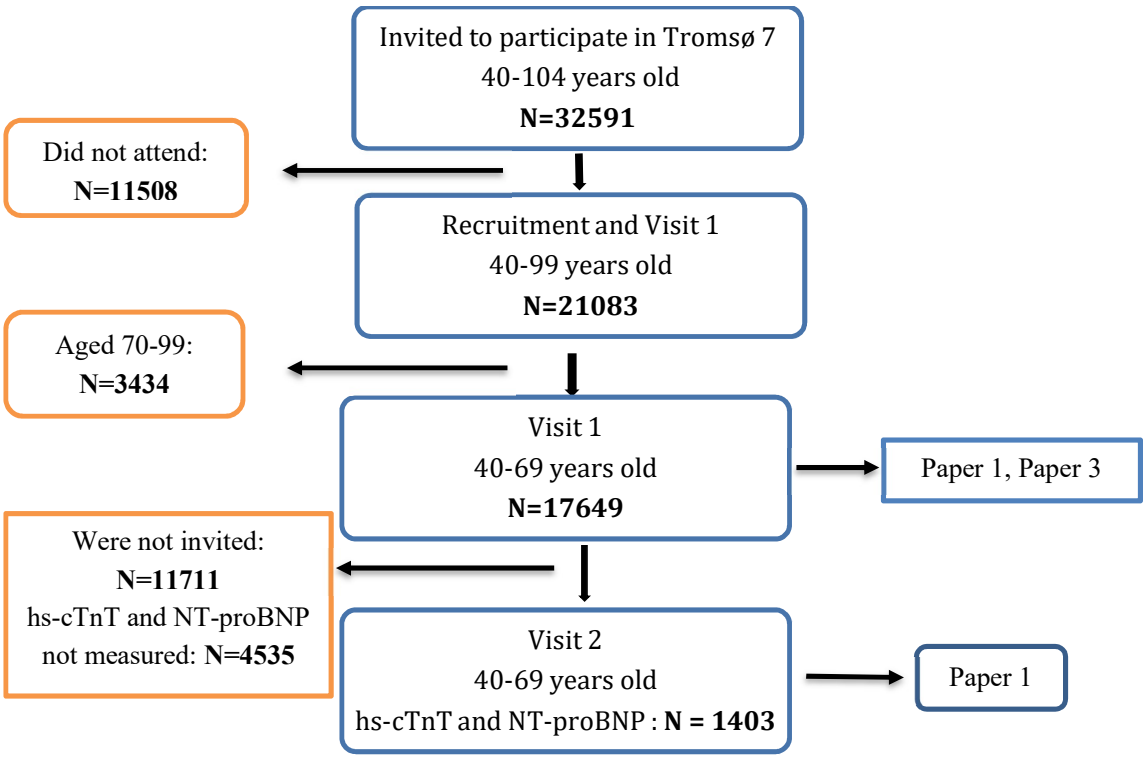

Figure 3. Flow chart for Tromsø 7

2.1.2. Know Your Heart study (Russia)

KYH study is a cross-sectional population-based study conducted in Russia in 2015-2018 (147). A random population-based sample of 35 – 69-year-old participants (n=5071) stratified by age, sex, and district was recruited in the cities of Arkhangelsk and Novosibirsk (Russia). Trained interviewers recruited and interviewed participants at home to ascertain information about their health, socio-demographic characteristics, and lifestyle (51.0% of approached agreed to participate). Participants were then invited to take part in a health check that usually occurred 1-2 weeks later in an outpatient clinic and 4512 (89%) attended.

At the same time, KYH recruited 278 patients from the Arkhangelsk Regional Psychiatric hospital with a primary diagnosis of alcohol problems (147). The latter group is referred to subsequently as the narcology clinic subsample consistent with Russian terminology. The inclusion criteria were: age 35-69 years, resident in the city of Arkhangelsk or Arkhangelsk region and admitted to the narcological department of the regional psychiatric hospital with a primary diagnosis related to alcohol drinking. A total of 278 patients were recruited out of 322 patients invited (85.4%).

The health check included a medical examination, questionnaire, and biological sample collection. The medical examination included blood pressure measurements, recording of weight and height, and a 12-lead ECG. TANITA BC 418 body composition analyser (TANITA, Europe GmbH) was used for weight measurements, systolic and diastolic blood pressure were measured with automated oscillometric upper arm blood pressure monitor Omron 705IT (HEM-759-E), ECG was performed using Cardiax digital device (IMED ltd, Hungary). The questionnaire collected data on health problems, lifestyle, and medication use. Participants were requested not to eat or drink alcohol in the 4 hours before their appointment. Within 2 hours

after venipuncture, blood was centrifuged, serum was frozen (-80C), shipped to a laboratory in Moscow, and analyzed in a single batch at the end of the fieldwork (147).

Paper 1 is based on data from 4046 participants aged 40-69 who attended the health check and provided a blood sample (Figure 4). Paper 2 is based on data from 2354 participants from general population in Arkhangelsk (35-69 years old) plus 271 persons from the narcology clinic subsample who attended the health check and for whom blood analyte concentrations were available (Figure 4). Paper 3 is based on 4121 participants aged 40-69 who attended the health check (Figure 4).

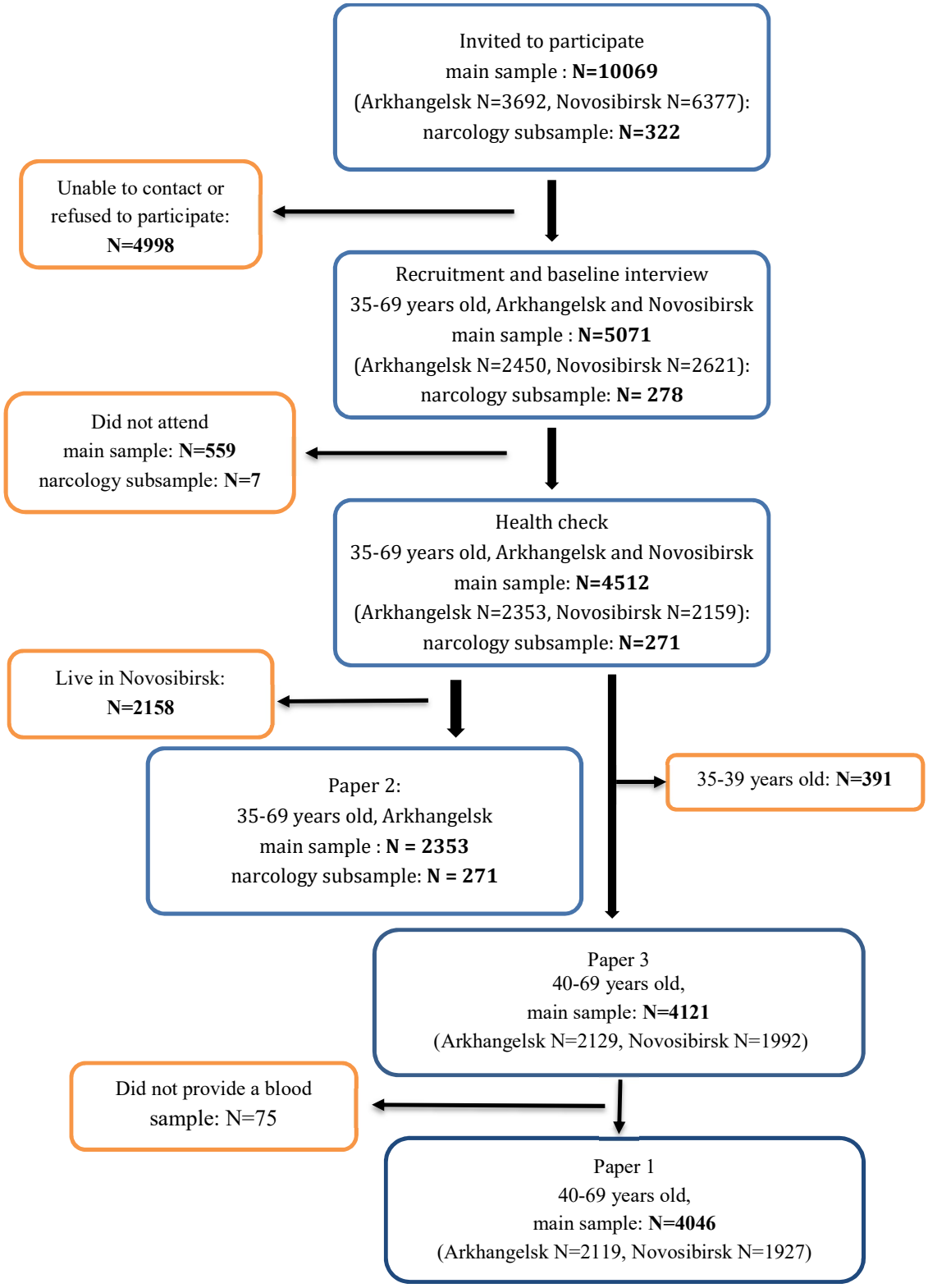

Figure 4: Flow chart for Know Your Heart study

2.2 Laboratory analysis and calibration of biomarkers.

All participants in KYH and Tromsø 7 with blood sample collected had the following biomarkers measured: lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), a marker of systemic inflammation (hsCRP), and glycosylated haemoglobin (HbA1c) (see Article 1, Supplemental Table S1 for a description of laboratory methods). A marker of cardiac damage (hs-cTnT) and a marker of cardiac wall stretch (NT-proBNP) were measured in all KYH participants with blood samples collected and in 1403 Tromsø 7 participants who were either selected randomly (81%) to attend the second visit or were invited because of their previous participation in the sixth wave of the Tromsø study (Figure 3).

Differences in the laboratory procedures in KYH and Tromsø 7 brings the potential for systematic differences in biomarker measurements between the two sites due to measurement error. This was addressed by a recalibration study with split sample testing (Article 1, Supplementary Methods M1). For this purpose, 100 serum samples and 50 whole blood samples from KYH participants were re-assayed in both the laboratory in Moscow and Department of Laboratory Medicine at the University Hospital of Northern Norway. The paired measurements were analyzed using Deming regression to assess any systematic differences in laboratory performance. The biomarker values for KYH were recalibrated using the resultant calibration coefficients for analyses in Paper 1 and Paper 3 (Article 1, Supplementary Table S7). To account for uncertainty in the estimation of the calibration coefficients in the subsequent comparative analysis we used a "double-bootstrap" approach, verified using a simulation study (Article 1, Supplementary Methods M2), to obtain 95% confidence intervals for the regression coefficients.

2.3. Definitions of other variables and their harmonization between studies

Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Mean systolic and diastolic blood pressure was calculated as the mean of 2nd and 3rd measurements. Waist circumference (WC) was measured at the narrowest part of the trunk in KYH, while in Tromsø 7 WC was measured at the umbilicus level. To ensure WC was comparable between the two studies, WC in Tromsø 7 was converted to the narrowest waist using a conversion equation (148). Waist-to-hip ratio (WHR) was calculated by dividing WC by hip circumference. Smoking status was categorized as current smokers, ex-smokers, and never-smokers. For current smokers the number of cigarettes smoked was specified as 1–10/day, 11–20/day, >20/day. Education level was classified into three categories: primary/ secondary, upper secondary, tertiary. Diabetes was defined as HbA1c concentration above 6.5%, and/or self-report of diabetes, and/or use of medication with ATC-code A10 (antidiabetics) according to the Anatomical Therapeutic Chemical (ATC) classification (149). Lipid-lowering drug use was determined according to recorded medications coded to the ATC classification as C10 (lipid-modifying agents) or self-reported use. Use of anti-hypertensives was determined according to recorded medications coded to the Anatomical Therapeutic Chemical (ATC) classification as either C02 (antihypertensives) or C03 (diuretics) or C07 (beta-blocking agents) or C08 (calcium channel blockers) or C09 (agents operating on the renin-angiotensin system). A small proportion of participants self-reported use of blood pressure lowering medication without a corresponding ATC code being found. These participants were also defined as being on anti-hypertensives. Renal function was assessed by measuring cystatin C and estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration cystatin C equation (150).

Time since last meal (fasting status) was determined from an interview question.

Pre-existing coronary heart disease was determined based on evidence of previous myocardial infarction on ECG, self-report of myocardial infarction (MI), or grade 2 angina pectoris. ECGs from both studies were coded according to the Minnesota code (MC 1.1-1.3) (151) using the same semi-automated system. Grade 2 angina was determined using the Rose angina questionnaire (short version) (152).

Alcohol use for Paper 2 was defined by two different variables based on available questionnaire and laboratory data. The first variable was binary and divided the study group into those from the narcology clinic subsample and those from the general population sample. The second variable further categorized the general population sample into groups based on self-report of various dimensions of alcohol consumption. Those who reported not drinking alcoholic beverages during last the 12 months at baseline interview and health check were classified as non-drinkers. The categories of harmful and hazardous drinkers were defined using three instruments: the validated Russian language translations of the Alcohol Use Disorders Identification Test (AUDIT) (153), the CAGE instruments (154), and questions on alcohol drinking pattern previously found to be highly predictive of mortality in Russia (74, 155). AUDIT is a screening test for hazardous and harmful drinking, while CAGE is used to screen for alcoholism in clinical settings. The CAGE score was adapted to have a reference period of the past 12 months rather than ever in a participant's lifetime, in keeping with a previous study from Russia, due to interest in alcohol use in the recent past (156). The scheme for categorizing the general population sample into four groups is described in detail in Paper 2. The resulting categories of alcohol consumption included (1) narcology clinic subsample (2) general population sample, harmful drinking pattern (3) general population sample, hazardous drinking pattern (4) general population sample, non-problem drinkers (5) general population sample, non-drinkers (ex-drinkers) (6) general population sample, non-drinkers (never-drinkers).

2.4 Statistical analysis

For the initial comparisons of means and proportions, age-standardization to the 2013 Standard European Population was used. Analysis stratified by sex was conducted in Paper 1 and Paper 3, but analyses for Paper 2 were not stratified by sex.

Regression modelling was used to explore the association between variables. Data from participants with complete information on all the covariates were used (complete case analysis). Biomarkers with skewed distributions (triglycerides, hsCRP, hs-cTnT, NT-proBNP) were ln-transformed before analysis and geometrical means were presented. To make the regression coefficients for ln-transformed biomarkers easier to interpret they were back-transformed and presented as a percent of difference in mean compared to the reference category (Paper 1 and Paper 2). Age was included in the model as a continuous variable. Quadratic and cubic terms were added to account for non-linearity and kept in the model if associated with an outcome at $p<0.05$.

For Paper 1 and Paper 2 sensitivity analysis excluded those with previous CVD (myocardial infarction and grade 2 angina) to see if elevated cardiac injury and cardiac wall stretch biomarkers were secondary to coronary heart disease.

Statistical analysis was performed using R version 3.6.0 and SAS software 9.4, SAS Institute Inc., Cary, NC, USA.

2.4.1. Statistical methods – summary of papers

Paper 1

Paper 1 aimed to compare mean levels of blood based CVD biomarkers between Russia and Norway by making comparisons of data from Tromsø 7 and KYH. Multivariable linear regression was used to assess if the differences in mean biomarker levels in the two studies

could be explained by differences in smoking prevalence, BMI, WHR, blood pressure, diabetes, education level (Model 2), and use of lipid-lowering drugs (in addition to variables in Model 2) (Model 3). Interaction between age and study was assessed by the inclusion of product term into the regression model, and results were presented separately for 40-54 and 55-69-year-olds.

Paper 2

Paper 2 aimed to assess the association between patterns of alcohol use and biomarkers of heart damage (hs-cTnT), cardiac wall stress (NT-proBNP), and general inflammation (hsCRP). Paper 2 is based on data from KYH only (participants from Arkhangelsk and narcology clinic subsample). First, we compared geometric means of CVD biomarkers in the narcology and general population samples. Next, we compared means of ln-transformed biomarkers across the categories of alcohol consumption: (1) narcology clinic subsample (2) general population sample, harmful drinking pattern (3) general population sample, hazardous drinking pattern (4) general population sample, non-problem drinkers (5) general population sample (ex-drinkers and never-drinkers).

The associations between alcohol use and ln-transformed biomarkers of cardiovascular disease (hs-cTnT, NT-proBNP, hsCRP) were assessed using multivariable adjusted linear regression models. Model 1 involved adjustment for age and sex. Model 2 adjusted for potential confounders (age, sex, smoking, education). Model 3 additionally included possible mediators (waist to hip ratio, BMI, lipids (LDL, HDL, ApoB/ApoA1 ratio), blood pressure (SBP and DBP), use of blood pressure medication, eGFR). A test for increasing linear trend in means of biomarkers across the categories of alcohol exposure was done with one degree of freedom.

Separating the category of non-drinkers into never drinkers and ex-drinkers for regression analysis did not reveal any specific differences in biomarkers between these two groups, therefore the results were presented keeping current non-drinkers as one group.

Paper 3

Paper 3 aimed to compare the prevalence of diabetes, undiagnosed diabetes, and prediabetes in Russia and Norway by making comparisons of data from Tromsø 7 and KYH. To examine if the prevalence differences between studies may be explained by different levels of diabetes risk factors we conducted mediation analysis using marginal structural models (157) which allows the decomposition of the total effect of exposure into that mediated by specific factors (indirect effect) the remaining (direct) effect. In our analysis, the study (KYH vs Tromsø 7) was considered the exposure, while diabetes risk factors were considered possible mediators. BMI and WC (both in quintiles) were included in the first step, controlling for age (158). A further model introduced smoking and hsCRP as additional mediators, the latter as it reflects proinflammatory status (158, 159).

2.5 Ethical approval

Ethical approval for the KYH study was received from the ethics committees of the London School of Hygiene & Tropical Medicine (approval number 8808, 24.02.2015, for sub-study involving patients in treatment for alcohol problems approval number 12018, 11/01/2017), Novosibirsk State Medical University (approval number 75, 21/05/2015), the Institute of Preventative Medicine (no approval number; 26/12/2014), Novosibirsk and the Northern State Medical University, Arkhangelsk (approval number 01/01-15, 27/01/2015, for sub-study involving patients in treatment for alcohol problems approval number 05/11-16, 02/11/2016). The Regional Committee for Research Ethics approved Tromsø 7 (REC North ref. 2014/940). Study has conformed to the principles embodied in the Declaration of Helsinki. All participants provided signed informed consent.

Chapter 3 Results – Summary of papers

3.1 Paper 1 "Why does Russia have such high cardiovascular mortality rates? Comparisons of blood-based biomarkers with Norway implicate non-ischemic cardiac damage"

Men in KYH were on average older, had higher blood pressure and lower BMI, and a higher proportion were current smokers, and had diabetes, compared to men in Tromsø 7. Women in KYH were on average older, had higher blood pressure and BMI, a higher proportion had diabetes, and a lower proportion were current or previous smokers, compared to women in Tromsø 7.

Having calibrated laboratory measurements in Russia and Norway, levels of total, low-density lipoprotein-, high-density lipoprotein-cholesterol and triglycerides were comparable in KYH and Tromsø 7 studies. NT-proBNP, hs-cTnT, hsCRP were higher in KYH compared with Tromsø 7. NT-proBNP was higher by 54.1% (95% CI 41.5% to 67.8%) in men and by 30.8% (95% CI 22.9% to 39.2%) in women; hs-cTnT—by 42.4% (95% CI 36.1% to 49.0%) in men and by 68.1% (95% CI 62.4% to 73.9%) in women; hsCRP—by 33.3% (95% CI 26.1% to 40.8%) in men and by 35.6% (95% CI 29.0% to 42.6%) in women. Adjustment for smoking, BMI, WHR, blood pressure, diabetes, education, and use of lipid-lowering drugs use had little effect on differences in lipids.

There was substantial attenuation of the differences in hsCRP due to adjustment by smoking, BMI, WHR, blood pressure, diabetes, and education but there remained evidence for differences between the two studies. For hs-cTnT and NT-proBNP, the adjustment did not change the estimate of the mean difference. The differences in hs-cTnT and NT-proBNP remained when the analysis was restricted to participants without previous CHD.

For most biomarkers, study differences were larger in women aged 55-69 years than 40-54 years. Among men, differences in hsCRP were more pronounced in the older age group (55-69 years), while differences in total and LDL-cholesterol were larger in younger men (40-54 years).

3.2 Paper 2 "Evidence for a direct harmful effect of alcohol on myocardial health: a large cross-sectional study of consumption patterns and cardiovascular disease risk biomarkers from Northwest Russia, 2015 to 2017"

The average age of the narcology clinic subsample was 48.5 years and that of the general population sample was 53.7. The narcology clinic sample were 76.8% men and the general population sample 41.7%. On average the narcology clinic subsample had lower systolic blood pressure (potentially due to clinical management during hospital admission), lower LDL and total cholesterol values, lower BMI and waist circumference, and lower eGFR compared to the general population sample. A much higher proportion of narcology clinic subsample compared to the general population sample were current smokers.

CVD biomarkers in the narcology clinic subsample vs the general population

After adjustment for age, sex, smoking, and education, the levels of all biomarkers (hs-cTnT, NT-proBNP, hsCRP) were higher in the narcology clinic subsample compared to the general population sample as a whole. Specifically, hs-cTnT was higher by 12.3% (95% CI: 5.9, 19.1) and NT-proBNP was higher by 43.9% (25.4, 65.1) while hsCRP was higher by 66.0% (41.7, 94.5).

CVD biomarkers across 5 categories of alcohol use

Consistent with the previous analysis, compared with non-problem drinkers in the general population sample, the narcology clinic sub-sample had much higher levels of hs-cTnT, NT-proBNP, and hsCRP. hs-cTnT was elevated by 10.3% (3.7, 17.4) in the narcology clinic subsample compared to the non-problem drinkers in the general population, controlling for gender, age, smoking, and education. However, hs-cTnT levels were lower in the group of harmful drinkers in the general population compared to non-problem drinkers. Adjustment for

the additional set of variables that are likely to be the mediators of the association between extremely heavy alcohol use and cardiac injury (determined via hs-cTnT) had only a minor effect on parameter estimates.

Harmful drinkers in the general population had an elevated concentration of NT-proBNP by 31.5% (95%CI: 3.4, 67.2) compared to non-problem drinkers, but to a lesser extent than in narcology clinic subsample: 46.7% (26.8, 69.8) controlling for age, sex, smoking, and education. Adjustment for potential mediators of the association between excessive alcohol use and cardiac wall stretch (measured by NT-proBNP) resulted in some attenuation of the effect estimate.

The elevation of concentration of low-grade systemic inflammation marker hsCRP by 69.2% (43, 100) was observed in narcology clinic subsample compared to non-problem drinkers in the general population sample, controlled for age, sex, smoking, and education. Intermediate elevations were also seen for harmful drinkers. Further adjustment for covariates that are likely to be on the mediation pathway between alcohol use and hsCRP led to increases in the regression coefficient.

Although we did not observe increased levels of cardiac biomarkers in the group of hazardous drinkers in general population, the trend test across all drinking categories (excluding non-drinkers) was significant for NT-proBNP and hsCRP with a concentration of biomarkers higher with a higher level of alcohol exposure.

In a sensitivity analysis, we excluded those with previous myocardial infarction, operations on the heart, and grade 2 angina (N=307, 11.73%) to see if elevated cardiac injury and cardiac wall stretch biomarkers were secondary to coronary heart disease. This had no substantial effect on the associations observed.

3.3 Paper 3 "What factors explain the much higher diabetes prevalence in Russia compared to Norway? Major sex-differences in the contribution of adiposity"

Age-standardized prevalence of diabetes was higher in KYH compared to Tromsø 7, in men (11.6% vs 6.2%) and in women (13.2% vs 4.3%). Age-standardized prevalence of undiagnosed diabetes was also higher in KYH than in Tromsø 7 in men (4.0% vs 1.2%) and in women (3.5% vs 0.5%).

In both studies men and women with diabetes had higher BMI and waist circumference, higher systolic and diastolic blood pressure and higher hsCRP levels than those who don't have diabetes. In KYH levels of total and LDL-cholesterol were similar in participants with and without diabetes, however, in Tromsø 7 total and LDL-cholesterol were lower in participants with diabetes. Smoking prevalence was similar in participants with and without diabetes in both studies. Substantial differences in mean risk factor levels were observed between KYH and Tromsø 7.

The age-adjusted odds of having diabetes in KYH was twice that in Tromsø 7 among men and more than three times higher among women. Treating BMI and WC and as mediators of the association between study and diabetes prevalence explained 46.0% (39.6, 53.8) of the diabetes differences between KYH and Tromsø 7 among women, but did not appear to explain any of the differences among men. Addition of smoking and hsCRP as mediating factors to the model increased to 55.5% (46.5, 66.0) the fraction of the difference in prevalence between studies in women. Among men, there was weak evidence of a much smaller percentage: 9.9% (-0.6, 20.8). It was notable that the residual (natural direct effect) effect not mediated by BMI and WC were similar in men and women in both studies.

Chapter 4 Methodological considerations

While conducting this research I had to face many methodological challenges that had to be solved or taken into account during the interpretation of the results. In the next section, I will describe the potential sources of bias and threats to validity of my findings. I will evaluate the success of the approaches taken to minimize the bias and what implication residual bias may have on the conclusions of the thesis. I will then provide a more comprehensive discussion of the findings than was possible in the limited format of the journal publications, putting it in the context of the high burden of CVD in Russia. Doing that I will summarize the findings of three papers presented and evaluate their contribution to answering a general research question about reasons behind the high CVD burden in Russia.

4.1. Study design

Two studies were used in this thesis for the comparison of CVD biomarkers to explain the differences in CVD burden between Russia and Norway: the KYH and Tromsø 7. KYH is a cross-sectional study, and Tromsø 7 was analysed as cross-sectional although the Tromsø Study as a whole had previous waves (Tromsø 1-6).

Inevitably current differences in CVD event rates / mortality between two populations are going to be most strongly related to the exposure profile to risk factors in the past rather than today. This is because of the time lag between levels and changes in risk factor exposure and subsequent CVD events and mortality. The risk of CHD and stroke starts to decline after smoking cessation in a short timeframe of fewer than 5 years although it may take up to 20 years for risk to reduce to that of non-smokers (160). Similarly with cholesterol and blood pressure most of the reductions in CVD risk occur within 5 years (12). Although, current CVD mortality is much higher in Russia than in Norway, in both countries a downward trend is

observed. Therefore, we are likely to still observe similar or somewhat smaller differences in CVD mortality in two countries in 5-10 years time, and they are going to reflect the currently observed levels of risk factors. Nevertheless, the results of Paper 1 and Paper 2 provide some important insights and explanations for the current differences in CVD mortality between countries with certain reservations due to the time lag.

Another concern is that one time measurement of blood pressure, adiposity (BMI and waist-to-hip ratio), hyperglycaemia, and lipid levels would not reflect long term exposure (or "usual level") to these risk factors when measured at a single point in time. Depending upon the underlying pathophysiological process linking exposure to an event (161), measurements at one point in time may provide an inadequate risk profile for a population even if in a particular period the cross-sectional levels are identical as the populations may have a different risk factor profile in the past.

A cross-sectional study design was used in Paper 2 to study the association between alcohol use and blood-based biomarkers of CVD. I suggest that the association may be causal although the temporality criteria for etiological inference cannot be fulfilled in the cross-sectional study design. Several considerations support a causal explanation (following Hill's criteria of causality):

1. Previous studies with the prospective study design support the association between heavy episodic drinking and cardiovascular mortality. Therefore the Hill's criterion of ***consistency*** is fulfilled: the causal association between heavy alcohol use and alcoholic cardiomyopathy has been known for decades for people with the diagnosis of alcoholism.
2. There is ***coherence*** with the results of the laboratory studies. The causal association between heavy alcohol use is ***plausible*** because many of the effects of alcohol on the

heart were reproduced in the animal models providing some ***experimental evidence*** (162).

3. There is evidence of ***biological gradient (dose-response relationship)***: the NT-proBNP and hsCRP concentrations are lowest in non-problem drinkers, higher in heavy drinkers, and the highest in narcology subsample.
4. We tried to avoid reverse causality by excluding ex-drinkers and never-drinkers from the reference group. Ex-drinkers might have quit drinking because of health problems experienced as the consequence of drinking. Never drinkers are considered a special group of people, especially in the context of Russia where alcohol use among men is normative behaviour. However, reverse causality cannot be excluded completely as an alternative explanation for the observed association. Some participants who used to drink heavily before and experience health problems now could reduce their drinking but not stop it completely. Therefore, they will end up in the reference category of non-problem drinkers, and consequently, the observed association between heavy alcohol use and biomarkers of heart damage would be smaller compared to the real association.

4.2 Internal validity

Briefly, internal validity implies the validity of inference for the source population of the study participants (26). Participants for KYH were sampled from the population of Arkhangelsk and Novosibirsk (Russia). The source population for the Tromsø Study was all residents of Tromsø municipality. Therefore the internal validity of studies presented in this thesis refers to the inference for populations living in these geographical areas. The violations of internal validity are usually classified into three categories: selection bias, information bias, confounding. In the

next sections, I will discuss possible threats to internal validity for the studies presented in this thesis.

4.2.1 Selection bias and response rate

Selection bias is a distortion of the study results due to procedures used to select participants and from factors that influence study participation (26).

The participants in KYH were selected randomly, but the response rate was not optimal. Therefore, we can suspect self-selection if participants who agreed to participate differ from those who did not. If those characteristics are relevant for the exposure-disease association, the bias in estimates of association occurs. Response rates in KYH study are characterized by Cook et al. (147). If the denominator for calculation of response percentage in KYH is restricted to addresses where it was determined that an eligible participant of the correct age and sex lived, it reflected the willingness and ability of households to engage and the skill of the interviewer in motivating them to do so. Response percentage equalled 51.0% (for Arkhangelsk – 68.2%, for Novosibirsk – 41.4%), percentages were higher in women compared to men, and among older compared to younger participants (147).

A subsequent health check was attended by 96% invited in Arkhangelsk, but only 83% in Novosibirsk. Men, younger age groups, and participants living further away from the clinic were also less likely to attend the health check (147). There is evidence that those who did not attend the health check differed from those who did. Adjusting for age, both men and women who did not attend the health check were more likely to have lower educational level, be unmarried, not be in regular paid employment, have a worse financial situation, be problem drinkers, smokers, less often visited a general practitioner (Appendix, Supplementary Table 3, Supplementary Table 4). Men, in particular, were less likely to report known hypertension or

taking blood pressure medication, which may mean that they did not approach the health care system to diagnose this disorder rather than that they don't have it (Appendix Supplementary table 4).

To assess the extent of sampling bias introduced by non-response the educational distribution of those with a baseline interview and health check was compared with the educational distribution for each city as determined at the 2010 Russian Census. For Arkhangelsk, participants and non-participants did not differ with respect to education levels, however, in Novosibirsk the age and sex-standardized proportion with higher education was higher for both completion of the baseline interview and attending the health check (147).

Tromsø 7 had a higher response rate than KYH: out of those invited (all residents of Tromsø municipality), 65.0 % participated. For the age range 40-69 years old, 69.9% of invited women and 62.1% of invited men participated. Those who participated were also on average 2 years older (53.7 vs 51.7 years old) compared to non-participants. Married residents were more likely to participate than unmarried (71.9% vs 59.5%). All participants of the previous wave of the Tromsø Study (Tromsø 6) who still lived in Tromsø municipality and were at least 40 years old were invited to participate in the Tromsø 7, but only 68.6% participated. Among the Tromsø 6 participants those lost to follow up differed slightly from those who participated in Tromsø 7 (both men and women): they were more likely to be daily smokers, had slightly lower levels of total and LDL-cholesterol, had higher self-reported diabetes, MI, or stroke, were less likely to be married (Appendix, Supplementary Tables 1 and 2).

During the second visit, 1403 Tromsø 7 participants (40 – 69 years old) had hs-cTnT and NT-proBNP measured; they were either selected randomly (81%) to attend the second visit or were invited because of their previous participation in Tromsø 6 (Figure 3). If these participants

systematically differed from all other Tromsø 7 study participants, it could have introduced an additional source of selection bias. I compared the main characteristics and CVD risk factors among these participants and the rest of Tromsø 7 participants and did not find any differences after adjustment for age and sex (Paper 1, Supplementary Table S2).

In summary, both KYH and Tromsø 7 studies appear to have both differentially included people who were older, female, married, did not smoked, and had received higher education. Because the bias is in the same direction in KYH and Tromsø 7, this gives us some confidence that differential selection bias is unlikely to undermine our central conclusions of Paper 1 and Paper 3 that compared the characteristics of the two populations. This is further supported by the fact that CVD risk factor profiles (blood lipids, blood pressure, smoking) found in KYH and Tromsø 7 are in agreement with the previous studies conducted in Russia and Norway. However, I cannot exclude that some of the results shown are due to differential health-related selection bias in the two studies.

Of particular relevance for the understanding of the results of Paper 2 are lower response rate and participation in health check among harmful and hazardous drinkers. It is known that participants with serious alcohol problems are harder to recruit to population-based studies (163, 164). Therefore, it was decided to approach patients of alcohol treatment facility (narcology clinic) to recruit an adequate number of participants who drink extremely heavily. They represent an end of the spectrum of alcohol use disorders, but are likely to be different from all people with alcohol use disorders in Russia: they have low income or no income at all, have lower socioeconomic status. Although heavy alcohol use is more prevalent in more socially disadvantaged groups, the low socioeconomic status of participants in the narcology clinic should be kept in mind when generalizing the results of Paper 2 to all heavy drinkers in Russia (see section external validity). Nevertheless, the inferences of the potential impact of

very heavy drinking are of aetiological nature and may be generalizable to very heavy drinking populations either in Russia or elsewhere.

It is still of relevance to study the population groups that may not qualify for the diagnosis of alcohol use disorder, but still consume large quantities of alcohol/ have hazardous drinking patterns, or experience significant life challenges due to alcohol consumption. Although KYH was able to recruit some participants with these characteristics from the general population, there were not many of them in the study sample. Having a low number of participants does not bias the measure of association but may mean that study is underpowered to detect the association between alcohol use patterns and heart damage biomarkers if the effect size is small.

4.2.2 Information bias

Information bias in epidemiological studies occurs due to measurement errors of exposure, outcome, or confounders. In this thesis, two particular sources of measurement error had to be considered: measurement error specific to particular variables and systematic differences in measurements conducted in two different studies when they are analysed together. For Paper 1 and Paper 3 which are comparison studies, the harmonization of data and calibration of measurements had to be conducted before data analysis.

Self-reported data

Smoking was self-reported in both KYH and Tromsø 7. There were slight differences in how the questions about current smoking, past smoking, smoking intensity were formulated in the two studies. For the purpose of harmonisation smoking status was categorized as current smokers (1–10 cigarettes/day, 11–20/day, >20/day), ex-smokers, and never-smokers. This approach does not account for smoking intensity among ex-smokers, but we did not have enough data to calculate the pack-years variable. In addition, smoking self-report is prone to

measurement errors and smoking is underestimated in the surveys (165). The observed differences in biomarkers between studies (Paper 1) or in diabetes prevalence (Paper 3) explained by smoking could have been bigger if the true distribution of smoking status and smoking intensity would have been known. Also, the association between alcohol use (narcology subsample, heavy and harmful drinkers) and biomarkers (Paper 2) could be attenuated if there were no residual confounding by smoking.

The harmonisation of the education variable between the studies was challenging because of differences in the education systems in Norway and Russia. As a result, we could reliably distinguish only three categories of education: primary/secondary, upper secondary, tertiary.

In Tromsø 7 self-reported use of medications for chronic conditions like hypocholesteraemia, hypertension, diabetes is considered of good quality. Combining the information on self-reported medication use in Tromsø 6 and the names of the medication provided in the questionnaire coded with ATC codes resulted in almost full agreement with the Norwegian Prescription Database (personal communication, Anne Elise Eggen). Because there is no national prescription registry in Russia it was not possible to undertake a parallel analysis in KYH. However, we were able to assess only internal agreement between two questions asked in KYH: the question if the blood pressure / cholesterol-lowering/ diabetes medication is used and a question about specific medications used that were subsequently coded using ATC codes. In most cases people taking medication with ATC code for antihypertensives, diuretics, beta-blocking agents, calcium channel blockers, agents operating on the renin-angiotensin system reported taking blood pressure medication. However, there was a poor agreement between answers to the question about the use of medication for cholesterol levels and ATC codes assigned to specific lipid-lowering medications taken by participants. We consider quality of data about blood pressure medication use in KYH satisfactory, however, data about the use of

lipid-lowering medications are of questionable quality. If there is substantial misclassification of lipid-lowering medication use, the adjustment in the regression model for the lipid-lowering medication use would only partially account for all the differences in blood lipids between KYH and Tromsø 7 (Paper 1).

The assignment of the participants into groups according to their level of alcohol consumption (Paper 2) was challenging due to the multidimensional nature of alcohol use and low validity of self-reported consumption. The self-reported volume of ethanol consumption in surveys is underestimated due to inaccurate recall and possible social desirability bias (166, 167). Methods that ask about both the frequency and amount consumed, for beer, wine, and liquor, separately (as was done in KYH) are likely to yield the most realistic levels of intake (167, 168). However, people who drink more heavily tend to underreport the volume and frequency of alcohol consumption more than people who drink moderately (non-proportional underreporting) (169). Other dimensions of alcohol consumption include measures of drinking pattern, measures of acute consequences of drinking such as drunkenness and hangover, measures of alcohol-related harm. Measurement of these dimensions can be affected by social desirability bias as well (163, 166) and by individual variation in alcohol tolerance and metabolism. However, they were strongly related to mortality in previous studies conducted in Russia (74).

Due to inherent problems with survey data on alcohol consumption, researchers have been looking for objective measures of alcohol use. Several alcohol biomarkers were suggested for use in research and clinical practice (170-172). Among them, gamma-glutamyl transferase (GGT) and carbohydrate deficient transferrin (CDT) are used most widely and were measured in KYH. GGT is not specific biomarker of alcohol use and levels are only modestly correlated with alcohol consumption (170). The elevation of GGT is not observed only after one episode of binge drinking unless a person has been drinking heavily previously (170). Sensitivity and

specificity of GGT as a screening tool for regular heavy drinking has been reported to be low, especially in non-clinical samples (170, 171). Serum concentration of CDT provides a good reflection of the recent chronic alcohol consumption when 60-80g of alcohol is consumed daily over at least 2 weeks (173, 174). CDT has a similar sensitivity for detecting heavy drinking (defined as >60g of ethanol per day or >280g per week) compared to GGT but its specificity is higher (175-177).

Neither alcohol biomarker reaches acceptable sensitivity or specificity for detecting heavy drinking but this was improved by using a combination of different biomarkers (178). Studies in clinical samples have shown that the combination of the biomarkers is more sensitive and specific for detecting heavy drinking, particularly combined GGT and %CDT (179, 180). However, the utility of biomarkers to detect heavy drinking in a general population is unclear. Alcohol biomarkers including CDT and GGT have low sensitivity and specificity to detect heavy drinking in a general population and correlate weakly with alcohol consumption (173, 181-184). In experimental studies alcohol use needed to be regular and sustained (over at least two weeks) to increase these biomarkers (170). Episodic heavy drinking which is characteristic for the population of Russia and is associated with detrimental consequences to health independent of the total volume of alcohol consumed would not be detected by alcohol biomarkers (66, 73). A biomarker reflecting hazardous drinking patterns has not been established (184).

Taking all this into account, using alcohol biomarkers alone to define alcohol use in general population of KYH was not justified. The initial hypothesis in Paper 2 was that patterns of alcohol use characterized by heavy episodic drinking are related to heart damage and general inflammation. Therefore, the goal of classifying the KYH sample by their drinking status was to separate the groups according to the intensity of such behaviour. To do that we used the

AUDIT and CAGE questionnaires and the questions on alcohol drinking patterns previously found to be highly predictive of mortality in Russia. Further, we validated the classification into harmful drinkers, hazardous drinkers, and non-problem drinkers using a combination of alcohol biomarkers (%CDT and GGT) (179), total volume of alcohol consumption during the previous year (185), reported consultations with narcologist/ social worker regarding drinking problems. Non-drinkers and ex-drinkers were identified by self-report and placed into separate categories to avoid reverse causality in the analysis. There is no gold standard to measure alcohol consumption in population-based studies, and this approach may be the most appropriate for the study aims in Paper 2. Any possible misclassification in the exposure variable in the general population sample could have caused underestimation of the association between alcohol use and CVD biomarkers.

I also attempted to harmonize variables of alcohol use in KYH and Tromsø 7 to compare alcohol consumption between the studies and relate it to the differences in blood-based CVD biomarkers. That was not possible to do reliably because standard questionnaires for harmful and hazardous alcohol use (AUDIT and CAGE) were never properly validated in Russia (186). The different size and direction of bias may be present when comparing the self-reported volume of alcohol use. A further limitation was that alcohol biomarkers were not measured in Tromsø 7.

Health check measurements

The health check measurements used in this thesis included blood pressure, BMI, waist and hip circumference. The measurements of systolic and diastolic blood pressure were performed according to the standardized protocol using validated instruments – Omron 705IT (HEM-759-E) in KYH and Dinamap (ProCare 300, GE Healthcare) in Tromsø 7. Both devices were validated against mercury reference sphygmomanometers with minimal differences from

the reference value (187-189) and calibrated by the manufacturer. It is implausible that differences in blood pressure between KYH and Tromsø 7 are due to differences in instruments used or differences in measurement procedures, however, I cannot completely exclude this possibility.

It is known that both routine and research study office blood pressure measurements are susceptible to the "white coat effect" (when BP measured in the general practitioner's office is higher than ambulatory blood pressure) and correlates relatively poorly with the awake ambulatory blood pressure and target organ damage (190). More recent guidelines recommend 24-h ambulatory blood pressure and home blood pressure for diagnosing hypertension. Also, the resent research on these subjects suggests using automated office blood pressure measurements where the patient is resting alone in a quiet room and fully automated oscillometric sphygmomanometers take multiple blood pressure readings. In Paper 1 blood pressure was used to explain the differences in Nt-proBNP and hs-cTnT. Neither KYH nor Tromsø 7 was able to completely eliminate errors related to the human factor in blood pressure measurements. If there are systematic differences in the "white coat effect" between the studies (physicians in Russia are known to have a more authoritative style when communicating to their patients), the distribution of blood pressure would be artificially shifted upwards in one study compared to another.

Similar to blood pressure, measures of adiposity (weight, height, waist and hip circumference) are taken using standardized protocols in both KYH and Tromsø 7 (147). The difference in protocols of waist circumference measurement due to different measurement sites (the narrowest part of the trunk in KYH and the umbilicus level in Tromsø 7) was adjusted for by a conversion equation (148).

Use of a single measurement of exposure typically underestimates the true association between long-term average (or "usual") levels of a particular risk factor with disease risk. This phenomenon is called regression dilution bias (RDB) (191, 192), and it arises from a combination of random measurement error, short-term biological variability and longer-term within-person variability (161). In this thesis, adjusting in the regression model for a risk factor that was measured on one occasion, for example, blood pressure (Paper 1) would not capture all the difference in the outcome explained by this risk factor. A similar issue may arise for other continuous exposures used in this thesis (BMI, waist circumference, hsCRP).

Blood-based biomarkers

The measurement error of biomarkers in both KYH and Tromsø 7 is generally low and within acceptable levels for clinical research studies. The coefficient of variation (CV) for each of the studied biomarkers is reported in Paper 1, Supplementary material. Only hs-cTnT has a quite high CV at the low values (below 10 ng/L) – 10% (193). That means a decreased precision for hs-cTnT measurements and underestimation of the strength of association for this biomarker in Paper 1 and Paper 2. Partly, that was addressed in the sensitivity analysis by categorizing hs-cTnT into a binary variable (below and above the top quintile in this study distribution (men - 11 ng/L, women - 8.07 ng/L)).

To prevent any systematic measurement errors due to differences between the two laboratories the calibration study was conducted which I designed and analysed. The design and results of the calibration study are described in Paper 1 (Supplementary material). There were minimal differences between the laboratories, but I took a rigorous approach and adjusted the biomarker measurements in KYH using the adjustment factors from the calibration study. Although many different sources of error may affect biomarker measurements in laboratory (batch differences

etc.) (194), I believe that the differences between the laboratories have not impacted on the conclusions of this study.

Estimated eGFR was assessed by using the Chronic Kidney Disease Epidemiology Collaboration cystatin C equation (150). This minimizes the problem of using creatinine to assess eGFR (150) which is a consequence of its concentration being affected by muscle mass (195) which was decreased in patients with alcohol use disorders (narcology clinic subsample) (Paper 2).

Definition of pre-existing coronary heart disease and diabetes

The definition of the variable "pre-existing coronary heart disease" (Paper 1 and Paper 2) in both KYH and Tromsø 7 is based either on self-reported MI, or on probable MI based on ECG findings, or grade 2 angina pectoris (based on short version of Rose angina questionnaire). All three approaches have low sensitivity and specificity for identifying people with coronary heart disease. In addition, questions about previous MI asked in KYH and Tromsø 7 were not identical, as well as their perception may be different between the two populations. The Rose angina questionnaire (short version) for exertional chest pain had a sensitivity of 51.8% and specificity of 89.4% against the gold standard of a primary care consultation for angina symptoms (152). The specificity of ECG to detect prior MI was reported to be in the range 76%-97%, but sensitivity was low – 21%-58% (196-198). The MI that disappeared over time and unrecognized non-Q wave MI cannot be identified with this detection method (199, 200). Nevertheless, I assumed that combination of information from these three variables allowed me to exclude most people with severe pre-existing CHD for analyses in Paper 1 and Paper 2.

Although the continuous variable that would characterise hyperglycaemia is more preferable for statistical analysis, current HbA1c levels would not reflect previous levels in persons with a diagnosis of diabetes and/or taking medications for diabetes (Paper 1 and Paper 3). Therefore, I harmonized the definition of diabetes in KYH and Tromsø 7 study and defined diabetes as a binary variable (HbA1c ≥ 6.5% and/or self-report of diabetes and/or diabetes medication use). Diabetes and/or diabetes medication use were shown to be reliable if reported in population-based studies (201, 202).

4.2.3 Confounding, mediation, interaction

Confounding occurs when the apparent effect of the exposure of interest is distorted because the effect of the extraneous factors is mistaken for – or mixed with – the actual exposure effect (26). The factor must fulfil all three criteria to be considered a confounder in any particular analysis (26):

1. A potential confounding factor is a risk factor where there is a consensus in the scientific community that it is likely to be causally related to the outcome.
2. A confounding factor must be associated with the exposure under study in the source population (the population at risk from which the cases are derived).
3. A confounding factor must not be causally affected by the exposure or the disease. In particular, it cannot be an intermediate step in the causal path between exposure and disease.

The approach which most epidemiologists take to identify confounders and mediators is a priori knowledge rather than data-driven selection. Directed acyclic graphs (DAG) is a useful tool to depict the relationship between the variables that are or are not measured in the study. The DAG describing the relationship between heavy drinking and CVD biomarkers is

presented in Paper 2, Supplementary material 1. This identified that only smoking and education were considered confounders, the other risk factors (blood pressure, blood lipids, eGFR, BMI, WHR) were treated as mediators.

Residual confounding is confounding that still present after adjustment. It can occur due to imperfect measurement of the confounder or inappropriate parametrisation of a confounder in a statistical model. Unmeasured confounding occurs when a potential confounder was not measured in the study and therefore it is not possible to control for it at the analysis stage. In Paper 2 physical activity, type of diet, and income were not measured and could potentially confound the association between heavy alcohol use and CVD biomarkers.

For Paper 1 and Paper 3 the primary exposure was the study which person participated in (KYH vs Tromsø 7). Because of the third criterion for confounding (confounders must not be affected by the exposure) all variables (except age) that explained the difference between outcomes in the two studies cannot be confounders but were instead formally considered mediators in these papers. For Paper 1 those were blood pressure, smoking, BMI, WHR, lipid-lowering medication use, diabetes, education. For Paper 3 those were BMI, waist circumference, hsCRP, smoking. Although alcohol use was often suggested as a potential explanatory factor for high CVD morbidity in Russia, lack of comparable information on alcohol consumption between studies and possible selection bias against participation of people with alcohol problems stopped me from considering it to explain the differences in CVD biomarkers between KYH and Tromsø 7.

There is an interaction between two exposures when the effect of one exposure on an outcome depends in some way on the presence or absence of another exposure (203). In Paper 1, I tested if differences in biomarker means between KYH and Tromsø 7 depend on the age of participants. Because the multiplicative term between age and study in the regression model

had a significant p-value, the differences were reported separately for two age groups: 40-55 and 56-69 years old. Testing for interaction requires sufficient power. However, because of small numbers, I did not test for an interaction between alcohol use and sex in Paper 2.

4.3. External validity

External validity (generalizability) implies the validity of the inferences as they pertain to people outside of the source population for the study sample (26). In the case of KYH (data collected in two cities – Arkhangelsk and Novosibirsk) it is too ambitious to assume that results should be generalizable to the whole population of Russia. The Russian Federation is a country with a population of 142.9 million (2010 Russian Census) with wide variations in ethnic composition and socioeconomic circumstances. The Tromsø municipality has a higher proportion of population with higher education compared to the national averages (204). Notably, the CVD mortality rate at the selected locations was similar to the national averages. However although KYH and Tromsø 7 studies cannot be fully generalizable to Russia and Norway respectively, I believe that comparison of CVD biomarkers in these two studies provides useful insights into the possible reasons behind different CVD burden in the two countries.

Patients of the alcohol treatment facility (narcology clinic) represent the extreme end of the spectrum of those with alcohol use disorders. However they may not be representative of all people with alcohol use disorders in Russia. Affluent people requiring treatment for alcohol use disorders in Russia are unlikely to attend the state narcology facilities and will instead use private treatment services. This should be kept in mind when generalizing the results obtained in this particular group to all people with alcohol use disorders because there may be an interaction between socioeconomic status and alcohol use on cardiovascular health.

4.4 Statistical considerations

4.4.1. Explaining the differences in means and prevalences between KYH and Tromsø 7

To quantify the contribution of variation in the distribution of the risk factors to the observed difference in means of CVD biomarkers (Paper 1) I used linear regression analysis. First, the unadjusted regression coefficients were calculated, and then the regression coefficients were adjusted for CVD risk factors. If there was a difference between coefficients from these two models, it would suggest that the differences in means of CVD biomarkers could be explained by differences in the distribution of the risk factors. The disadvantage of this approach is that it does not allow calculation of the confidence intervals around the percentage of the difference mediated by the risk factor.

In Paper 3 we used mediation analysis to quantify what proportion of the difference in diabetes prevalence is explained by known risk factors for diabetes: adiposity (BMI and waist circumference), hsCRP, and smoking. To our knowledge, this approach was not used in the comparison studies before. Statistical mediation analysis generally refers to the collection of tools designed to quantify mechanisms specific causal pathways that link cause and effect (205). Therefore, I tried to apply this approach to quantify the possible mechanisms that drive the differences in diabetes prevalence between KYH and Tromsø 7.

The first step of any mediation analysis is to describe pre-existing beliefs about the causal structure in which the mediation analysis is to be conducted. Directed acyclic graphs (DAGs) are usually used for that purpose.

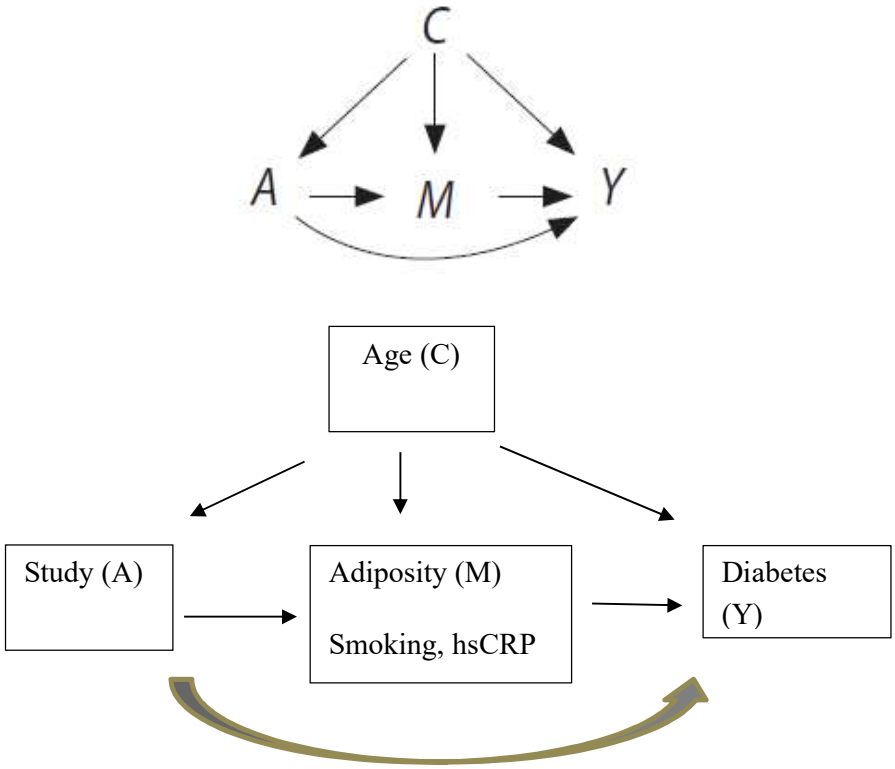

Figure 5. DAG for a relationship between A and Y, where M is a mediator and C is a confounder.

In Figure 1, being a participant of either KYH or Tromsø 7 is assumed to determine the adiposity status, smoking status, hsCRP concentration, which in turn affects diabetes status; this is called an indirect effect. There might also be other mechanisms that link the study and diabetes status; this is called a direct effect. In mediation analysis, we describe what would happen if a) the indirect pathway was the only causal pathway between exposure and outcome and b) the indirect pathway could be deactivated completely. To mathematically define the corresponding parameters to be estimated the counterfactual variables must be introduced (205). Nested counterfactual for the expected value of Y (outcome) permits a precise

mathematical definition of mediation by defining the natural indirect effect and natural direct effect. The natural indirect effect is the effect seen by changing the mediator as if you had changed the exposure without actually changing the exposure itself. Likewise, the natural direct effect is the effect you see by changing the exposure, but keeping the mediator fixed at whatever level it would be had you not changed the exposure. Thus there is a set of assumptions that must be met to allow mediation analysis: no uncontrolled confounding, positivity, consistency, identification of the natural effects (205).

To operationalize the estimation of natural direct and indirect effects I used the class of natural effect models (NEMs) originally introduced by Lange et al. (157) and implemented in the R package Medflex [2]. In natural effect models mediation analysis is approached as a multiple regression problem, thereby a) parameterizing the quantities of interest, b) allowing the choice of outcome model to follow the convention for that type of outcome (i.e., logistic regression for binary outcomes), and c) using the existing software to implement the regression. NEMs are built on duplicate the original data set, when an artificial exposure, A* is created, which takes on different values in the 2 replications of each observation. Then, an auxiliary model is used to link the artificial observations (i.e., those where $A \neq A^*$) to the mediators, which is either done through weighting or imputation. Once this is done, the NEM can be estimated by fitting the regression in the extended data set and using both A and A*, possibly along with C, as the model specification. After taking the average of the log-OR estimates, the OR for natural effect estimates can be obtained along with confidence intervals. Also, the mediated proportions with confidence intervals can be obtained.

4.4.2. Calibration study and "double bootstrap" for the laboratory biomarkers

To calibrate CVD biomarkers between the two studies the calibration study was conducted (Paper 1, Supplementary Material). The relationship between two measurements of the same sample done in two different laboratories was analysed using Deming regression. Deming Regression accounts for errors in both the dependent and independent variables and is routinely used in laboratory comparison studies (206).

Because the calibration study was conducted based on a small sample, there is inherent uncertainty in the biomarker values that take account of the results of the calibration study. This statistical uncertainty should be accounted for when calculating the standard errors and confidence intervals (CI) for the regression estimates of the main study (Paper 1). At the present time there is no widely tested or accepted methodology for doing this. I therefore used a bootstrapping approach to obtain confidence intervals for the regression estimates (Paper 1, Supplementary Material).

Before applying this approach to my data I undertook a simulation study that showed that the standard error obtained using "double bootstrap" procedure is close to the empirical standard error. However, the standard error obtained without considering error of the calibration study (standard approach) is smaller than the empirical standard error, indicating that the standard approach underestimates the uncertainty in the estimated mean difference. The underestimation is stronger when the calibration sample is small. The standard approach is satisfactory when the size of the calibration study increases. If calibration samples are relatively small in sample size it is more appropriate to estimate standard errors and confidence intervals using the proposed "double bootstrap" approach.

4.5. Study strengths

This study several important strengths that made it possible to look into the important public health and epidemiological question about the reasons behind the high CVD burden in Russia. First, it was designed as a cross-country comparison study. A comparison with a country with a particularly low CVD burden like Norway provided the best way to identify any underlying differences in biomarkers and risk factors. There are very few studies that took this approach before and used mediation analysis to quantify the proportion of differences explained by a particular risk factor. Second, I designed and analysed the calibration study of blood biomarkers. The application of calibration coefficients to biomarker values was necessary to achieve the comparability between Know Your Heart and Tromsø 7. Third, this study brings in the novel findings by measuring the population level of CVD biomarkers of heart damage (NT-proBNP and hs-cTnT) in Russia where they have not been measured before. Last, in Paper 2 I was able to compare the biomarkers of heart damage in the sample of very heavy drinkers (narcology clinic subsample) to general population sample. It was an important contribution to the research of alcohol effects on cardiac health as it is one of the few studies with such measurements done in a sample with extreme drinking patterns.

Chapter 5. Discussion of the main results

In this work I attempted to use blood-based biomarkers to identify factors that could explain the high CVD rates in Russia. Since CVD mortality is so much higher in Russia compared to countries in Western Europe, one might expect lipid levels (including total cholesterol and LDL-cholesterol) to be higher in Russia too. As was stated in the introduction section, the previous research did not indicate that lipid levels are particularly high in Russia, in fact, several studies have showed that total cholesterol levels in Russia were low compared to Western

countries (34, 106, 107, 109, 144, 207). In Paper 1 of this thesis, I compared two population-based studies conducted in Russia and Norway ensuring the comparability of the laboratory analysis by calibrating the results based on the derived calibration equations. My analyses confirmed previous studies in showing that even today total cholesterol and LDL are not drivers of the high levels of CVD in Russia. In fact even though Norway has much lower CVD mortality rates, the lipid levels in Tromsø 7 sample were slightly higher than in KYH. Moreover I have been able to show that conventional CVD risk factors and use of lipid-lowering medications does not explain this result. However, in my study I was not able to measure and assess the impact of all factors that can influence cholesterol levels in the population with nutrition probably being the most important (208, 209).

These results look rather paradoxical at a first glance since adverse lipid levels have been shown to be strongly associated with CVD events and explain the decline in CVD mortality in Western countries (10, 11, 49, 50), but are consistent with previous studies going back to the 1970s. That led us to the hypothesis that some other pathophysiological pathways may be contributing to CVD mortality in Russia. While cholesterol levels are contributing to development of atherosclerosis, coronary heart disease, most MI events, and occlusive stroke, they are not contributing to non-atherosclerotic pathways to CVD (40, 41). Non-atherosclerotic pathways to CVD lead to systolic and diastolic dysfunction of the heart, which ultimately results in heart failure (45). Besides, cardiac arrhythmias (i.e. atrial fibrillation), hemorrhagic stroke, some MI events (Type II MI) do not have atherosclerotic process in the pathogenesis (41, 47) . The main risk factors for CVD of non-atherosclerotic origin overlap with those of atherosclerotic origin and include high blood pressure, obesity, diabetes, and excess alcohol use (46).

The hypothesis of prevalent CVD of non-atherosclerotic origin in Russian population was supported by the results of Paper 1. Biomarkers of cardiac damage (hs-cTnT) and cardiac wall stretch (NT-proBNP) have higher concentrations in KYH than in Tromsø 7 among both men

and women. In population-based samples, low-grade elevation in NT-proBNP was shown to be an early marker of heart failure (210), predicts atrial fibrillation (97), and stroke (211). Similarly, elevated hs-cTnT in a population-based sample is recognized as a marker of replacement (scarring) fibrosis and heart failure rather than atherosclerosis and ischemia (91, 92, 212) . Therefore, higher population means of these two biomarkers in KYH can be considered as evidence of stronger involvement of non-atherosclerotic component in structure of CVD in Russia than in countries such as Norway. When interpreting the findings of Paper 1 I had to keep in mind that heart failure can also occur as a long-term consequence of myocardial infarction that resulted in heart damage (45). However, differences in NT-proBNP and hs-cTnT between KYH and Tromsø 7 remained significant even after exclusion of participants with previous coronary heart disease from the analytic sample (Paper 1).

It was unexpected that the differences in heart damage biomarkers between studies were not explained by blood pressure, smoking, BMI, WHR, and diabetes (Paper 1). This might be the result of measurement error and inability to measure long term "usual" exposure. Alternatively, there are some other factors that were not assessed in the study and caused heart damage in the Russian population. While it is not fully clear if blood pressure can explain the observed elevated mean levels of heart damage and cardiac wall stretch biomarkers in KYH, alcohol use is the most likely candidate that was implicated in increased CVD mortality in Russia in many previous studies. However, we could not check this hypothesis by comparing levels of alcohol use in KYH and Tromsø 7 because the measures of alcohol use in these two studies were not comparable.

The particularly hazardous and harmful patterns of alcohol use that are relatively common in Russia provided an excellent opportunity to look at the association between patterns of alcohol use and cardiac biomarkers in KYH. In Paper 2 I have shown that markers of cardiac injury hs-

cTnT and cardiac wall stretch NT-proBNP are substantially elevated among those receiving treatment for alcohol problems at the narcology clinic compared to the general population. Most importantly there was a significant linear increasing trend of NT-proBNP across four groups of drinkers: non-problem drinkers, hazardous drinkers, harmful drinkers, narcology clinic sample. Other studies that had inconsistent findings were limited by the fact that the populations they studied had much lower levels of alcohol consumption (213-215). The prospective study in UK that was published after Paper 2 demonstrated that only recent heavy drinkers rather than earlier heavy drinkers had higher levels of NT-proBNP (216). This suggests that damaging effects of alcohol on the heart may be reversible. Drinking cessation is also related to improvement in heart function in people with a diagnosis of alcoholic cardiomyopathy (217). A literature search for other studies that looked at the association between alcohol consumption and hs-cTnT identified only reports with a relatively moderate level of alcohol use, which reported either decreased or the same hs-cTnT levels in some groups of drinkers compared to non-drinkers (213-215, 218).

The elevated levels of both NT-proBNP and hs-cTnT in the group of extremely heavy drinkers from the narcology subsample are consistent with heavy alcohol drinking leading to non-ischemic damage of the heart. Elevated NT-proBNP in harmful drinkers from general population provides further evidence for this. Furthermore, exclusion of individuals who had a previous diagnosis of coronary heart disease did not have an impact on the substantive results. Chronic heavy drinking is an established mechanism of alcoholic cardiomyopathy (ACM), which is characterized by systolic and diastolic dysfunction and ultimately leads to heart failure (79). To confirm the results of Paper 2 it was desirable to assess the structure and function of the heart. In the heart imaging study conducted on the same population, mean left ventricular end-diastolic diameter and mean left atrial systolic diameter was increased in the narcology clinic subsample compared to the population-based sample (219). Also, left ventricular ejection

fraction was decreased in narcology clinic subsample compared to the population-based sample (219).

It was also of interest to answer the question about the mechanism of alcohol damage of the heart. Among the potential mechanisms that are suggested to mediate the harmful action of alcohol on the heart are increased blood pressure and a direct effect of the toxic alcohol metabolites on the heart muscle. Blood pressure has a linear dose-response relationship with volume of alcohol consumed (70) and is an established risk factor for CVD. Toxic alcohol metabolites affect heart muscle by increasing oxidative stress, triggering cell apoptosis (220). In Paper 2 adjustment for the possible mediators of the association between alcohol use and biomarkers (blood pressure, blood lipid indices, BMI, WHR, eGFR) led to some attenuation of the regression coefficients for NT-proBNP but not for hs-cTnT. Controlling for blood pressure in the regression model did not substantially change the estimates of the association between alcohol use and echocardiographic abnormalities (219). This suggests that hypertension may not play a large role in mediating the observed relationship between heavy alcohol use and consequent heart damage (represented by increased NT-proBNP, hs-cTnT, and echocardiographic abnormalities).

The results reported in Paper 2 help to explain why heavy alcohol drinking has been related to excess mortality in Russia if considered in the context of previous studies exploring causes of high cardiovascular mortality in Russia (74). Cardiomyopathic effects of extremely heavy drinking patterns may contribute to non-atherosclerotic CVD in a population even when the levels of officially diagnosed alcoholic cardiomyopathy may be relatively low. Although alcohol use is declining in Russia especially in younger age groups, it can remain an important contributor to CVD in some population groups that are characterized by low socioeconomic status and extremely risky patterns of alcohol use.

Other factors that increase the risk of CVD of non-atherosclerotic origin are diabetes and obesity. These two factors are predictors of left ventricular remodeling and heart failure especially among women (52). In Paper 3 I report a much higher prevalence of diabetes in Russia compared to Norway both among women and men. The prevalence of diabetes was higher in women than in men in the Russian sample, which is the opposite of what is observed in Norway and other countries (133, 137). When we looked for an explanation of the differences in the prevalence of diabetes between the two countries, adiposity measured by BMI and WC could explain up to 46% of the difference in diabetes prevalence between studies in women but did not explain the differences between studies observed in men. The high prevalence of obesity and diabetes in women in Russia is in agreement with more pronounced differences in hs-cTnT and NT-proBNP between studies among women in the older age group (55-69 years old) (Paper 1).

Following adjustment for adiposity the prevalence of diabetes in men and women was very similar in KYH. Adjustment for further factors did not explain in full why the prevalence of diabetes differs in Norway and Russia. Including hsCRP and smoking in the model in addition to BMI and WC increased from 46% to 55.5% the proportion explained in women but explained almost none of the difference for men. It is consistent with increased hsCRP and general inflammation associated with visceral adiposity. Alternatively, association with hsCRP may reflect an ongoing atherosclerotic process facilitated by diabetes (reverse causality).

One more circulating biomarker that is known to be associated with CVD is hsCRP. It is a non-specific marker of systemic inflammation which is associated with coronary plaque burden (103) and atherosclerosis (101). The higher mean hsCRP levels in KYH compared to Tromsø (Paper 1) might in part reflect more intense atherosclerotic processes in KYH. This interpretation is supported by evidence of higher carotid plaque burden in KYH compared to

Tromsø 7, although neither adiposity nor established CVD risk factors explained the difference (221). Elevated hsCRP levels in KYH may reflect both atherosclerosis, as well as a higher prevalence of CVD risk factors, like obesity, smoking, alcohol use, or environmental exposures (environmental pollutants). In fact, differences in hsCRP between studies were appreciably attenuated by adjustment for conventional CVD risk factors (Paper 1). While I was not able to adjust for alcohol use in Paper 1 (no comparable data on alcohol use), the association between alcohol use and hsCRP was assessed in Paper 2. The elevation of hsCRP in the narcology clinic sample and the trend for elevated hsCRP across harmful and hazardous drinkers in the population-based sample may be secondary to inflammatory process caused by harmful and hazardous drinking. Other explanations cannot be excluded, like toxic effects of alcohol and its metabolites on the liver ranging from fatty liver to steatosis, process of detoxification, and exposure to the specific medications during treatment for alcohol problems.

In summary, the results of biomarker comparisons included in this thesis suggest that CVD of atherosclerotic origin still contribute to a significant proportion of the total CVD burden in Russia and differences between Russia and Norway. While high LDL-cholesterol may not be the key factor driving the differences in CHD between Russia and Norway, smoking among men, obesity among women, high diabetes prevalence, high blood pressure are those conventional risk factors that may be responsible for the differences. A sufficient-component cause model of disease causation provides a very approximate conceptual representation of the causal process in this case. The sufficient cause is a complete causal mechanism that is sufficient for an outcome to occur (26). Very high levels of blood total and LDL-cholesterol may not be a necessary cause for CVD of atherosclerotic origin. In case of the presence of other component causes (like high blood pressure, smoking, obesity) the medium levels of cholesterol may still be sufficient to produce the outcome.

In the last several decades non-atherosclerotic pathways to CVD have received more attention. Although the incidence of heart failure is stable or falling in many European countries due to better treatment of risk factors, decrease in incidence of MI, and better survival of patients with MI, the absolute numbers of heart failure cases are increasing due to aging of populations (222, 223). While CVD mortality in Russia is currently decreasing, it is still much higher than in many European countries. The prevention and treatment of CVD of atherosclerotic origin in Russia have not reached the optimal levels yet and cohort effects with early life exposures will remain an important contributor to increased CVD mortality for some decades. Based on biomarker profile observed in KYH, the structure of CVD in Russia is characterized by high prevalence and co-occurrence of both CVD of atherosclerotic and non-atherosclerotic origin.

The observed biomarker profile in KYH reported in this thesis also points to a high risk of multimorbidity in Russia. Multimorbidity is defined as the co-existence of two or more chronic conditions. A study based on SAGE data defined and compared multimorbidity patterns across different regions, where the cardio-metabolic class with the excess prevalence of diabetes, hypertension, myocardial infarction, or angina and stroke was the most prevalent in Russia (224). Besides, the proportion of undiagnosed diabetes is high in Russia (Paper 3), therefore the prevalence of cardiometabolic multimorbidity may be underestimated if using self-reported data. Any combination of MI, stroke, or diabetes is associated with multiplicative mortality risk, life expectancy is substantially lower in people with multimorbidity (225). Based on Emerging Risk Factors Collaboration analysis a history of any 2 of these conditions at the age of 60 years, was associated with 12 years reduced life expectancy and a history of all 3 of these conditions was associated with 15 years reduced life expectancy (225).

Chapter 6 Conclusion and future research

1. Non-atherosclerotic pathways may take a significant share of CVD morbidity in Russia compared to other countries which is supported by evidence of higher levels of cardiac wall stretch (NT-proBNP) and heart damage (hs-cTnT) biomarkers in Russia.
2. The elevated levels of NT-proBNP and hs-cTnT in the group of extremely heavy drinkers in Russian addiction treatment centers are consistent with heavy alcohol drinking leading to non-ischemic damage of the heart.
3. There were no substantial differences in lipid profiles between KYH and Tromsø 7. A higher pro-inflammatory status which is reflected by higher mean hsCRP, and higher diabetes prevalence in KYH compared to Tromsø 7 may partly explain the higher mortality due to coronary heart events in Russia compared to Norway.
4. Higher levels of hsCRP in extremely heavy drinkers compared to non-problem drinkers in Russia can be evidence of alcohol contributing to the atherosclerotic process.
5. Russia has a much higher prevalence of diabetes than Norway based on results from KYH and Tromsø 7. The prevalence of undiagnosed and untreated diabetes mellitus is also high in Russia.
6. Obesity (measured as BMI and waist circumference) can explain a substantial proportion of differences in diabetes prevalence between KYH and Tromsø 7 among women but not among men.

The potential future research direction related to the topics explored in this thesis are:

- To assess systolic and diastolic function of the heart in Russia using comparable heart imaging methods. Consider more precise methods like MRI to characterize heart structure and function in heavy alcohol users.

- A substantial proportion of unexplained differences in diabetes prevalence between KYH and Tromsø 7 requires further investigation. For example, researchers should assess a volume of visceral adipose tissue to explain the high risk of diabetes in Russia. Other potential explanations can include diet (56, 226), level of physical activity (58), differences in frequency of alleles that carry a genetic risk for diabetes (227), and exposure to environmental pollutants.
- Finally, researchers should consider clusters of conditions instead of single diseases when planning further studies on CVD in Russia, because of the high prevalence of multimorbidity indicated by biomarker profile.

While this thesis is focusing on circulating biomarkers of CVD in the general population and patients of addiction treatment centers, it did not look into many other potential explanations for differences in CVD mortality between Russia and Norway. Many other research directions including health policy, hearth care services, pharmacoepidemiology, other biomarkers, and risk factors of CVD should be followed to in order to fully explain the very high burden of CVD in Russia.

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Paper 1

OPEN ACCESS

Why does Russia have such high cardiovascular mortality rates? Comparisons of blood-based biomarkers with Norway implicate non-ischaemic cardiac damage

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ABSTRACT

Background Russia has one of the highest rates of mortality from cardiovascular disease (CVD). At age 35–69 years, they are eight times higher than in neighbouring Norway. Comparing profiles of blood-based CVD biomarkers between these two populations can help identify reasons for this substantial difference in risk.

Methods We compared age-standardised mean levels of CVD biomarkers for men and women aged 40–69 years measured in two cross-sectional population-based studies: Know Your Heart (KYH) (Russia, 2015–2018; n=4046) and the seventh wave of the Tromsø Study (Tromsø 7) (Norway, 2015–2018; n=17 646). A laboratory calibration study was performed to account for inter-laboratory differences.

Results Levels of total, low-density lipoprotein-, high-density lipoprotein-cholesterol and triglycerides were comparable in KYH and Tromsø 7 studies. N-terminal pro-b-type natriuretic peptide (NT-proBNP), high-sensitivity cardiac troponin T (hs-cTnT) and high-sensitivity C-reactive protein (hsCRP) were higher in KYH compared with Tromsø 7 (NT-proBNP was higher by 54.1% (95% CI 41.5% to 67.8%) in men and by 30.8% (95% CI 22.9% to 39.2%) in women; hs-cTnT—by 42.4% (95% CI 36.1% to 49.0%) in men and by 68.1% (95% CI 62.4% to 73.9%) in women; hsCRP—by 33.3% (95% CI 26.1% to 40.8%) in men and by 35.6% (95% CI 29.0% to 42.6%) in women). Exclusion of participants with pre-existing coronary heart disease (279 men and 282 women) had no substantive effect.

Conclusions Differences in cholesterol fractions cannot explain the difference in CVD mortality rate between Russia and Norway. A non-ischemic pathway to the cardiac damage reflected by raised NT-proBNP and hs-cTnT is likely to contribute to high CVD mortality in Russia.

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INTRODUCTION

Russia has one of the highest rates of mortality from cardiovascular disease (CVD) in the world,[1] although it has been falling since 2005.[2] The causes of this high CVD mortality are not fully understood. Comparison of blood-based biomarkers and other risk factors in Russia relative to other countries with lower CVD risk should throw light on the likely drivers of these differences in mortality. A small number of such studies have been conducted with blood-based biomarkers restricted to lipid profiles (total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides).[3–6] These have generally found no major differences between Russia and other countries.

Biomarkers such as high-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro-b-type natriuretic peptide (NT-proBNP) provide information on actual cardiovascular morbidity and are not simply risk predictors. They have been increasingly used in population-based research where they have been shown to be independent predictors of CVD events.[7–9] Outside of acute ischaemic cardiac events, hs-cTnT elevation is associated with future risk of heart failure, which is supported by structural and functional studies of the heart.[10] NT-proBNP is used in diagnostics of heart failure and is predictive of heart failure in population-based cohorts,[11] along with atrial fibrillation and stroke.[12] While some controversy exists about the role of high-sensitivity C-reactive protein (hsCRP) in CVDs,[13] it is associated with coronary heart disease, stroke and vascular death independently of the traditional risk factors.[14] In fact, studies of large population-based cohorts identified hsCRP, hs-cTnT and NT-proBNP as the blood biomarkers that are the most predictive of cardiovascular events.[15]

In this paper, data from the Know Your Heart (KYH) study (Russia) and the Tromsø study (Norway) are compared to establish the differences in major cardiovascular biomarkers measured in blood among men and women aged 40–69 years. Norway has a CVD mortality rate approximately eight times lower than that in Russia in this middle-aged group[16]; thus, it provides a good contrast for comparing CVD biomarker levels.

METHODS

Study populations

Know Your Heart (Russia). A random population-based sample of participants aged 35–69 years (n=5107) stratified by age, sex and district were recruited in the cities of Arkhangelsk and Novosibirsk (Russia).[16] Trained interviewers recruited and interviewed participants at home to

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ascertain information about their health, socio-demographic characteristics and lifestyle (51% of approached agreed to participate). Participants were then invited to take part in a health check at an outpatient clinic and 4543 (89%) attended. Our analysis is based on 4046 participants aged 40–69 years who attended the health check and provided a blood sample. The health check included blood pressure measurements, recording of weight and height, a 12-lead ECG and biological sample collection. The additional questionnaire collected data on health problems, lifestyle and medication use. Within 2 hours after venipuncture (non-fasting samples), blood was centrifuged, serum was frozen (−80℃), and analysed in a single batch at the end of the fieldwork in Moscow.[16]

The *Tromsø Study (Norway)*. In Tromsø 7, all inhabitants of the municipality of Tromsø aged 40 years and above were invited and 21 083 participated (65%). The subset of 17 646 participants aged 40–69 years was included in our analysis. All participants completed questionnaires and examinations including biological sampling. The questionnaire covered lifestyle, medication use and medical history. A random subsample (5965 participants) attended a second visit. Blood samples (non-fasting) at both visits were processed immediately after collection and the laboratory assays of the biomarkers were performed the same day at the Department of Laboratory Medicine, University Hospital of Northern Norway (ISO certification NS-EN ISO 15 189:2012).

Study measurements

All participants in KYH and Tromsø 7 with blood sample collected had measured lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), a marker of systemic inflammation (hsCRP) and glycosylated haemoglobin (HbA1c)—Supplementary Table S1. A marker of cardiac damage (hs-cTnT) and a marker of cardiac wall stretch (NT-proBNP) were measured in all KYH participants and in 1403 Tromsø 7 participants who were either selected randomly (81%) to attend the second visit or were invited because of their previous participation in the sixth wave of the Tromsø study. The characteristics of those in Tromsø study with measured cardiac biomarkers are very similar to that of the total study sample (Supplementary Table S2).

Body mass index (BMI) was calculated as weight (kilograms) divided by height (metres) squared. Mean systolic and diastolic blood pressure was calculated as the mean of second and third measurements. Waist circumference (WC) was measured at the narrowest part of the trunk in KYH, while in Tromsø 7, WC was measured at the umbilicus level. To ensure WC was comparable between the two studies, WC in Tromsø 7 was converted to the narrowest waist using a conversion equation.[17] Waist-to-hip ratio (WHR) was calculated by dividing WC by hip circumference. Smoking status was categorised as current smokers, ex-smokers and never-smokers. For current smokers, the number of cigarettes smoked was specified as 1–10/day, 11–20/day and >20/day. Education level was classified into three categories: primary/secondary, upper secondary and tertiary. Diabetes was defined as HbA1c concentration above 6.5%, or self-report of diabetes, or use of medication with ATC-code A10 (antidiabetics) according to the Anatomical Therapeutic Chemical (ATC) classification.[18] Lipid-lowering drugs use was determined according to recorded medications coded to the ATC classification as C10 (lipid-modifying agents) or self-reported use.

The pre-existing coronary heart disease was determined as evidence of previous myocardial infarction (MI) on ECG, self-report of MI or grade 2 angina pectoris. ECGs from both studies were coded according to the Minnesota code (MC 1.1–1.3)[19] using the same semi-automated system. Grade 2 angina was determined using the Rose Angina Questionnaire (short version).[20]

Calibration of laboratory data

Differences in the laboratory procedures in KYH and Tromsø 7 bring the potential for systematic differences in biomarker measurements between the two sites due to measurement error. This was addressed by a recalibration study with split sample testing (Supplementary Methods M1, Supplementary Tables S3–S5, Supplementary Figures S1–S10). For that purpose, 100 serum samples and 50 whole blood samples from KYH participants were re-assayed in both the laboratories in Moscow and Tromsø. The paired measurements were analysed using Deming regression to derive the calibration equations.

Statistical analysis

Mean biomarker levels among men and women were compared having age-standardised to the 2013 Standard European Population. Biomarkers with skewed distributions (triglycerides, hsCRP, hs-cTnT, NT-proBNP) were ln-transformed before analysis and geometrical means were presented. Multivariable linear regression was used to assess if the differences in mean biomarker levels in the two studies could be explained by differences in age (Model1), smoking prevalence, BMI, WHR, blood pressure, diabetes, education level (in addition to age) (Model 2) and use of lipid-lowering drugs (in addition to variables in Model 2) (Model 3). For triglycerides, models were also adjusted for the fasting status. The regression models for hs-cTnT and NT-proBNP were repeated for study participants without previous MI or grade 2 angina. For the regression modelling, data from participants with complete information on all the covariates were used. For skewed biomarkers, the regression coefficients were back-transformed to be interpreted as a per cent difference between studies. Based on finding evidence of an interaction between age and study, the differences in biomarkers between studies were presented separately for 40–54 and 55–69 year olds.

All analyses were done using recalibrated biomarkers (Supplementary Table S5). To account for uncertainty in the estimation of the calibration coefficients in the subsequent comparative analysis, we used a 'double-bootstrap' approach, verified using a simulation study (Supplementary Methods M2, Supplementary Tables S6–S7), to obtain 95% CIs for the regression coefficients. Statistical analysis was performed using R version 3.6.0 and SAS software 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Descriptive characteristics presented in table 1 show that men in KYH were on average older, had higher blood pressure and lower BMI, and a higher proportion were current smokers, and had diabetes, compared with men in Tromsø 7. Women in KYH were on average older, had higher blood pressure and BMI, a higher proportion had diabetes, and a lower proportion were current or previous smokers, compared with women in Tromsø 7 (table 1). The similar proportion of participants reported using lipid-lowering drugs that could be identified by ATC code in KYH and Tromsø 7; however, self-reported use of lipid-lowering drugs was higher in KYH (table 1).

The age-standardised means of CVD biomarkers are compared in table 2. The geometric means for hs-cTnT, NT-proBNP and hsCRP were significantly higher in KYH compared with Tromsø 7 among both men and women. It is notable that KYH had

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Table 1 Characteristics of the study sample (participants aged 40–69 years with blood sample collected): KYH (N=4046) and Tromsø 7 (N=17 555)

	Men		Women	
	KYH†	Tromsø 7‡	KYH	Tromsø 7
Age, mean (SD)	56.2 (8.5)	53.8 (8.5)	55.9 (8.7)	53.6 (8.4)
SBP, mean (SD)	138.6 (19.8)	130.8 (17.1)	129.9.0 (19.6)	123.4 (18.7)
DBP, mean (SD)	86.5 (11.3)	78.8 (9.7)	81.3 (11)	72.6 (9.6)
Hypertension§	1102 (63.6)	3462 (41.5)	1337 (56.0)	2759 (29.7)
Smoking, N (%)				
Current smoker >20/day	110 (6.5)	47 (0.6)	21 (0.9)	21 (0.2)
Current smoker 11–20/day	376 (22.2)	477 (5.8)	154 (6.6)	353 (3.9)
Current smoker 1–10/day	136 (8.0)	1083 (13.3)	200 (8.6)	1399 (15.3)
Ex-smoker	640 (37.8)	3329 (40.7)	370 (15.8)	4109 (44.8)
Never smoked	432 (25.5)	3226 (39.5)	1595 (68.1)	3279 (35.8)
BMI, mean (SD)	27.7 (4.8)	27.9 (4.0)	28.9 (6.2)	26.8 (4.9)
WHR, mean (SD)	0.95 (0.07)	0.94 (0.07)	0.85 (0.08)	0.79 (0.07)
HbA1c ≥6.5%, N (%)	195 (11.6)	404 (4.9)	262 (11.3)	264 (2.9)
Use of diabetes medication, N (%)	76 (4.5)	350 (4.2)	170 (7.3)	271 (2.9)
Diabetes, N (%)	217 (12.8)	515 (6.2)	353 (15.1)	393 (4.3)
Lipid-lowering drugs (ATC code C10 and/or self-report), N (%)	266 (15.7)	1090 (13.1)	468 (20.0)	837 (9.1)
Lipid-lowering drugs (ATC code C10), N (%)	169 (10.8)	913 (10.9)	212 (9.9)	720 (7.74)
Education level				
Primary/secondary	147 (8.7)	1624 (19.6)	134 (5.7)	1699 (18.36)
Upper secondary	878 (51.7)	2570 (31.0)	1280 (54.6)	2409 (26.0)
Tertiary	675 (39.7)	4108 (49.5)	932 (39.7)	5145 (55.6)
Pre-existing coronary heart disease	238 (14.0)	471 (5.7)	259 (11.0)	215 (2.3)

†Missing data in KYH: SBP/DBP—334 (8.3%), smoking—12 (0.3%), BMI—12 (0.3%), WHR—2 (0.1%), diabetes—18 (0.4%), HbA1c—51 (1.3%), diabetes medication—419 (10.4%).
‡Missing data in Tromsø 7: SBP/DBP—45 (0.3%), smoking—273 (1.6%), BMI—41 (0.2%), WHR—65 (0.4%), HbA1c—135 (0.8%).
§Hypertension was defined as SBP >140 mmHg and/or DPB >90 mmHg and/or use of antihypertensive medication (ATC codes C02 (antihypertensives), C03 (diuretics), C07 (beta-blocking agents), C08 (calcium channel blockers), or C09 (agents operating on the renin-angiotensin system) and/or self-reported use.
ATC, Anatomical Therapeutic Chemical; BMI, body mass index; DBP, diastolic blood pressure; KYH, Know Your Heart; SBP, systolic blood pressure; WHR, waist-to-hip ratio.

a higher proportion of participants with detectable hs-cTnT: 98.6% compared with 64.4% in Tromsø 7.

Table 3 shows the conditional differences in mean biomarker levels between the studies from three regression models. The age-adjusted model shows that men and women in KYH had slightly lower LDL- and HDL-cholesterol than in Tromsø 7, while triglyceride levels in women were higher in KYH. Adjustment for smoking, BMI, WHR, blood pressure, diabetes, education, and use of lipid-lowering drugs use had little effect on these differences (Model 2 and Model 3).

In the age-adjusted model, hsCRP in KYH was 33.3% (95% CI 26.1% to 40.8%) higher in men and 35.6% (95% CI 29.0% to 42.6%) higher in women compared with Tromsø 7 (untransformed coefficients in table 3). The corresponding values for NT-proBNP were 54.1% (95% CI 41.5% to 67.8%) and 30.8% (95% CI 22.9% to 39.2%), and for hs-cTnT—42.4% (95% CI 36.1% to 49.0%) and 68.1% (95% CI 62.4% to 73.9%). There was substantial attenuation of the differences in hsCRP due to adjustment by smoking, BMI, WHR, blood pressure, diabetes and education, but there remained evidence for differences between the two studies (Model 2). For hs-cTnT and NT-proBNP, adjustment did not change the estimate of the mean difference.

Table 2 Age-standardised means† of CVD biomarkers in KYH and Tromsø 7

	KYH		Tromsø 7		P value for difference
	N	Mean (95% CI)	N	Mean (95% CI)	
Men					
Total cholesterol (mmol/L)	1700	5.26 (5.21, 5.31)	8302	5.46 (5.44, 5.48)	<0.001
HDL-cholesterol (mmol/L)	1700	1.34 (1.32, 1.36)	8301	1.37 (1.36, 1.38)	0.002
LDL-cholesterol (mmol/L)	1700	3.44 (3.39, 3.48)	8302	3.70 (3.67, 3.72)	<0.001
Triglycerides (mmol/L)‡	1700	1.45 (1.41, 1.49)	8302	1.54 (1.52, 1.55)	<0.001
hsCRP (mg/L)‡	1700	1.42 (1.35, 1.49)	8302	1.06 (1.04, 1.08)	<0.001
NT-proBNP (pg/ml)‡	1700	54.7 (52.4, 57.2)	650	35.3 (32.5, 38.4)	<0.001
hs-cTnT (ng/L)‡	1700	7.59 (7.42, 7.77)	645	5.23 (5.01, 5.46)	<0.001
Women					
Total cholesterol (mmol/L)	2346	5.50 (5.46, 5.54)	9253	5.53 (5.51, 5.55)	0.138
HDL-cholesterol (mmol/L)	2346	1.61 (1.59, 1.63)	9253	1.72 (1.71, 1.73)	<0.001
LDL-cholesterol (mmol/L)	2346	3.54 (3.50, 3.58)	9253	3.56 (3.54, 3.58)	0.569
Triglycerides (mmol/L)‡	2346	1.30 (1.27, 1.32)	9253	1.18 (1.16, 1.19)	<0.001
hsCRP (mg/L)‡	2346	1.37 (1.32, 1.43)	9253	1.03 (1.01, 1.05)	<0.001
NT-proBNP (pg/mL)‡	2342	71.0 (68.9, 73.1)	762	56.5 (53.3, 59.8)	<0.001
hs-cTnT (ng/L)‡	2342	5.93 (5.83, 6.02)	758	3.58 (3.47, 3.69)	<0.001

†Standardised to the Standard European Population 2013.
‡Geometric means are presented.
CVD, cardiovascular disease; HDL, high-density lipoprotein, hsCRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; KYH, Know Your Heart; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-b-type natriuretic peptide.

The differences in hs-cTnT and NT-proBNP remained when the analysis was restricted to participants without previous MI, or grade 2 angina (table 4).

The differences in biomarker levels between the two studies differed by age group (table 5). For most biomarkers, study differences were larger in women aged 55–69 years than 40–54 years. Among men, differences in hsCRP were more pronounced in the older age group (55–69 years), while differences in total and LDL-cholesterol were larger in younger men (40–54 years).

Sensitivity analysis

As hs-cTnT assays are known to show appreciable imprecision at the low values seen in the general population,[21] we conducted a sensitivity analysis using logistic regression with hs-cTnT categorised into values below and above the top quintile in this study distribution (men—11 ng/L, women—8.07 ng/L). The results were consistent with hs-cTnT analysed as a continuous outcome (Supplementary Tables S8–S9). Adjustment for lipid-lowering drugs based only on ATC codes in the regression model produced similar results to the main analysis which defined lipid-lowering drugs based on ATC code and self-reported use.

DISCUSSION

This comparison study shows that, after adjustment for sex and age, the lipid profile was comparable in KYH (Russia) and in Tromsø 7

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Table 3 Differences† in mean biomarker levels in KYH vs Tromsø 7 adjusted for CVD risk factors

	N	Model 1 (adjusted for age)	Model 2 (adjusted for age, smoking, BMI, WHR, SBP, DBP, diabetes, education)	Model 3 (adjusted for age, smoking, BMI, WHR, SBP, DBP, diabetes, education, lipid-lowering drugs)
Men				
Total cholesterol	9669	−0.22 (−0.29, −0.1)	−0.31 (−0.39, −0.19)	−0.30 (−0.38, −0.17)
HDL	9669	−0.05 (−0.07, −0.02)	−0.05 (−0.07, −0.02)	−0.05 (−0.07, −0.02)
LDL	9679	−0.26 (−0.34, −0.22)	−0.32 (−0.41, −0.28)	−0.31 (−0.39, −0.27)
Triglycerides‡§	9454	0.03 (0.00, 0.07)	0.02 (−0.01, 0.06)	0.02 (−0.01, 0.06)
hsCRP‡	9669	0.29 (0.23, 0.35)	0.16 (0.10, 0.22)	0.17 (0.11, 0.22)
NT-proBNP‡	2192	0.44 (0.36, 0.53)	0.37 (0.27, 0.47)	0.37 (0.27, 0.46)
hs-cTnT‡	2197	0.36 (0.31, 0.40)	0.37 (0.32, 0.42)	0.37 (0.32, 0.42)
Women				
Total cholesterol	11 189	−0.07 (−0.15, 0.04)	−0.13 (−0.21, −0.01)	−0.09 (−0.17, 0.03)
HDL	11 189	−0.13 (−0.16, −0.11)	−0.02 (−0.04, 0.01)	−0.02 (−0.04, 0.01)
LDL	11 189	−0.03 (−0.10, 0.01)	−0.13 (−0.21, −0.09)	−0.09 (−0.17, −0.05)
Triglycerides‡	10 859	0.10 (0.07, 0.12)	0.03 (0.00, 0.06)	0.03 (0.00, 0.06)
hsCRP‡	11 189	0.31 (0.26, 0.35)	0.04 (−0.01, 0.10)	0.05 (0, 0.11)
NT-proBNP‡	2876	0.27 (0.21, 0.33)	0.33 (0.25, 0.39)	0.32 (0.24, 0.38)
hs-cTnT‡	2880	0.52 (0.48, 0.55)	0.49 (0.45, 0.53)	0.49 (0.45, 0.53)

†Values in KYH minus those in Tromsø 7 and 95% CIs, all models based on cases without missing data on adjustment variables.
‡Analysis is based on ln-transformed values.
§The models for triglycerides were additionally adjusted for fasting time because of differences in mean fasting time in the two studies. Fasting time was recorded from participants' self-report.
BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; HDL, high-density lipoprotein, hsCRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; KYH, Know Your Heart; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-b-type natriuretic peptide; systolic blood pressure; WHR, waist-to-hip ratio.

(Norway) despite the much higher cardiovascular mortality in Russia. In contrast, biomarkers of cardiac damage have higher concentrations in KYH than in Tromsø 7 even after excluding participants with previous coronary artery disease. These results are not explained by the higher prevalence of hypertension and smoking in Russia, suggesting that mechanisms in addition to coronary heart disease contribute to cardiovascular mortality in Russia.

Cholesterol fractions

All cholesterol fractions were slightly lower in KYH than in Tromsø 7 among both men and women, although the magnitude of this difference was small and would not translate into large differences in risk of vascular events. This is notable given that Russia has one of the highest CVD mortality rate and a large proportion of CVD death in country's mortality statistics are attributed to coronary heart disease. These findings are consistent with previous studies comparing lipid levels in Russia with other countries, concluding that blood lipid profiles were similar in Russia and Western countries.[3–6,22–24]

The differences in cholesterol measures between studies are not explained by differences in prevalence of classic risk factors and use of lipid-lowering drugs.

NT-proBNP

Levels of the cardiac wall stretch biomarker NT-proBNP were higher in KYH compared with Tromsø 7 among both men and women. The differences were not explained by classic CVD risk factors (blood pressure, smoking, BMI, WHR, diabetes). Among women, we found difference between studies only in the older age group (55–69 years old).

Elevated NT-proBNP is a biomarker of cardiac dysfunction related to several pathological processes in the cardiovascular system: heart failure,[25] atrial fibrillation[26] and stroke.[12] We suggest that elevated NT-proBNP in KYH compared with Tromsø 7 may be explained by higher heart damage due to non-ischaemic pathways to heart disease. Although heart damage and the development of chronic heart failure can be facilitated by MI or stable coronary heart disease, our conclusions were robust after exclusion of participants with a history of coronary heart disease.

High-sensitivity cardiac troponin T

Similar to NT-proBNP, we found higher mean levels of hs-cTnT in KYH compared with Tromsø 7 among both men and women. This was not explained by a different prevalence of classic CVD risk factors (smoking, BMI, WHR, blood pressure, diabetes), but among women, the difference was more

Table 4 The difference in mean levels of NT-proBNP and hs-cTnT in KYH compared with Tromsø 7, adjusted for age.

	Without coronary heart disease		With coronary heart disease	
	N	Mean difference (95% CI)	N	Mean difference (95% CI)
Men				
NT-proBNP†	1913	0.42 (0.33, 0.51)	279	0.34 (−0.02, 0.68)
hs-cTnT†	1918	0.35 (0.30, 0.40)	279	0.35 (0.17, 0.52)
Women				
NT-proBNP†	2594	0.24 (0.18, 0.30)	282	0.49 (0.13, 0.81)
hs-cTnT†	2598	0.52 (0.49, 0.55)	282	0.38 (0.12, 0.58)

Analysis is stratified by pre-existing coronary heart disease (ECG or self-reported MI, grade 2 angina).
†Analysis is based on ln-transformed values.
hs-cTnT, high-sensitivity cardiac troponin T; KYH, Know Your Heart; MI, myocardial infarction; NT-proBNP, N-terminal pro-b-type natriuretic peptide.

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Table 5 Age-stratified differences† in mean biomarker levels in KYH compared with Tromsø 7 by sex, adjusted for age (within strata)

	Men			Women		
	40–54 years old	55–69 years old	P value for interaction	40–54 years old	55–69 years old	p-value for interaction
Total cholesterol	−0.27 (−0.36, −0.19)	−0.17 (−0.25, −0.09)	0.077	0.05 (−0.01, 0.12)	−0.18 (−0.25, −0.12)	<0.001
HDL cholesterol	−0.01 (−0.04, 0.02)	−0.08 (−0.11, −0.05)	0.002	−0.03 (−0.06, 0.00)	−0.22 (−0.25, −0.19)	<0.001
LDL cholesterol	−0.33 (−0.41, −0.25)	−0.20 (−0.27, −0.13)	0.02	0.01 (−0.06, 0.07)	−0.06 (−0.12, 0.00)	0.157
Triglycerides‡§	−0.01 (−0.06, 0.04)	0.07 (0.02, 0.11)	0.012	0.12 (0.09, 0.16)	0.19 (0.15, 0.22)	0.016
hsCRP‡	0.18 (0.10, 0.25)	0.38 (0.31, 0.45)	<0.001	0.19 (0.13, 0.26)	0.41 (0.34, 0.47)	<0.001
NT-proBNP	0.33 (0.19, 0.48)	0.49 (0.39, 0.59)	0.081	0.07 (−0.03, 0.17)	0.40 (0.32, 0.47)	<0.001
hs-cTnT‡	0.35 (0.27, 0.43)	0.36 (0.31, 0.42)	0.788	0.44 (0.38, 0.49)	0.57 (0.53, 0.61)	<0.001

†Values in KYH minus those in Tromsø 7.
‡Analysis is based on ln-transformed values.
§The models for triglycerides were additionally adjusted for fasting time because of differences in mean fasting time in two studies.
HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; KYH, Know Your Heart; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-b-type natriuretic peptide.

pronounced in the older age group (55–69 years old). Our study is the first to measure hs-cTnT in a general population in Russia. Several studies in the US and Western Europe used hs-cTnT measurements in population samples free of known CVD to predict future CVD.[15 27] High hs-cTnT was recognised as an indicator of heart failure rather than ischaemic damage.[9 10] Biochemical evidence of myocyte injury was associated with subsequent imaging evidence of replacement fibrosis both in the sample of asymptomatic individuals[9] and in symptomatic non-ischaemic heart disease populations.[28–30] Even in patients with chronic coronary artery disease, hs-cTnT was associated with death and heart failure but not MI.[31] It is notable that exclusion of participants with pre-existing coronary heart disease in our study did not change the estimates of the differences in hs-cTnT substantially, neither did adjustment for hypertension and smoking.

High-sensitivity C-reactive protein

This marker of systemic inflammation was higher in KYH than in Tromsø 7. The differences are of similar magnitude among men and women, are more pronounced in older age among men and are appreciably attenuated by adjustment for classical CVD risk factors (smoking, BMI, WHR, blood pressure, diabetes). Several previous studies have investigated predictors of increased hsCRP levels in Russian populations but did not report mean levels or systematically compare them with western studies.[6 32]

Raised levels of hsCRP have been found to be predictive of future CVD events[14 33] and were associated with coronary plaque burden[34] and atherosclerosis[35]; however, the relationship is not considered to be causal.[13] Low-grade elevation of hsCRP is non-specific and may reflect exposure to pro-inflammatory influences including smoking, particulate air pollutants, aspects of diet, medications, oral cavity health, obesity and metabolic syndrome.[35] While elevated hsCRP levels in KYH indicate higher general inflammatory status in the participants, this may reflect both atherosclerosis and higher prevalence of CVD risk factors, like obesity and smoking. Although this study does not permit inferences about the prevalence of atherosclerosis, elevated hsCRP may indicate greater risk of future CVD outcomes in the Russian sample.

Strengths and limitations

We analysed biomarker levels in recently obtained population-based samples of men and women within the same age range in the two studies. Similar methodology was used for data and sample collection. A key strength is that a calibration study was done to ensure the comparability of the laboratory essays for biomarkers. Furthermore, an innovatory approach to calculate CIs of the regression coefficients obtained using calibrated measures was developed to ensure 95% coverage.

Because the study was conducted in three cities, and response rates in KYH were not optimal, we should be cautious to generalise the findings to the whole of Norway and Russia. The age distribution of the populations of Novosibirsk and Arkhangelsk was similar to the national average in both cities.[16] Tromsø and Novosibirsk have higher proportion of population with higher education compared with respective national averages.[16 36] However, it should be noted that the selected locations have CVD mortality rates that are similar to the national averages.[16]

Considering the ongoing changes in cardiovascular mortality in Russia, there are many other factors that may explain recent reduction, including improvements in treatment for acute CVD events.[2] However, in this paper, we were focusing on circulating biomarkers in the general population rather than particular high-risk groups.

CONCLUSIONS

By comparing the blood biomarker profiles in comparable population-based studies conducted in Russia and Norway, the latter a country with much lower CVD mortality rates, we attempted to identify the distinguishing features of CVD epidemic in contemporary Russia that make it unique to the rest of the world. We have found the evidence that non-ischaemic pathways beyond lipid-related mechanisms may take a significant share of CVD morbidity in Russia. The higher levels of NT-proBNP and hs-cTnT in Russia may indicate that this population is at higher risk of dilated cardiomyopathy, heart failure, atrial fibrillation and cardioembolic stroke. Very minor differences in lipid levels are not enough to explain the much high mortality due to coronary heart events in Russia compared with Norway. However, higher pro-inflammatory status reflected by hsCRP and contribution of higher levels of hypertension, BMI and WHR (among women); smoking (among men); and diabetes are very likely to contribute to explaining the high coronary heart disease mortality in Russia.

To further explore heart damage, more in-depth characterisation of heart structure and function with echocardiography and carotid ultrasound is required. Exploration of alcohol use as

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a potential explanation of biomarker differences should be a potential future research direction.[37]

The results of this study are important from a prevention perspective. As we suggest a substantive proportion of CVD in Russia occurring due to non-ischaemic pathways, additional efforts are needed to detect and treat people with early structural and functional changes in the heart.

What is already known on this subject

- Russia has one of the highest rates of mortality from cardiovascular disease (CVD) in the world with the reasons for that not fully understood. A small number of studies measured blood-based biomarkers of CVD in Russia but included only lipid profiles. Comparison of lipid profiles to other countries did not find major differences.

What this study adds

- Levels of total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were similar in two population-based studies conducted in Russia and Norway: Know Your Heart and Tromsø 7. This finding is paradoxical given high cardiovascular mortality rates in Russia. However, markers of cardiac damage and general inflammation were considerably higher in Russian compared with Norwegian study. Non-ischaemic pathways to cardiac damage reflected by raised NT-proBNP and hs-cTnT are likely to contribute to high CVD mortality in Russia.

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Competing interests None declared.

Patient consent for publication Not required.

Ethical approval Ethical approval for the study was received from the ethics committees of the London School of Hygiene & Tropical Medicine (approval number 8808, 24/02/2015), Novosibirsk State Medical University (approval number 75, 21/05/2015), the Institute of Preventative Medicine (no approval number; 26/12/2014), Novosibirsk and the Northern State Medical University, Arkhangelsk (approval number 01/01-15, 27/01/2015). The Regional Committee for Research Ethics approved Tromsø 7 (REC North ref. 2014/940), and The Norwegian Data Inspectorate licensed the data. Study has conformed to the principles embodied in the Declaration of Helsinki.

Data sharing statement Data are available upon reasonable request.

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Supplementary material

Why does Russia have such high cardiovascular mortality rates? Comparisons of blood-based biomarkers with Norway implicate non-ischemic cardiac damage

Contents

Supplementary Table S1. The list of the analytic methods used for the analysis of biomarkers in Know Your Heart and Tromsø study

	Method Lytech (Know Your Heart)	Instrument	Inter-essay CV (concentration - %)	Method UNN (Tromsø 7)	Instrument	Inter-essay CV (concentration - %)	Biological sample
Total cholesterol	Enzymatic Colour Test	AU 680 Chemistry System / Beckman Coulter	3.88 mmol/L - 1.6%	Enzymatic colorimetric test	Cobas 8000 / Roche	4.8 mmol/L - 1.1%	Serum
HDL cholesterol	Enzymatic Colour Test	AU 680 Chemistry System / Beckman Coulter	0.96 mmol/L - 1.61%	Homogeneous enzymatic colorimetric test	Cobas 8000 / Roche	1.3 mmol/L - 1.6%	Serum
LDL cholesterol	Enzymatic Colour Test	AU 680 Chemistry System / Beckman Coulter	4.31 mmol/L - 4.26%	Homogeneous enzymatic colorimetric test	Cobas 8000 / Roche	3.1 mmol/L – 0.77%	Serum
Triglycerides	Enzymatic Colour Test	AU 680 Chemistry System / Beckman Coulter	1.63 mmol/L - 5.6%	Enzymatic colorimetric test	Cobas 8000 / Roche	1.5 mmol/L - 1.37%	Serum
High sensitivity CRP	Immuno-turbidimetric Test	AU 680 Chemistry System / Beckman Coulter	14.52 mg/L - 2.32%	Particle enhanced immunoturbidimetric assay.	Cobas 8000 / Roche	1.03 mg/L – 5.07%	Serum
HBA1c (Glycated haemoglobin)	Immuno-turbidimetric Test	AU 680 Chemistry System / Beckman Coulter	3.88%	Capillary electrophoresis	Capillarys 3 tera	<3%	Whole blood (EDTA)
Hs Troponin T	Electrochemi-luminescence Immunoassay	Cobas e411 / Roche	136 ng/L - 8.23%	Electrochemiluminescense Immunoassay	Cobas 8000 / Roche	12 ng/L - 6.3%	Serum
Nt-Pro-BNP	Electrochemiluminescense Immunoassay	Cobas e411 analyser / Roche	92.85 pg/ml - 8.15%	Electrochemiluminescense Immunoassay	Cobas 8000 / Roche	238 pg/ml - 4.2%	Serum

Supplementary Table S2. Differences in main study variables between Tromsø 7 study participants with NT-Pro-BNP measured (N=1403) and rest of Tromsø 7 study participants, Visit 1 (N=16243) in age group 40-69 years, adjusted for age and sex

	NT-Pro-BNP measured (N=1403)	Visit 1 participants (N=16243)	P-value
Total cholesterol (mmol/L), mean (sd)	5.47 (1.05)	5.50 (1.04)	0.313
HDL-cholesterol (mmol/L), mean (sd)	1.59 (0.50)	1.55 (0.47)	0.008
LDL- cholesterol (mmol/L), mean (sd)	3.58 (0.99)	3.62 (0.97)	0.124
Triglyserids, (mmol/L), GM	1.29	1.34	0.027
CRP, (mmol/L), GM	1.04	1.04	0.822
BMI, mean (sd)	27.1 (4.45)	27.3 (4.58)	0.116
Waist to hip ratio, mean (sd)	0.86 (0.10)	0.86 (0.10)	0.165
SBP, mean (sd)	127 (19.0)	127 (18.2)	0.373
DBP, mean (sd)	74.8 (9.92)	75.6 (10.1)	0.004
Education less than college level, % (N)	49.5 (708)	50.0 (7546)	0.462
Current smoker, % (N)	19.4 (266)	19.6 (3127)	0.740
Diabetes, % (N)	4.9 (76)	5.3 (814)	0.191
Lipid lowering medication, % (N)	10.2 (222)	10.2 (1716)	0.832
MI detected on ECG, % (N)	4.6 (56)	4.6 (175)	0.840
Heart failure (self-report), % (N)	1.7 (27)	1.4 (155)	0.102
Heart attack (self-report), % (N)	2.7 (45)	2.7 (334)	0.929
Grade 2 angina, % (N)	0.8 (16)	0.8 (138)	0.713

Supplementary Methods M1

Recalibration of Blood Biomarker Measurements in Know Your Heart Study for Comparisons with Tromsø 7 study.

Background

Comparisons of biomarker data obtained in different studies may be biased due to the differences in pre-analytic and analytic stages in the laboratory. The similarity of pre-analytic stage has to be ensured during study setup, while the analytic stage bias may be controlled by a calibration study where measurements of one of the studies are recalibrated to the measurements made in another study. In the situation of multicentre or longitudinal studies with laboratory measurements recalibration is needed to correct for laboratory differences in time or space (assay type, assay manufacturer, analytic platform) [1].

The intrinsic quality of a manufacturer's assay or test system might be confounded by the laboratory using the system [2]. An investigation of the comparability of assays produced by different manufacturers showed that assays sometimes do not meet the optimal bias limits and there are considerable calibration differences between manufacturers/assays [2]. Even small biases that occur with use of different assays, instruments or procedures may have considerable implications for the conclusions of research studies and affect comparability in the research setting [3]. At the population level, small, systematic differences shift the entire distribution of a biomarker, resulting in biased estimates of mean values and prevalence of a condition under study defined in terms of a cut-off level [1]. Epidemiologic studies must carefully assess the calibration and reproducibility of their biomarker measurements to ensure equivalence across study sites.

The goal of this calibration study is to derive a calibration equation that reflects the bias (systematic difference) in the measurement of biomarkers in Know Your Heart (KYH) relative

to Tromsø 7 study due to the laboratory analytic stage. The University Hospital of Northern Norway (UNN) Department of Laboratory Medicine was assigned as the «reference laboratory». Representative samples of properly stored vials of serum and blood samples from Know Your Heart study were re-measured there.

Methods

Eight analytes were included into the calibration study: total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, high sensitivity C-reactive protein (hsCRP), HbA1c (Haemoglobin A1c), high sensitivity cardiac Troponin T (hs-cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP). We obtained stratified random sampling of 102 KYH study participants on the basis of 3 age groups (35–46, 47–58, 59–69) and genders (male/female). For calibration of HbA1c measurements, 50 whole blood samples were selected using uniform sampling procedure. All 102 serum and 50 whole blood samples were split and reassayed at both Lytech laboratory (Russia) and UNN (Norway) in December 2018. The type of the laboratory assay, platform and the coefficient of variation for both laboratories (Lytech laboratory and UNN) are summarized in Supplementary Table S1.

Quality control procedures

Both UNN, Department of Laboratory Medicine, and Lytech have internal and external quality control procedures that assure the reliability of the measurements of common clinical analytes. The external quality control procedures involve analysis of standard serum distributed in the country's network of laboratories participating in the program. Inter-assay coefficient of variation was calculated based on analyses of commercial control samples. UNN Department of Laboratory Medicine is reference laboratory for Northern Norway, accredited according to ISO 15189.

Data Analysis

Recalibration

Initially, we compared the biomarker measures from UNN and Lytech graphically by examining scatter plots and Bland–Altman plots (differential plots). Before further data analysis, outliers were excluded: observations >3 SDs from the mean difference were defined as outliers and removed (Supplementary Table S3). After exclusion of outliers, Pearson's correlation coefficient was computed and a Cusum test (Passing-Bablock) was performed to assess the linearity of relationship between UNN and Lytech values [4] . The Cusum test indicated a non-linear relationship between the two sets of biomarker measures for hsCRP and HbA1c. For those two analytes calibration equations were fitted separately in different ranges, with the break points determined using iterative procedure [5].

The calibration function for the relationship between split-sample measurements conducted in University Hospital of Northern Norway (UNN) Department of Laboratory Medicine and Lytech laboratory (Moscow) was determined using Deming regression, which accounts for errors in both the dependent and independent variables [6]. The regression equation UNN = Intercept+Slope*Lytech represents the regression relationship between paired values was assumed to be of the form UNN = Intercept+Slope*Lytech. Unweighted or weighted Deming regression methods were used in this calibration study.[9] The choice between the unweighted and weighted methods was made based on the distribution of the data points on the differential plot [6]. Weighted Deming regression was used if the coefficient of variation (CV) was constant while standard deviation changes proportionally to the concentration [6]. Statistical calculations were performed in R using the packages "mcr" (1.2.1), "VDSPCalibration" (1.0), and "segmented" (0.5-4.0).

Use of calibration study results

The resulting regression coefficients (intercept and slope) were used to recalibrate Know Your Heart study values so that they are comparable with Tromsø 7 study measurements. There is uncertainty in the estimation of the regression coefficients in the calibration models, which should be carried through to the subsequent analyses in which the recalibrated values are used in regression analyses. To account for this we used a "double-bootstrap" approach. This allows estimation of the confidence intervals for the regression coefficients in the main regression analyses (representing adjusted mean difference between recalibrated biomarker levels between the two studies), taking into account the uncertainty at both stages of the analysis by using bootstrapping for the calibration study sample and for the main study sample. The double bootstrap approach is described in more detail in Supplementary Methods M2 and we conducted a simulation study to demonstrate the validity of this approach for these purposes (Supplementary Methods M2).

Results:

Development and application of calibration equations

In general, the calibration study showed very good correlation between UNN Department of Laboratory Medicine values and Lytech values for most analytes (Supplementary Table S3). The exception was hs-cTnT, which showed Pearson's correlation of 0.883.

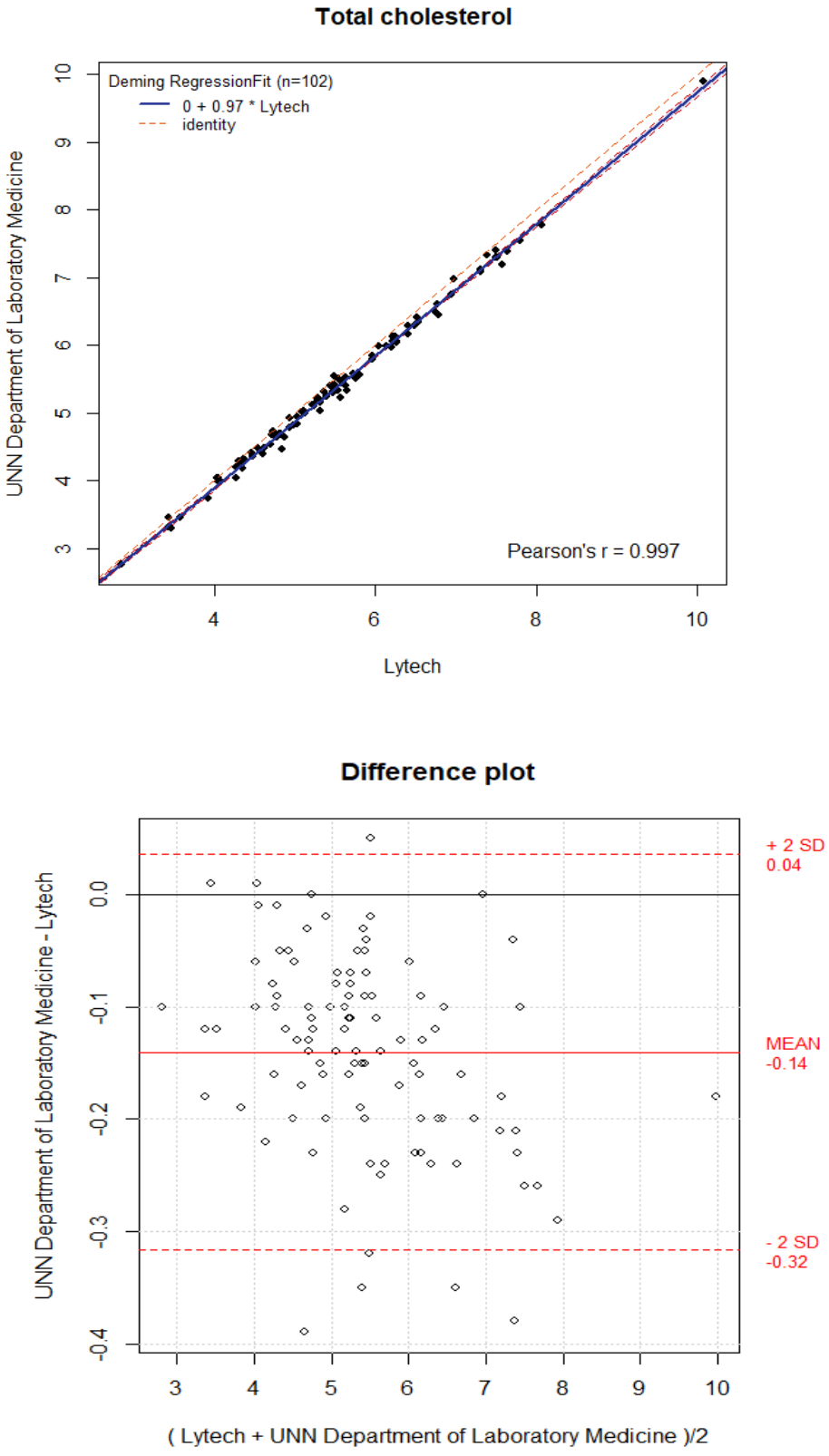
Also, the relationship between UNN Department of Laboratory Medicine values and Lytech values was linear for many analytes. The differential (Bland-Altman) plots and scatter plots are shown in Supplementary Figure 1. The regression equation coefficients are summarized in Supplementary Table S3. Departure from linearity was found for hsCRP and HbA1c. Therefore, different calibration equations were developed separately for each segment. The estimated break points for hsCRP are 1.45 mg/L and 5.57 mg/L, and for HbA1c - 7.48 %.

Because hs-cTnT test has high CV at low values [7], and its limit of quantification is at 13 ng/L it is not feasible to reliably calibrate this test as quantitative measure of Troponin T concentration. Therefore, values were compared above and below a threshold of top quintile (11 ng/L). Using the binary threshold, Lytech laboratory misclassified about 4 % of values relative to UNN Department of Laboratory Medicine.

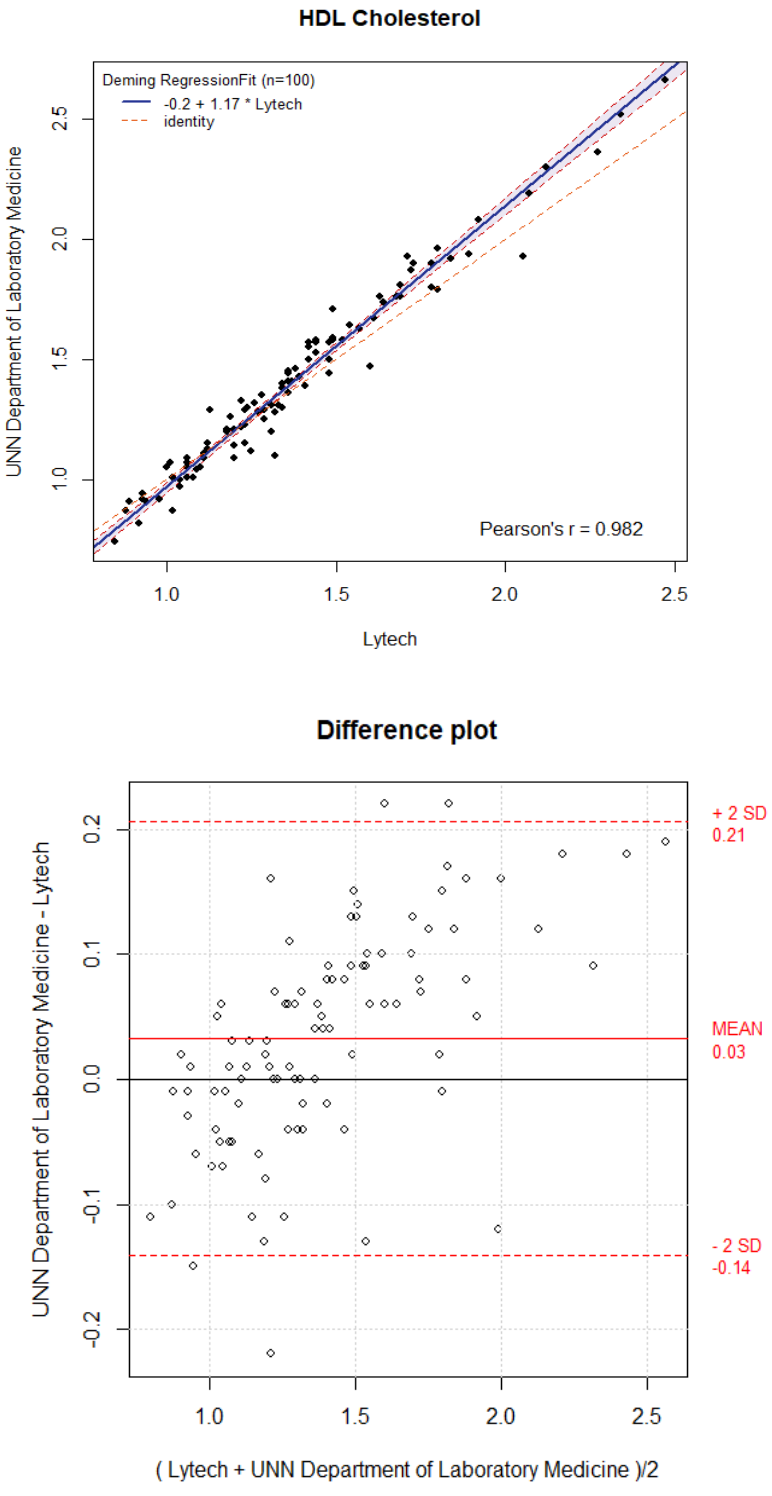
Supplementary Table S3. The relationship between UNN Department of Laboratory Medicine values and Lytech values described via Pearson's R^2 and Deming regression.

Analyte	No of excluded outliers	R^2	Intercept, 95% CI	Slope, 95% CI
Total cholesterol, mmol/L	0	0.997	0.00 (-0.08, 0.08)	0.97 (0.96, 0.99)
HDL cholesterol, mmol/L	2	0.982	-0.20 (-0.26,-0.14)	1.17 (1.13, 1.22)
LDL cholesterol, mmol/L	2	0.986	-0.66 (-0.82,-0.50)	1.11 (1.07, 1.15)
Triglycerides, mmol/L	2	0.999	0.05 (0.03, 0.06)	0.99 (0.99, 1.00)
High sensitivity CRP, mg/L	1	0.996		
hsCRP, < 1.45 mg/L*			0.07 (0.05, 0.09)	0.70 (0.67, 0.73)
hsCRP, 1.445 - 5.57 mg/L*			0.35 (-0.40, -0.29)	0.96 (0.93, 0.98)
hsCRP, > 5.57 mg/L*			1.13 (0.74, 1.51)	0.68 (0.64, 0.73)
HbA1c, % (Glycated haemoglobin)	2	0.997		
HbA1c <7.48 %*			-0.99 (-1.37,-0.62)	1.22 (1.16, 1.30)
HbA1c >7.48 %*			0.63 (0.04, 1.222)	1.01 (0.95, 1.08)
Hs-cTnT, ng/L	0	0.883	-	-
Nt-proBNP, pg/mL	0	0.998	6.41 (4.69-8.13)	0.62 (0.59-0.64)

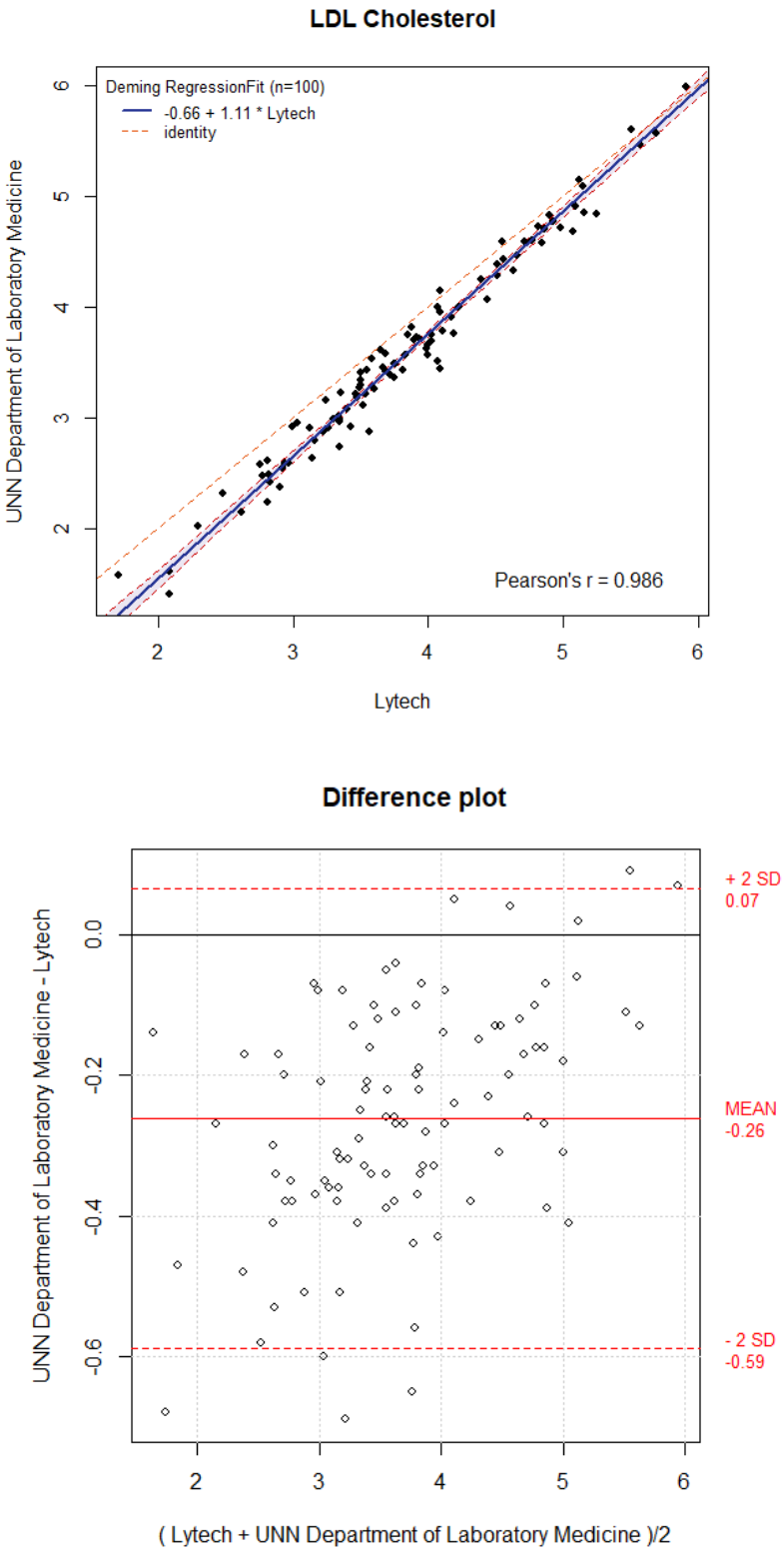
*Weighted Deming regression was used to develop calibration equations



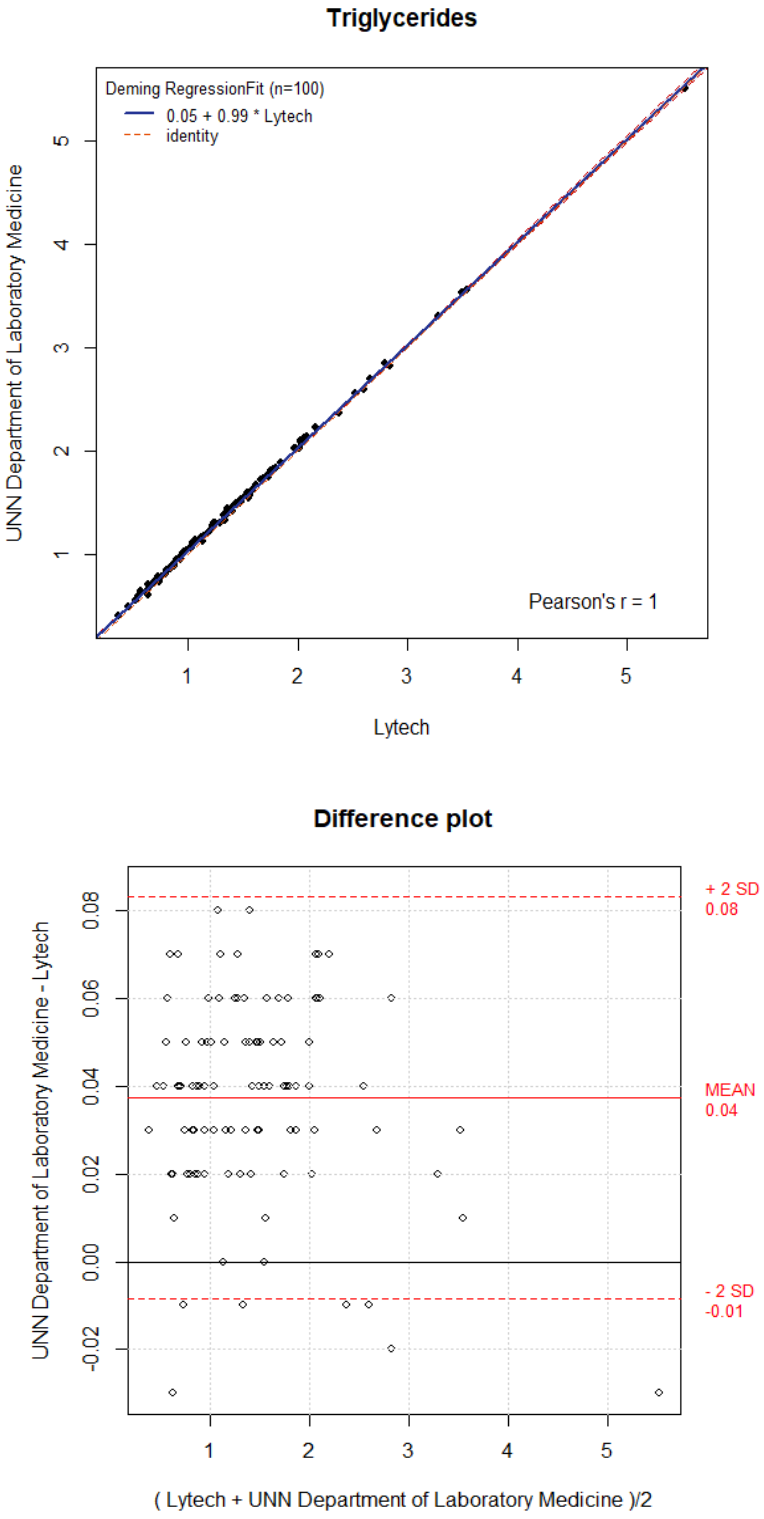
Supplementary Figure S1. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed total cholesterol in the KYH recalibration subsample. Yellow dotted line on the scatter plot represents the identity line for measurements, while two red dotted lines represent the 95% Confidence Intervals for the biomarker values in the calibration sample.



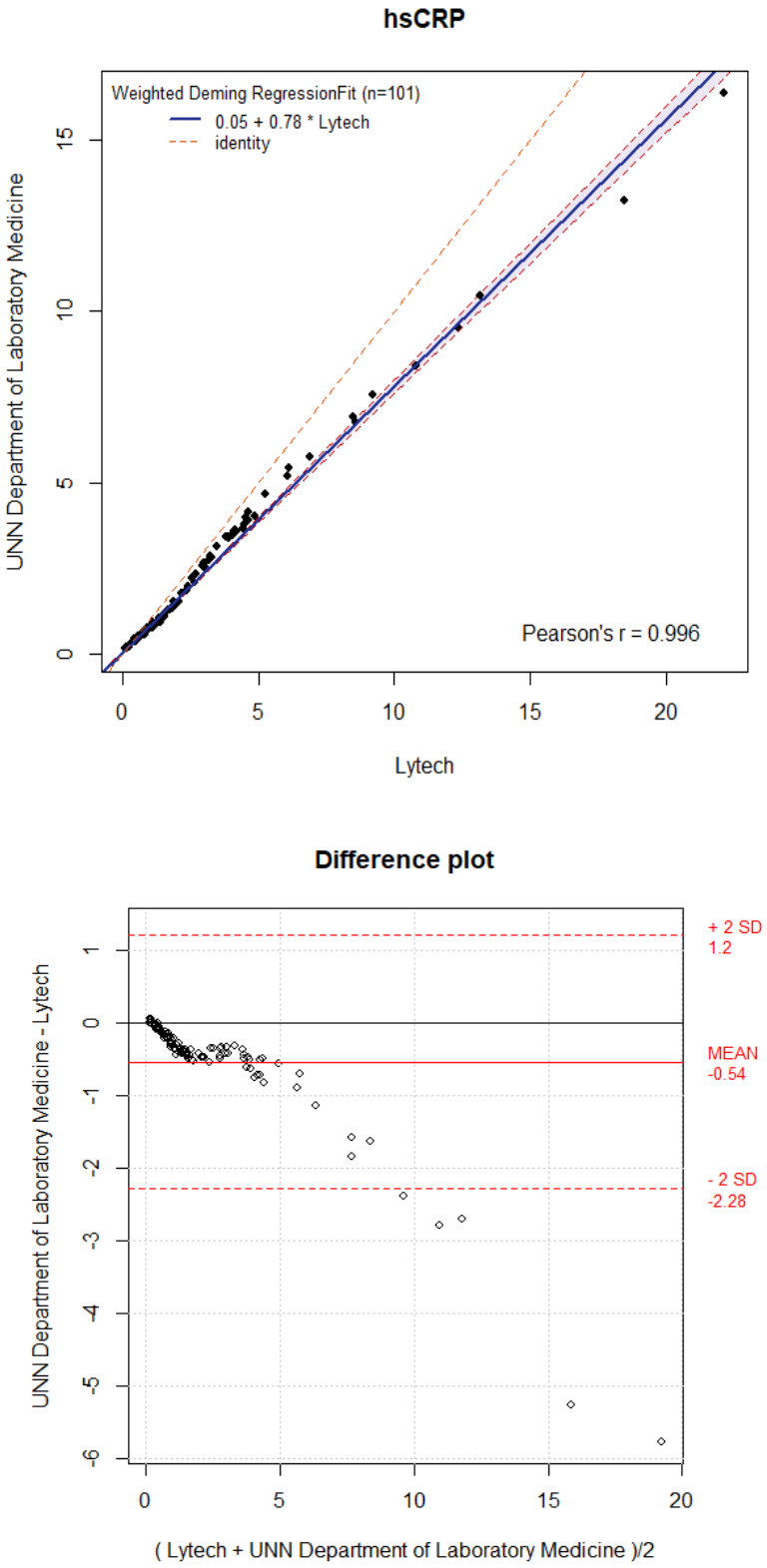
Supplementary Figure S2. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed HDL-cholesterol in the KYH recalibration subsample



Supplementary Figure S3. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed LDL-cholesterol in the KYH recalibration subsample

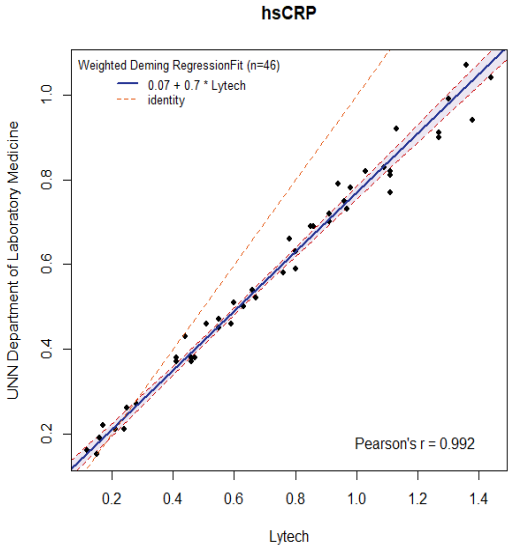


Supplementary Figure S4. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed tryglycerides in the KYH recalibration subsample

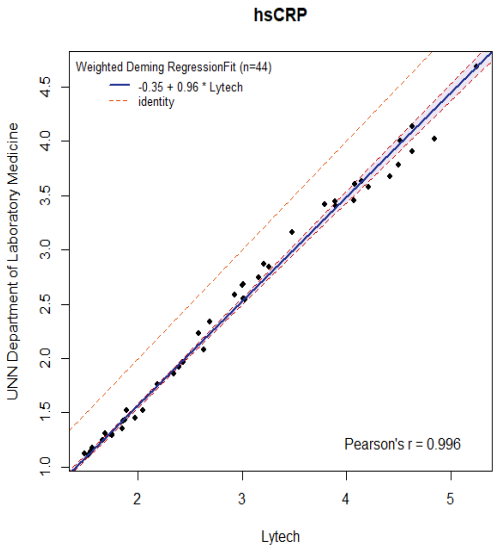


Supplementary Figure S5. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed hsCRP in the KYH recalibration subsample.

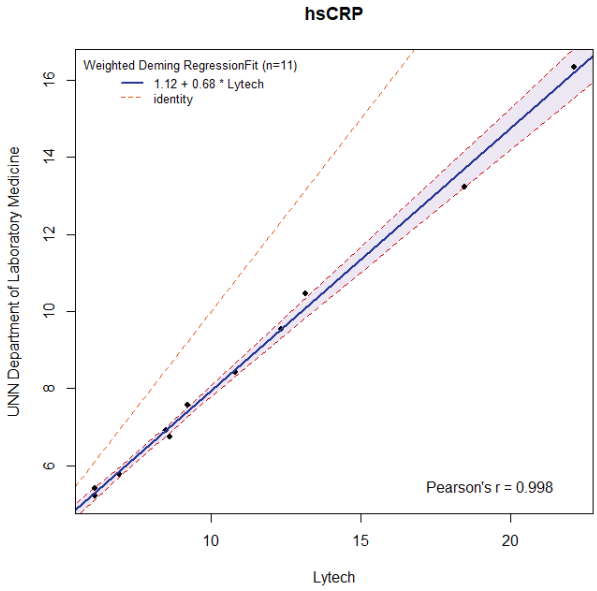
A) hsCRP < 1.45 mg/L



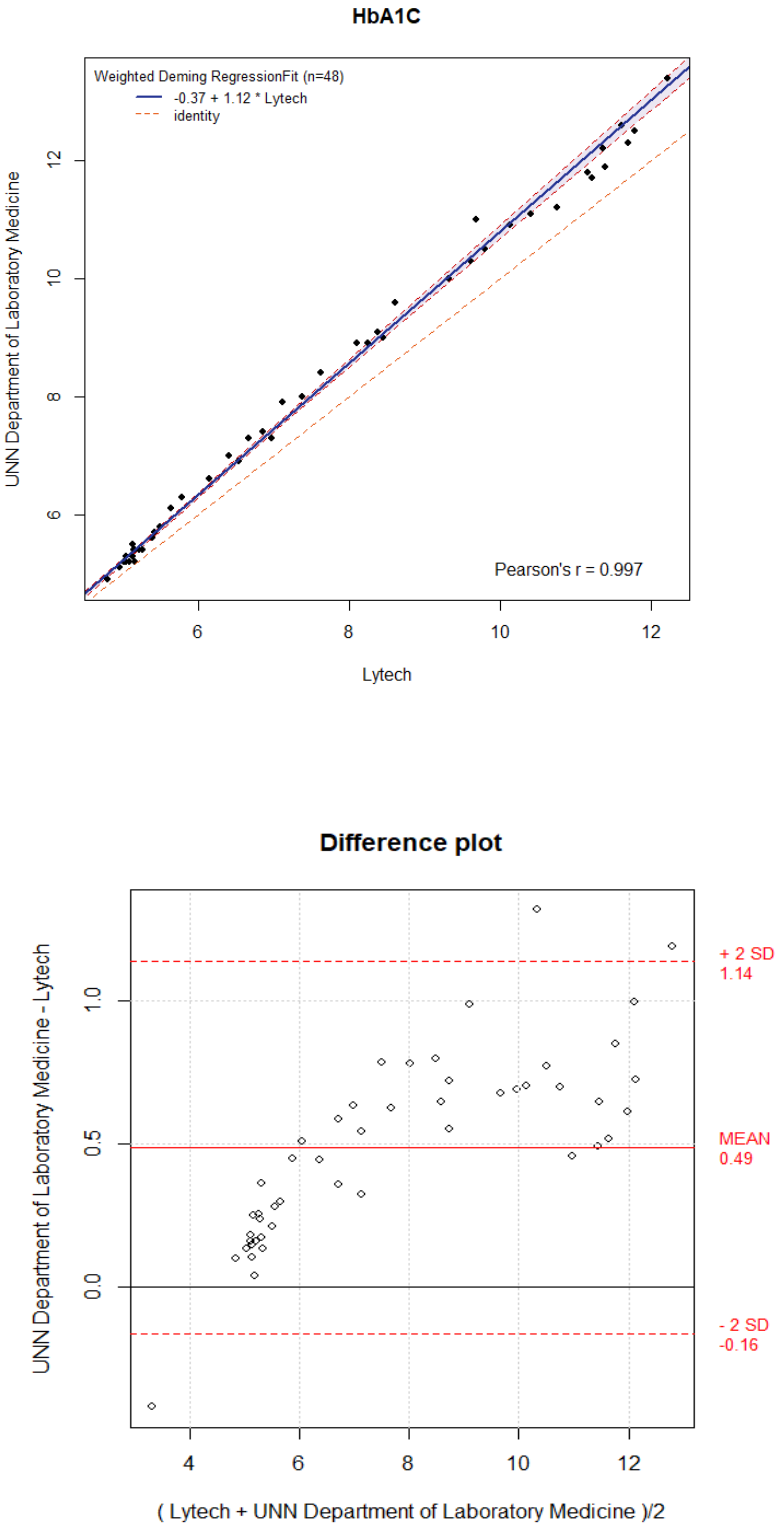
B) hsCRP 1.45 - 5.57 mg/L



C) hsCRP > 5.57 mg/L

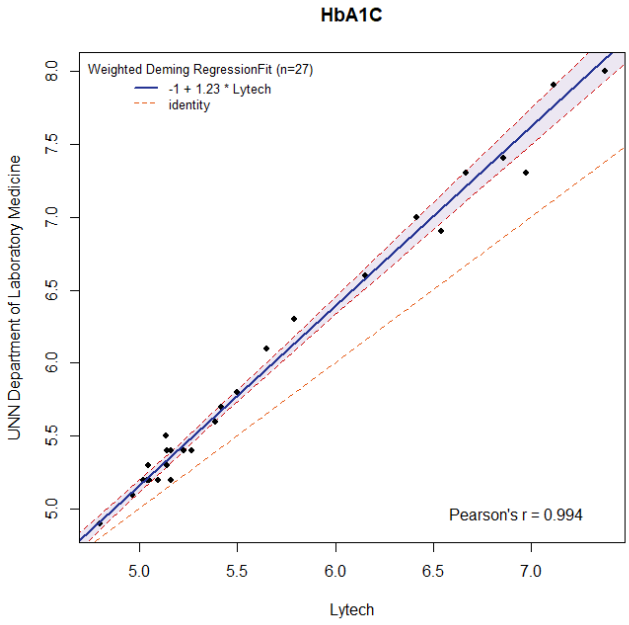


Supplementary Figure S6. Scatter plots of Lytech versus UNN assayed hsCRP in the KYH recalibration subsample with regression line split into three segments. A) hsCRP < 1.45 mg/L; B) hsCRP 1.45 - 5.57 mg/L; C) hsCRP > 5.57 mg/L

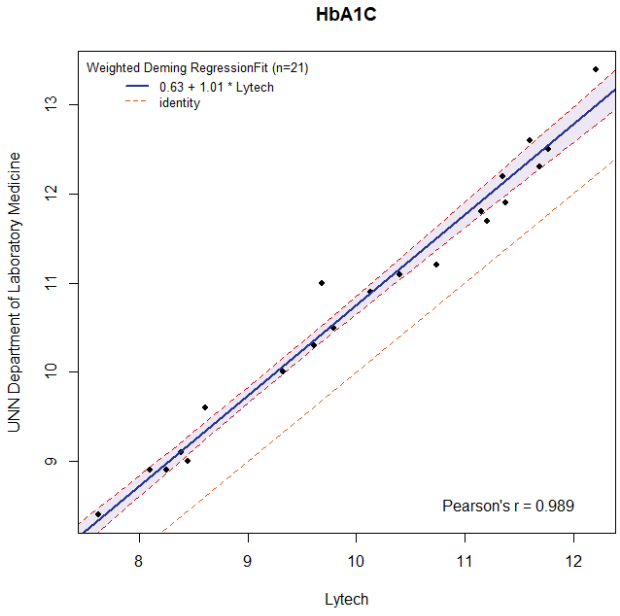


Supplementary Figure S7. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed HbA1c in the KYH recalibration subsample

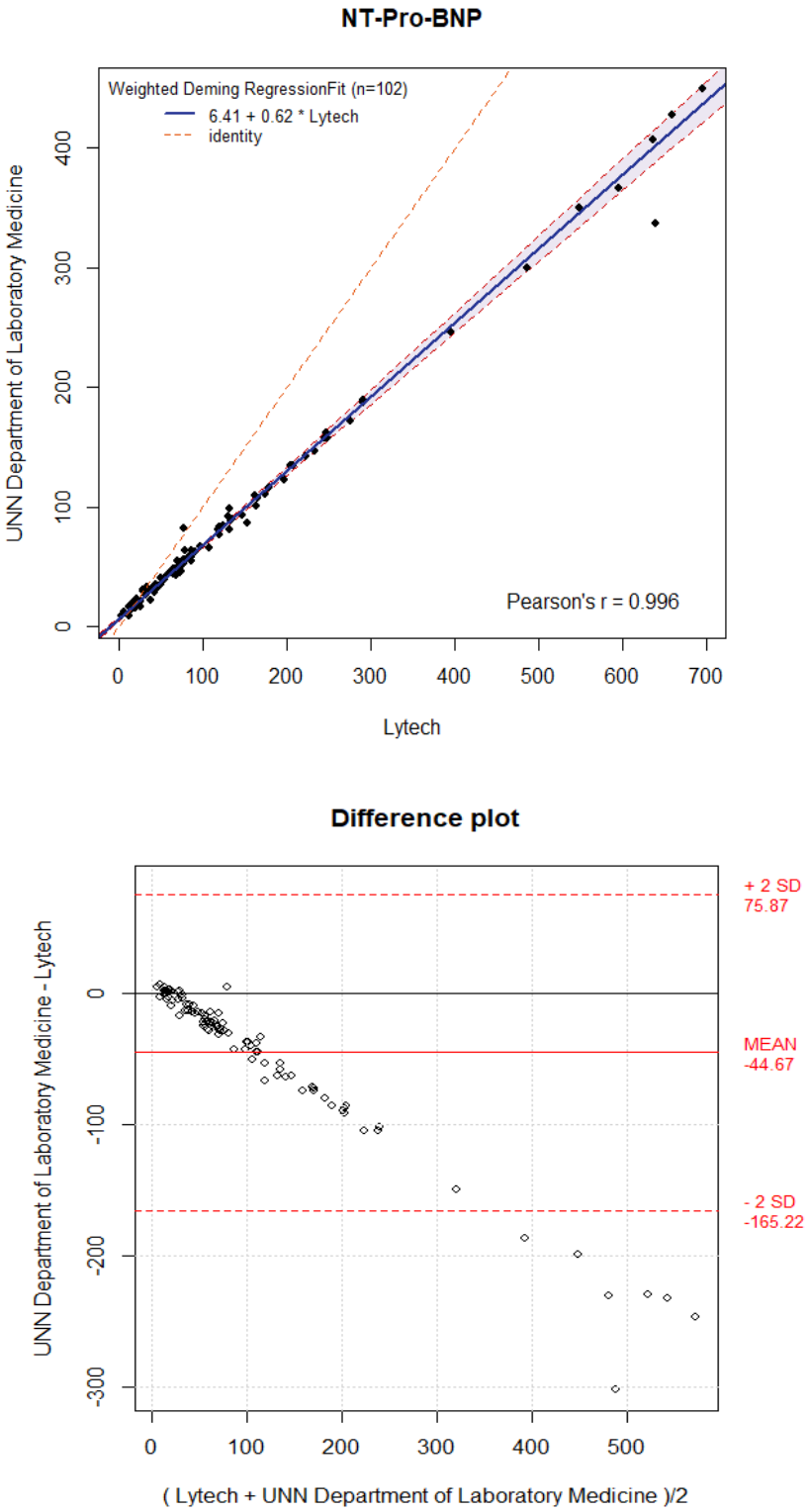
A) HbA1C < 7.48 %



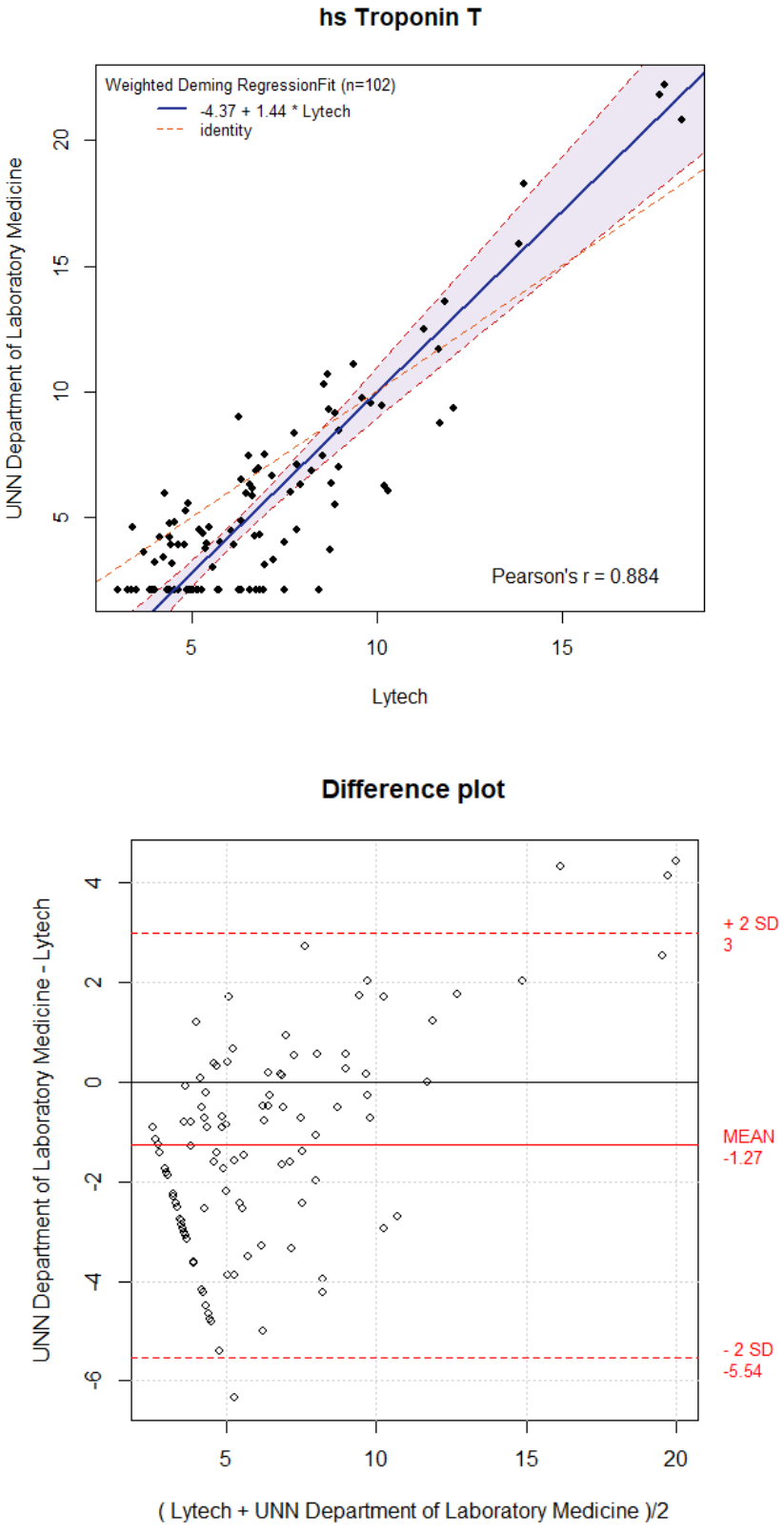
B) HbA1C > 7.48 %



Supplementary Figure S8. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed HBA1c in the KYH recalibration subsample with regression line split into two segments: A) HbA1c < 7.48 %; B) HbA1c > 7.48 %



Supplementary Figure S9. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed NT-proBNP in the KYH recalibration subsample



Supplementary Figure S10. Scatter and differential (Bland-Altman) plots of Lytech versus UNN assayed hs-cTnT in the KYH recalibration subsample

Supplementary Table S4. Concordance of hs-cTnT measurements in UNN Department of Laboratory Medicine and Lytech laboratory by threshold of 11 ng/L and 8.07 ng7L

hs-cTnT	UNN Department of Laboratory Medicine	
Lytech laboratory		
	< 11 ng/L	> 11 ng/L
< 11 ng/L, N (%)	93 (95.9%)	4 (4.1%)
> 11 ng/L, N (%)	0 (0%)	5 (100%)
	< 8.07 ng/L	> 8.07 ng/L
< 8.07 ng/L, N (%)	81 (92.1)	7 (7.9)
> 8.07 ng/L, N (%)	0	14 (100)

Use of calibration study results and limitations

The calibration function/equation represents the systematic bias in the KYH measurements relative to the Tromsø 7 measurements, due to the measurements being made in two different laboratories (Supplementary Table S5). The slope in the calibration equation represents the proportional error, and the intercept represents the constant error. Further statistical analysis involving comparisons of two populations using KYH and Tromsø 7 data should use the calibrated values. For example, when one is interested in comparing total cholesterol levels in Know Your Heart and Tromsø 7, it is important to remove the difference due to differences in analytic procedures in two laboratories.

However, although the appropriate calibration can account for the systematic bias, the recalibrated values have some uncertainty because the regression coefficients in the calibration equation are estimated (using the calibration study data) rather than being known exactly. This uncertainty should be accounted for in subsequent analyses using the recalibrated values for valid statistical inference. It is possible to do that by calculating confidence intervals of the estimates using a double-bootstrap procedure (Supplementary Material S2).

Due to appreciable imprecision of hs-cTnT assays at the low values seen in the general population, the development of calibration equation for this biomarker was not possible. Therefore, we compared hs-cTnT values between UNN Department of Laboratory Medicine and Lytech laboratory by binary threshold. The high values of hs-cTnT in Lytech are also classified as high in UNN, and low values are mostly (95.9%) also classified as low in UNN (Supplementary Table S4). Therefore, if further analysis of hs-cTnT is planned which involves both Tromsø 7 and Know Your Heart data, it should include the sensitivity analysis using with hs-cTnT as a binary variable.

Supplementary Table S5. Recalibration recommendations to maximize comparability of laboratory assays in Know Your Heart and Tromsø 7 study.

	Mean*			
	Lytech	UNN	Recommended calibration equation**	Comment
Total cholesterol, mmol/L	5.50	5.36	0 + 0.97*KYH	Small proportional bias, UNN measures lower
HDL cholestrol, mmol/L	1.38	1.41	-0.2 + 1.17*KYH	
LDL cholesterol, mmol/L	3.82	3.56	-0.66 + 1.11*KYH	UNN values are lower
Triglycerides, mmol/L	1.41	1.45	0.05 + 0.99*KYH	
High sensitivity CRP, mg/L	2.84	2.30	***<1.45 mg/L:*** 0.07+ 0.7*KYH ***1.45 - 5.569 mg/L:*** -0.35 + 0.96*KYH ***>5.57 mg/L:*** 1.12 + 0.68*KYH	The recalibration of KYH values should be done using 3 equations for 3 segments: <1.445 mg/L, 1.445 - 5.569 mg/L, >5.569 mg/L.
HBA1c, % (Glycated haemoglobin)	7.5	8.00	***<7.48 %*** -0.99 + 1.22*KYH ***>7.48 %*** 0.63+1.01*KYH	The recalibration of KYH values should be done using 2 equations for 2 segments: <7.48 %, >7.48 %
hs-cTnT, ng/L	7.00		Not applicable	The analysis of hs-cTnT data should be performed using threshold of top quantile of biomarker distribution, recalibration as a quantitative variable is not possible.
NT-proBNP, pg/mL	132.2	87.6	6.41 + 0.62*KYH	UNN measures lower

*Means are after exclusion of outliers, UNN Department of Laboratory Medicine

**Recommendation should be applied to Know Your Heart sample if the comparisons with Tromsø 7 study are planned

Supplementary Methods 2

Simulation Study

Aims:

1) to assess whether the "double-bootstrap" method for obtaining standard errors and confidence intervals for the mean differences based on recalibrated biomarker measurements is correct;
2) to compare the results obtained with "double-bootstrap" method with standard method which ignores uncertainty in the estimation of the regression coefficients in the calibration model;

Data-generating mechanisms:

Three samples will be simulated:

(1) "Study A" – a sample of size N_A. Biomarker values are simulated from a Normal distribution with mean E(A) and standard deviation SD(A);

(2) "Study B" – a sample of size N_B. Biomarker values are simulated from a Normal distribution with mean E(B) and standard deviation SD(B).

(3) External calibration sample – a sample size N_c with paired data (x, y); where x is corresponds to measurements by the instrument used in the Study A and y corresponds to measurements by the instrument used in the Study B. Paired biomarker values (x, y) are generated, with x generated from a normal distribution with mean E(x) and standard deviation SD (x), and y generated from a conditional normal distribution with mean b0 +b1*x and standard deviation s, i.e. y= b0 +b1*x + e, where e is normally distributed with mean 0 and standard deviation s. Both values in the validation sample are assumed to be error-prone

measures of an underlying true value: variable x has measurement error CVx and variable y has measurement error CVy (error ratio = CV_1^2/CV_2^2).

The main analysis uses data from studies A and B. Study B is considered as the reference study for the purposes of calibration. The real mean difference in biomarkers level between Study A and Study B is E(Ac)- E(B), where Ac denotes the recalibrated biomarker values obtained using the calibration coefficients obtained in the validation sample: b0 and b1.

Several scenarios were assessed in this simulation study, those are selected according to the characteristics of the real data that are available to researcher and need to be analysed (Supplementary Table S6).

Supplementary Table S6. Scenarios for the simulation study based on eight biomarkers of interest.

	Scenario							
Parameter	1	2	3	4	5	6	7	8
N_A	1700	1700	1700	1700	1700	1700	1700	1700
N_B	8302	8302	8302	8302	8302	2712	8302	8302
E (A)	5.42	1.31	3.69	1.70	94.59	0.97	5.42	5.48
E (B)	5.46	1.37	3.69	1.78	81.76	1.04	5.46	5.60
SD (A)	1.14	0.33	0.91	1.38	28.10	0.37	1.14	0.48
SD (B)	1.04	0.39	0.97	1.12	14.37	0.15	1.04	0.40
E(Ac)-E(B)	-0.20	-0.04	-0.25	-0.04	3.16	-0.01	-0.20	0.095
N_c	100	100	100	100	100	100	500	25
E(x)	5.36	1.41	3.55	1.45	76.03	0.91	5.36	5.99
SD(x)	1.14	0.39	0.92	0.79	14.27	0.16	1.14	0.94
b0	0	-0.2	-0.66	0.05	-29.42	0.06	0	-0.99
b1	0.97	1.17	1.11	0.99	1.21	1	0.97	1.22
s	0.0817,	0.0736,	0.1516,	0.0224,	2.695,	0.0194	0.0817	0.1069
CV_1^2/CV_2^2	2.1	2.0	30.6	16.7	13.8	2.7	2.1	1.7

Methods:

I. Standard

The calibration coefficients are estimated based on simulated validation study using Deming regression. The Study A simulated values are multiplied by the calibration coefficients (b0 and b1). The calibrated values in simulated dataset are regressed on the variable "Study" to estimate the mean difference, standard error of the difference (SE), and 95% confidence interval (95% CI).

II. "Double bootstrap"

The steps are as follows:

(i) Take M random samples with replacement from the calibration study data.

(ii) Perform the Deming regression analysis for each of the M calibration study samples, to obtain M sets of estimates of the calibration model parameters (intercept and slope).

(iii) Take M random samples with replacement within the main study data, with the sampling being stratified by study (A and B).

(iv) In bootstrap sample m $(m = 1, \dots, M)$ from the main study, use the calibration model parameters from bootstrap sample m of the calibration study data to obtain recalibrated biomarker measures in study A.

(v) In sample m $(m = 1, \dots, M)$ of the main study, the calibrated biomarker values regress on the variable "Study" to obtain the mean difference. The standard deviation of the B estimates provides an estimate of the standard error for the mean difference, and the 2.5% and 97.5% percentiles of B estimates gives the percentile-based bootstrap 95% confidence intervals.

We used B=500 and the simulation was repeated 1000 times.

Estimands:

We focus on the standard error for the mean difference and coverage of the confidence intervals of the mean difference between Study A and Study B obtained using the standard approach and the proposed double-bootstrap method.

Performance measures:

The "true" standard error (empirical standard error, EmpSE) is estimated by the standard deviation of the 1000 estimates of the mean difference. The means of the standard errors (ModSE) obtained with 1000 repetitions of standard (ModSE) and double-bootstrap methods (BootSE) are compared with the true standard error. Coverage of the confidence intervals obtained with the bootstrapping approach and the standard approach is given by the percentage of 95% confidence intervals for the mean difference (over the 100 simulations) that contain the true mean difference. Results are shown in Supplementary Table S7.

Supplementary Table S7. Results from simulation study: Estimates of the performance measures of interest for the range of scenarios.

Scenarios	EmpSE*	ModSE* (standard)	BootSE* (bootstrap)	Coverage (%) (standard)	Coverage (%)(bootstrap)
1	0.032	0.028	0.030	93	95
2	0.013	0.010	0.013	83	90
3	0.032	0.026	0.031	89	94
4	0.035	0.031	0.035	92	95
5	0.983	0.517	0.972	66	92
6	0.012	0.009	0.012	87	94
7	0.028	0.028	0.029	94	95
8	0.029	0.012	0.029	56	94

*EmpSE – Empirical standard error; ModSE – Model standard error; BootSE – bootstrap standard error

In all explored scenarios standard error obtained using double-bootstrap procedure is close to the empirical standard error. However, the standard error obtained using standard procedure is smaller than the empirical standard error, indicating that the standard approach underestimates the uncertainty in the estimated mean difference. The underestimation is severe in some scenarios, for example when the validation sample is small. Similarly, the coverage of

percentile-based bootstrap confidence intervals is close to the nominal level of 95% while under-coverage is observed when confidence intervals are obtained using standard method, with the under-coverage again being severe in some scenarios. The standard approach would be expected to improve as the size of the calibration study increases. However, in a situation of calibration samples of realistic (i.e. relatively small) sample size it is appropriate to estimate standard errors and confidence intervals using the proposed “double bootstrap” approach.

Supplementary Table S8. Age-standardized proportion of participants with hs-cTnT value above top quantile of distribution in KYH and Tromsø 7.

	KYH	Tromsø 7	p-value
	Men		
Hs-cTnT > 11.0 ng/L	0.19 (0.17, 0.21)	0.12 (0.09, 0.15)	<0.001
	Women		
Hs-cTnT > 8.07 ng/L	0.22 (0.20, 0.23)	0.06 (0.03, 0.08)	<0.001

Supplementary Table S9. The odds of hs-cTnT being in the top quantile of distribution in KYH study compared to Tromsø 7, explained by adjustment for classical CVD risk factors: smoking, BMI, WHR, SBP and DBP, diabetes, education.

	N	Model 1 (adjusted for age) OR (95% CI)	Model 2 (adjusted for age, smoking, BMI, WHR, SBP, DBP, diabetes, education) OR (95% CI)
Men			
Hs-cTnT > 11 ng/L	2196	1.95 (1.5, 2.52)	1.88 (1.41, 2.52)
Women			
Hs-cTnT > 8.07 ng/L	2881	5.93 (4.34, 8.1)	4.85 (3.4, 6.91)

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Paper 2

ORIGINAL RESEARCH

Evidence for a Direct Harmful Effect of Alcohol on Myocardial Health: A Large Cross-Sectional Study of Consumption Patterns and Cardiovascular Disease Risk Biomarkers From Northwest Russia, 2015 to 2017

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***Background*—**Alcohol drinking is an increasingly recognized risk factor for cardiovascular disease. However, there are few studies of the impact of harmful and hazardous drinking on biomarkers of myocardial health. We conducted a study in Russia to investigate the impact of heavy drinking on biomarkers of cardiac damage and inflammation.

***Methods and Results*—**The Know Your Heart study recruited a random sample of 2479 participants from the population of northwest Russia (general population) plus 278 patients (narcology clinic subsample) with alcohol problems. The general population sample was categorized into harmful drinkers, hazardous drinkers, nonproblem drinkers, and nondrinkers, according to self-reported level of alcohol consumption, whereas the narcology clinic sample was treated as the separate group in the analysis. Measurements were made of the following: (1) high-sensitivity cardiac troponin T, (2) NT-proBNP (N-terminal pro-B-type natriuretic peptide), and (3) hsCRP (high-sensitivity C-reactive protein). The narcology clinic subsample had the most extreme drinking pattern and the highest levels of all 3 biomarkers relative to nonproblem drinkers in the general population: high-sensitivity cardiac troponin T was elevated by 10.3% (95% CI, 3.7%–17.4%), NT-proBNP by 46.7% (95% CI, 26.8%–69.8%), and hsCRP by 69.2% (95% CI, 43%–100%). In the general population sample, NT-proBNP was 31.5% (95% CI, 3.4%–67.2%) higher among harmful drinkers compared with nonproblem drinkers. Overall, NT-proBNP and hsCRP increased with increasing intensity of alcohol exposure (test of trend $P<0.001$).

***Conclusions*—**These results support the hypothesis that heavy alcohol drinking has an adverse effect on cardiac structure and function that may not be driven by atherosclerosis. **(*J Am Heart Assoc*. 2020;9:e014491. DOI: 10.1161/JAHA.119.014491.)**

Key Words: alcohol use • CRP (C-reactive protein) • NT-proBNP (N-terminal pro-B-type natriuretic peptide) • troponin T

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Alcohol drinking is increasingly recognized as a risk factor for cardiovascular disease (CVD).[1] Alcohol, even when consumed in moderation, is associated with complex changes in blood biochemistry, involving changes in many biomarkers for cardiometabolic risk.[2] Binge drinking is associated with alcoholic cardiomyopathy, high blood pressure, increased risk of myocardial infarction, arrhythmias, and fatal cardiac arrest and stroke.[3] However, the causal nature of many of the associations between heavy alcohol use and CVD biomarkers as well as the mediation pathways between alcohol use and cardiovascular outcomes are not fully understood. In particular, it is unclear whether any effect is through alcohol's effect on the atherosclerotic process in vessels as distinct from direct toxic damage to the myocardium.

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Accompanying Table S1 and Figures S1 through S3 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.014491

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Clinical Perspective

What Is New?

- In the population-based study, we observed elevated levels of markers of heart damage, cardiac wall stretch, and general inflammation among heavy alcohol users compared with nonproblem drinkers.
- Heavy drinking was confirmed as an important risk factor of cardiovascular disease, with probable direct effect on cardiac structure and function.

What Are the Clinical Implications?

- Prevention of cardiovascular diseases in the general population should include screening and intervening on harmful and hazardous alcohol use.

Levels of blood-based cardiovascular biomarkers can be used as proxy measures of cardiovascular health. High-sensitivity cardiac troponin T (hs-cTnT) and NT-proBNP (N-terminal pro-B-type natriuretic peptide) were both developed for use in clinical cardiology and are now increasingly used in population-based studies of CVD. When evaluating general population cohorts, any concentration of hs-cTnT >3 ng/L has been associated with subclinical CVD and has adverse prognostic implications.[4] Cardiac wall stretch biomarker NT-proBNP has been mostly used for diagnosis of heart failure and for prognosis in the setting of heart failure.[5] However, in population-based samples, low-grade elevation in NT-proBNP was shown to be an early marker of cardiac injury that is not yet clinically evident.[6] Assessment of natriuretic peptides can predict first-onset heart failure or improve prediction of coronary heart disease in people without known CVD.[7] In addition, NT-proBNP concentration predicted stroke as strongly as a diagnosis of coronary heart disease. This could partly be explained by associations between NT-proBNP concentration and stroke risk factors: left ventricular hypertrophy and atrial fibrillation.[7–9]

There has been substantial interest in CRP (C-reactive protein) as a risk predictor related to the underlying inflammatory nature of atherosclerosis,[10] although there is evidence that it is in itself not causal but may instead be a marker of a general inflammatory disease process.[11] A 1000-fold elevation of CRP is indicative of acute inflammation,[12] whereas lower persistent elevation of hsCRP (high-sensitivity C-reactive protein) may be caused by low-grade systemic inflammatory processes associated with atherosclerosis.[13] Increased levels of hsCRP have been predictive of future cardiovascular events[14,15] and have been associated with coronary plaque burden.[16]

Previous research has looked at the relationship of biomarkers with classic risk factors for CVD, among them smoking, obesity indexes, blood pressure, and lipid profiles.[13,17–20] However, there has been little work on the association of alcohol with biomarkers of heart damage, cardiac wall stretch, and systemic low-grade inflammation. Most of the published work was done in the populations with relatively moderate levels of alcohol consumption,[18,21–23] with the exception of one study that showed prospectively an association between heavy drinking and heart failure in vulnerable men with underlying myocardial ischemia.[24] As noted elsewhere, there is a gap in the research literature on heavy drinking patterns affecting cardiovascular outcomes.[25]

Russia is one of the countries that has had a tradition of heavy drinking of spirits and has been characterized as having a particularly harmful drinking profile.[1] Studies of CVD biomarkers in the Russian population make it possible to achieve 2 goals: (1) to investigate the mechanisms by which hazardous and harmful patterns of alcohol use increase the risk of cardiovascular outcomes and (2) to help clarify the role of heavy alcohol use in explaining why Russia has one of the highest CVD rates of any country.[26] In this study, we used measures of hs-cTnT, NT-proBNP, and hsCRP to assess the damage to myocardium, cardiac wall stretch, and general low-grade inflammation in heavy drinking individuals recruited through state-run facilities for treatment of alcohol use disorders and in a large general population sample in Russia categorized according to level and pattern of alcohol use.

Methods

Requests to access the data set from bona fide researchers may be sent to the International Project on Cardiovascular Disease in Russia.[27]

Study Design

The Know Your Heart study recruited 2479 participants from the general population of the city of Arkhangelsk in northwest Russia from 2015 to 2018. A detailed account of the rationale and description of the methods of the study has been published previously.[28] At the same time, we recruited 278 patients from the Arkhangelsk Regional Psychiatric Hospital with a primary diagnosis of alcohol problems.[28] The latter group is referred to subsequently as the narcology clinic subsample, consistent with Russian terminology. The study sample was almost exclusively of European descent.

Ethical Approval

All procedures performed were in accordance with the ethical standards of the institutional research committee (ethics committees of the London School of Hygiene and Tropical Medicine [London, UK] and the Northern State Medical

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University [Arkhangelsk, Russia]) and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants included in the analysis gave signed informed consent.

Study Participants

The general population sample from Arkhangelsk was recruited at random (stratified by age, sex, and district of residence) using the regional health insurance fund register as the sampling frame. Trained interviewers visited the addresses selected and invited the appropriate resident at each address to take part in the study. A minimum of 3 attempts were made to get a response from each address. When successful, an interview was conducted about circumstances, health, and behaviors of the participant. The response rate was 68% of the addresses where contact with a person of the target age and sex was established, and 96% of those interviewed took part in a subsequent health check.[28]

In addition, a sample of heavy drinkers (narcology clinic subsample) were recruited from inpatients at the regional psychiatric hospital. The inclusion criteria were as follows: age of 35 to 69 years, resident in the city of Arkhangelsk or Arkhangelsk region, and admitted to the narcological department of the regional psychiatric hospital with a primary diagnosis related to alcohol drinking. People with ≥1 of the following characteristics were excluded:

1. Experiencing alcohol withdrawal symptoms or during the first week of alcohol detoxification;
2. Behavior that suggested that an individual could pose a threat to the safety of the clinic staff or other participants during the survey;
3. Current or past misuse of drugs other than nicotine or alcohol;
4. Unable to give informed consent for participation in the study (eg, severe cognitive deficit or acute psychiatric illness).

Clinicians at the hospital used their judgement to decide which participants should or should not be invited. Signed informed consent was obtained. A total of 278 patients were recruited of 322 patients invited (85.4%).

We analyzed data on 2354 participants from the general population in Arkhangelsk plus 271 individuals from the narcology clinic subsample who attended the health check and for whom blood analyte concentrations were available.

Data and Sample Collection

The baseline interview was administered by a trained interviewer using a tablet computer-assisted personal interviewing device. For the general population sample, the interview was done in people's homes in nearly all cases. For the narcology clinic subsample, it was done at the Arkhangelsk Regional Psychiatric Hospital by the same set of trained interviewers. Information was collected on medical history and socioeconomic circumstances, education, and lifestyle.

The subsequent health check comprised a physical examination (including blood pressure, height, waist and hip circumference, and weight) and blood sample collection. Participants were requested not to eat or drink alcohol in the 4 hours before their appointment. Participants in the narcology clinic subsample were transported to the research clinic for the health check, accompanied by a nurse. A second interview was conducted at this stage that recorded medical history, use of medications, alcohol use, and smoking.

A total of 50 mL of blood was taken from each participant. Samples were centrifuged, and serum was transferred to barcoded 1.8-mL cryovials and frozen (−80°C) within 2 hours after venipuncture. These were subsequently shipped to a laboratory in Moscow, where they were stored at −80°C and then analyzed in a single batch at the end of the fieldwork. The laboratory staff were blind to all characteristics of participants, including whether serum was from the narcology clinic subsample or the general population sample.

Outcome Variables

hs-cTnT and NT-proBNP were measured using a high-sensitivity electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Hitachi, Japan) on a Cobas e411 analyzer. hsCRP was measured using a high-sensitivity immunoturbidimetric test on AU 680 Chemistry System Beckman Coulter. The lower limit of detection for hs-cTnT test was 3 ng/L, and 54 participants (2.07%) with values below the limit of detection had their values recoded to 2.9 ng/L. The limit of detection of NT-proBNP test was 5 ng/L, and NT-proBNP values of 19 participants (0.7%) with values below the limit of detection were recoded to 4.9 ng/L. Because we were interested in low-grade inflammation that is not caused by acute infection, 38 participants with hsCRP values >99th percentile for the general population (30 mg/L) were excluded from the statistical analysis of hsCRP.

Exposure Variables

We defined 2 categorical exposure variables. The first was binary and divided the study group into those from the narcology clinic subsample and those from the general population sample. The second exposure variable further categorized the general population sample into groups based on self-report of various dimensions of alcohol consumption. Those who reported not drinking alcoholic beverages during

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the past 12 months at baseline interview and health check were classified as nondrinkers. The categories of harmful and hazardous drinkers were defined using 3 instruments: the validated Russian-language translations of the Alcohol Use Disorders Identification Test,[29] the Cut down, Annoyed, Guilty, Eye Opener (CAGE) instrument,[30] and questions on alcohol drinking pattern previously found to be highly predictive of mortality in Russia.[31,32] The Alcohol Use Disorders Identification Test is a screening test for hazardous and harmful drinking, whereas CAGE is used to screen for alcoholism in clinical settings. The CAGE score was adapted to have a reference period of the past 12 months rather than ever in a participant's lifetime, in keeping with a previous study from Russia because of interest in alcohol use in the recent past.[33] The scheme for categorizing the general population sample into 4 groups is presented in the Figure.

To validate the approach chosen for classification of the general population sample into drinking categories, we used the following: (1) information on alcohol volume consumed during the past 12 months, calculated using the standard quantity frequency approach[34]; (2) history of asking for help with alcohol problems from social workers or physicians; and (3) blood biomarkers of alcohol use. γ-Glutamyl transferase and carbohydrate-deficient transferrin (CDT) are biomarkers of excessive drinking.[35] We used a previously developed approach[36] to calculate a combined biomarker value of ɣ-%CDT=[0.8×ln(γ-glutamyl transferase)]+[1.3×ln(%CDT)], with a cutoff value of 4.0 for heavy drinking. γ-Glutamyl transferase was measured in all study participants. Because of cost, CDT was not assayed in everyone. It was measured in all 271 patients receiving treatment for alcohol problems, all 400 problem drinkers (Alcohol Use Disorders Identification Test score ≥8 or CAGE score ≥2), all 143 nonproblem drinkers drinking >5 L per year, and 244 randomly selected nondrinkers and nonhazardous drinkers. The combined ɣ-%CDT was thus only available for 1032 participants. As this biomarker was only used to establish the face validity of the alcohol categorization, the fact that it was only available for a subset of study subjects did not affect the numbers used in the main analyses.

Other Covariates

Information was available on classic risk factors, including those that are on the potential causal pathway between alcohol use and CVD biomarkers. We constructed directed acyclic graphs to identify the minimal sufficient adjustment set of variables for estimating the total effect of alcohol use (Figures S1 through S3). These were age, sex, smoking, and education. Education was classified into 4 categories: incomplete secondary or lower; secondary or professional school; incomplete higher or specialized secondary (eg, medical,

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Category of drinker	Drinking alcoholic beverages during last 12 month	AUDIT>=8	CAGE>=2	Harmful Russian drinking pattern *
General population sample				
Harmful drinker	✓	✓	✓	✓
Hazardous drinker	✓	✓ for one or two instruments		
Non-problem drinker	✓	✗	✗	✗
Non-drinker	✗	✗	✗	✗

Figure. The assignment scheme of the general population sample into categories by drinking status: (1) harmful drinkers, (2) hazardous drinkers, (3) nonproblem drinkers, and (4) nondrinkers. *Twice weekly or more frequency of hangover and/or excessive drunkenness and/or sleeping in clothes at night because of drunkenness and/or failing their family or personal obligations because of drinking and/or drinking nonbeverage alcohols (sources of ethanol not intended for drinking, such as medicinal tinctures) and/or ≥1 episodes of zapoi (a period of ≥2 days of being drunk, during which a participant is withdrawn from normal social life).[32] AUDIT indicates Alcohol Use Disorders Identification Test; GAGE, Cut down, Annoyed, Guilty, Eye Opener.

teacher training college, or technical); and higher (university). Professional schools include institutions that provide professional training but no degree. Smoking status was categorized as current smokers, ex-smokers, and never smokers. For current smokers, the number of cigarettes smoked was specified as 1 to 10/day, 11 to 20/day, and >20/day.

Other variables used in the analysis included potential mediators. These included systolic and diastolic blood pressure (mean of second and third measurements) and use of antihypertensives, determined according to recorded medications coded to the Anatomical Therapeutic Chemical classification as C02 (antihypertensives), C03 (diuretics), C07 (β-blocking agents), C08 (calcium channel blockers), or C09 (agents operating on the renin-angiotensin system). A small proportion of participants self-reported use of blood pressure–lowering medication without a corresponding Anatomical Therapeutic Chemical code being found. These participants were also defined as being on antihypertensives.

Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist/hip ratio was the mean of 2 measurements of waist divided by the mean of 2 measurements of hip. The blood lipid profile was measured and included total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, apolipoprotein A1, and apolipoprotein B. Renal function was assessed by measuring cystatin C and estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration cystatin C equation.[37]

Statistical Analyses

Descriptive tabulations of participant characteristics were age and sex standardized to the Standard European Population 2013. hs-cTnT, NT-proBNP, and hsCRP had right-skewed distributions and were ln transformed for analysis.

We assessed the association between alcohol use and biomarkers of CVD (hs-cTnT, NT-proBNP, and hsCRP) by comparing geometric mean levels of biomarkers in the narcology and general population samples. Next, we compared means of ln-transformed biomarkers across the categories of alcohol consumption: (1) narcology clinic subsample; (2) general population sample, harmful drinking pattern; (3) general population sample, hazardous drinking pattern; (4) general population sample, nonproblem drinkers; and (5) general population sample, nondrinkers. This approach allowed a nuanced assessment of CVD biomarkers, depending on the drinking pattern, separating nonproblem drinkers as a comparison group and determining if there was an increasing trend with increased intensity of alcohol use.

The sociodemographic characteristics and CVD risk factors were compared by the categories of main exposure variable using heterogeneity tests adjusting for age and sex in generalized linear models. The associations between alcohol use and ln-transformed biomarkers of CVD (hs-cTnT, NT-proBNP, and hsCRP) were assessed using multivariable adjusted linear regression models. Age was included in the model as a continuous variable. Quadratic and cubic terms were added to account for nonlinearity and kept in the modelif associated with an outcome at $P<0.05$. Model 1 involved adjustment for age and sex. Model 2 adjusted for potential confounders (age, sex, smoking, and education). Model 3 additionally included possible mediators (waist/hip ratio, BMI, lipids [low-density lipoprotein, high-density lipoprotein, and apolipoprotein B/apolipoprotein A1 ratio], blood pressure [systolic and diastolic], use of blood pressure medication, and estimated glomerular filtration rate). This final model provided an estimate for the direct effect of alcohol on cardiac damage, cardiac wall stretch, and low-grade inflammation. A test for increasing linear trend in means of biomarkers across the categories of alcohol exposure was done with $df=1$. To make the regression coefficients more interpretable and comparable, they were back transformed and presented as percentage of difference in mean compared with the reference category (nonproblem drinkers). Statistical analysis was performed using SAS software 9.4 (SAS Institute Inc, Cary, NC).

Results

The descriptive characteristics of the narcology clinic subsample and the general population sample (age and sex standardized) are presented in Table 1. The average age of the narcology clinic subsample was 48.5 years and that of the general population sample was 53.7 years. The narcology clinic sample was 76.8% men, and the general population sample was 41.7% men. On average, the narcology clinic subsample had lower systolic blood pressure (potentially because of clinical management during hospital admission), lower low-density lipoprotein and total cholesterol values, lower BMI and waist circumference, and lower estimated glomerular filtration rate compared with the general population sample. A much higher proportion of narcology clinic subsample compared with the general population sample were current smokers. Detectable hs-cTnT was observed in 98% of participants, whereas the equivalent figure for NT-proBNP was 99%. The geometric means for hs-cTnT, NT-proBNP, and hsCRP were significantly higher in the narcology clinic subsample compared with the general population sample.

The face validity of our categorization of alcohol use is demonstrated in Table 2. This shows indicators of drinking for each of the drinking categories derived from self-reported alcohol use in the general population and the narcology clinic sample. Almost all of the alcohol measures show a clear trend

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Table 1. Age- and Sex-Standardized Means and Proportions With 95% Confidence Intervals (n=2625)

Variables	Narcology Clinic Subsample (n=271)	General Population Sample (n=2354)	*P* Value, Test of Heterogeneity
Current drinkers*	1.00	0.91 (0.89–0.92)	<0.001
Harmful Russian drinking pattern†	0.89 (0.85–0.93)	0.08 (0.07–0.09)	<0.001
AUDIT score ≥8	0.95 (0.90–1.00)	0.16 (0.15–0.18)	<0.001
CAGE score ≥2	0.92 (0.87–0.98)	0.15 (0.14–0.16)	<0.001
Current smoking	0.75 (0.68–0.82)	0.26 (0.25–0.28)	<0.001
Use of antihypertensive medication	0.33 (0.26–0.40)	0.39 (0.37–0.41)	0.102
BMI, mean, kg/m^2	25.3 (24.5–26.2)	27.6 (27.3–27.8)	<0.001
Waist/hip ratio, mean	0.90 (0.89–0.91)	0.89 (0.88–0.89)	0.028
Waist, mean, cm	86.9 (84.8–88.9)	91.2 (90.7–91.7)	<0.001
Systolic blood pressure, mean, mm Hg	127 (124–130)	132 (131–132)	0.006
Diastolic blood pressure, mean, mm Hg	83.6 (81.8–85.4)	83.5 (83.1–84.0)	0.95
Total cholesterol, mean, mmol/L	5.15 (4.98–5.32)	5.37 (5.33–5.42)	0.012
LDL cholesterol, mean, mmol/L	3.43 (3.29–3.57)	3.63 (3.60–3.67)	0.006
HDL cholesterol, mean, mmol/L	1.44 (1.38–1.50)	1.43 (1.42–1.45)	0.891
Apolipoprotein B/apolipoprotein A1 ratio, mean	0.71 (0.67–0.74)	0.72 (0.72–0.73)	0.353
eGFR (cystatin C), mean, mL/min per 1.73 m^2	74.1 (72.0–76.1)	80.1 (79.6–80.6)	<0.001
hs-cTnT, GM, ng/L	7.09 (6.63–7.58)	6.43 (6.32–6.54)	0.006
NT-proBNP, GM, pg/mL	112 (95.7–131)	72.6 (69.7–75.6)	<0.001
hsCRP, GM, mg/L	3.06 (2.55–3.68)	1.51 (1.44–1.58)	<0.001
Triglycerides, GM, mmol/L	1.37 (1.25–1.50)	1.24 (1.21–1.27)	0.043

Data are standardized to the standard European population 2013. AUDIT indicates Alcohol Use Disorders Identification Test; BMI, body mass index; eGFR, estimated glomerular filtration rate; GM, geometric mean; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CAGE, Cut down, Annoyed, Guilty, Eye Opener.

*All participants from the narcology clinic sample are current drinkers, but they were not drinking during the period of admission to the narcology clinic.

†Twice weekly or more frequency of hangover and/or excessive drunkenness and/or sleeping in clothes at night because of drunkenness and/or failing their family or personal obligations because of drinking and/or drinking nonbeverage alcohols (sources of ethanol not intended for drinking, such as medicinal tinctures) and/or ≥1 episodes of zapoi (a period of ≥2 days of being drunk, during which a participant is withdrawn from normal social life).

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across the categories. The concentration of alcohol use biomarkers (CDT and γ-glutamyl transferase) and the proportion of the participants with an elevated combined biomarker of alcohol use are highest in harmful drinkers, intermediate in hazardous drinkers, and lowest in nonproblem drinkers. Similarly, the volume of alcohol consumed by drinkers during the past year and the amount of alcohol consumed per day are highest in harmful drinkers, intermediate in hazardous drinkers, and lowest in nonproblem drinkers. Of the 227 nondrinkers, 82 were former drinkers, as distinct from lifelong nondrinkers.

CVD Biomarkers in the Narcology Clinic Subsample Versus the General Population

After adjustment for age, sex, smoking, and education, the levels of all biomarkers (hs-cTnT, NT-proBNP, and hsCRP) were higher in the narcology clinic subsample compared with the general population sample as a whole (Table 3). Specifically, hs-cTnT was higher by 12.3% (95% CI, 5.9%–19.1%) and NT-proBNP was higher by 43.9% (95% CI, 25.4%–65.1%), whereas hsCRP was higher by 66.0% (95% CI, 41.7%–94.5%).

CVD Biomarkers Across 5 Categories of Alcohol Use

Consistent with the previous analysis, compared with nonproblem drinkers in the general population sample, the narcology clinic subsample had much higher levels of hs-cTnT, NT-proBNP, and hsCRP (Table 4).

hs-cTnT was elevated by 10.3% (95% CI, 3.7%–17.4%) in the narcology clinic subsample compared with the nonproblem drinkers in the general population, controlling for sex, age, smoking, and education. However, hs-cTnT levels were lower in the group of harmful drinkers in the general population compared with nonproblem drinkers. Adjustment for the

Table 2. Descriptive Measures of Alcohol Use by Categories of Alcohol Use

Variables	Narcology Clinic Sample (n=271)	General Population Sample				*P* Value†
		Harmful Drinkers (n=71)	Hazardous Drinkers (n=424)	Nonproblem Drinking (n=1632)	Nondrinkers* (n=227)	
Combined biomarker of heavy alcohol use (GGT and CDT) ≥4, N (%)	135 (50.9)	27 (38.6)	55 (14.0)	23 (5.2)	0	<0.001
Have asked for help of narcologist or social worker for drinking problem, N (%)	271 (100)	26 (36.6)	27 (6.4)	12 (0.8)	19 (23.2)§	<0.001
Drinking >40 g of alcohol per day, N (%)‡	62 (23.7)	26 (36.6)	48 (11.3)	12 (0.7)	0	<0.001
Binge drinking (60 g of alcohol per drinking occasion) at least once a month, N (%)	189 (70.5)	49 (69.0)	215 (51.9)	76 (4.8)	0	<0.001
Alcohol consumed per year, mean, L‡	15.0	19.0	8.5	1.9	0	<0.001
Alcohol consumed per day, mean, g‡	33.45	40.09	18.41	4.04	0.00	<0.001
GGT, U/L	68.02	44.39	38.48	25.03	23.69	<0.001
CDT, %	1.64	1.60	0.94	0.74	0.53	<0.001

CDT indicates carbohydrate-deficient transferrin; GGT, γ-glutamyl transferase.
*Nondrinkers include lifetime abstainers and ex-drinkers.
†Test for linear trend, adjusted for age and sex.
‡Alcohol consumption recorded for the past 12 months.
§Among ex-drinkers.

additional set of variables that are likely to be the mediators of the association between extremely heavy alcohol use and cardiac injury (determined via hs-cTnT) had only a minor effect on parameter estimates (Table 4).

Harmful drinkers in the general population had an elevated concentration of NT-proBNP by 31.5% (95% CI, 3.4%–67.2%) compared with nonproblem drinkers, but to lesser extent than in the narcology clinic subsample (46.7%; 95% CI, 26.8%–69.8%), controlling for age, sex, smoking, and education. Adjustment for potential mediators of the association between excessive alcohol use and cardiac wall stretch (measured by NT-proBNP) resulted in some attenuation of the effect estimate (Table 4).

The elevation of low-grade systemic inflammation marker hsCRP by 69.2% (95% CI, 43%–100%) was observed in the narcology clinic subsample compared with nonproblem drinkers in the general population sample, controlled for age, sex, smoking, and education. Intermediate elevations were also seen for harmful drinkers. Further adjustment for covariates that are likely to be on the mediation pathway between alcohol use and hsCRP leads to increases in the regression coefficient (Table 4).

Although we did not observe increased levels of cardiac biomarkers in the group of hazardous drinkers in the general population, the trend test across all drinking categories (excluding nondrinkers) was significant for NT-proBNP and hsCRP, with concentration of biomarkers higher with higher level of alcohol exposure (Table 4).

Sensitivity Analysis

hs-cTnT assays are known to show appreciable imprecision at the low values seen in the general population.[38] The interassay coefficient of variation for values below the limit

Table 3. Percentage Differences in hs-cTnT, NT-proBNP, and hsCRP Between Narcology Clinic Subsample and General Population

Narcology Clinic Subsample vs General Population	% Difference (95% CI), Adjusted for Age and Sex	% Difference (95% CI), Additionally Adjusted for Smoking and Education	% Difference (95% CI), Additionally Adjusted for Mediators*
hs-cTnT (n=2595)	8.2 (2.6–14.3)	12.3 (5.9–19.1)	12 (5.7–18.7)
NT-proBNP (n=2595)	63.3 (43.8–85.5)	43.9 (25.4–65.1)	30.9 (14.6–49.6)
hsCRP (n=2562)	107.2 (78.8–140.2)	66.0 (41.7–94.5)	98.3 (71.2–129.8)

Dependent variable was ln transformed, and the regression coefficients were back transformed and presented as percentage difference in mean in comparison to the reference group. hsCRP indicates high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.
*Possible mediators included were systolic and diastolic blood pressure, use of blood pressure medication, lipid profile (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, apolipoprotein A1, and apolipoprotein B), renal function (estimated glomerular filtration rate), body mass index, and waist/hip ratio.

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Table 4. Percentage Differences in hs-cTnT, NT-proBNP, and hsCRP Between Levels of Alcohol Use

Alcohol Use	% Difference (95% CI), Adjusted for Age and Sex	% Difference (95% CI), Additionally Adjusted for Smoking and Education	% Difference (95% CI), Additionally Adjusted for Mediators*
hs-cTnT (n=2595)			
Narcology clinic subsample	6.6 (0.7 to 12.8)	10.3 (3.7 to 17.4)	10.3 (3.7 to 17.3)
Harmful drinkers, general population sample	−14.4 (−22.6 to −5.3)	−11.5 (−20.1 to −2)	−9.6 (−18.2 to −0.1)
Hazardous drinkers, general population sample	−3.3 (−7.7 to 1.4)	−2.6 (−7.1 to 2.1)	−1.8 (−6.3 to 2.8)
Nonproblem drinking, general population sample	0 (Reference group)	0 (Reference group)	0 (Reference group)
Nondrinkers, general population sample	2.1 (−3.6 to 8.2)	1.6 (−4.1 to 7.7)	−0.6 (−6.1 to 5.1)
P value for linear trend (among drinkers)	0.272	0.068	0.047
P value for heterogeneity	<0.001	<0.001	<0.001
NT-proBNP (n=2595)			
Narcology clinic subsample	68.6 (47.6 to 92.6)	46.7 (26.8 to 69.8)	34.9 (17.1 to 55.4)
Harmful drinkers, general population sample	45.6 (14.9 to 84.6)	31.5 (3.4 to 67.2)	30.1 (3.5 to 63.5)
Hazardous drinkers, general population sample	1.5 (−9.1 to 13.3)	−3.5 (−13.7 to 7.8)	1.9 (−8.4 to 13.3)
Nonproblem drinking, general population sample	0 (Reference group)	0 (Reference group)	0 (Reference group)
Nondrinkers, general population sample	10.3 (−3.7 to 26.4)	6.6 (−6.9 to 22.1)	0.9 (−11.3 to 14.8)
P value for linear trend (among drinkers)	<0.001	<0.001	<0.001
P value for heterogeneity	<0.001	<0.001	<0.001
hsCRP (n=2562)			
Narcology clinic subsample	117.1 (86.1 to 153.2)	69.2 (43 to 100.2)	99.7 (70.9 to 133.4)
Harmful drinkers, general population sample	33.9 (2.2 to 75.4)	13.4 (−13.7 to 49)	28.4 (0.2 to 64.6)
Hazardous drinkers, general population sample	14.7 (1.1 to 30.2)	6.9 (−5.8 to 21.4)	0.5 (−10.5 to 12.9)
Nonproblem drinking, general population sample	0 (Reference group)	0 (Reference group)	0 (Reference group)
Nondrinkers, general population sample	−9 (−22.1 to 6.4)	−12.2 (−24.8 to 2.5)	−10.7 (−22.4 to 2.8)
P value for linear trend (among drinkers)	<0.001	<0.001	<0.001
P value for heterogeneity	<0.001	<0.001	<0.001

Dependent variable was ln transformed, and the regression coefficients were back transformed and presented as percentage difference in mean in comparison to the reference group. hsCRP indicates high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*Possible mediators included were systolic and diastolic blood pressure, use of blood pressure medication, lipid profile (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, apolipoprotein A1, and apolipoprotein B), renal function (estimated glomerular filtration rate), body mass index, and waist/hip ratio.

of quantification (13 ng/L) was 15%. To ensure the robustness of conclusions about hs-cTnT, we conducted a sensitivity analysis using logistic regression, with hs-cTnT categorized into values below and above the top quintile in the general population sample (9.34 ng/L). The results of this analysis were consistent with analyses presented above (Table S1).

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Separating the category of nondrinkers into never drinkers and ex-drinkers for regression analysis did not reveal any specific differences in biomarkers between these 2 groups; therefore, the results were presented keeping current nondrinkers as one group.

In a further sensitivity analysis, we excluded those with previous myocardial infarction, operations on the heart, and grade 2 angina (N=307 [11.73%]) to see if elevated cardiac injury and cardiac wall stretch biomarkers were secondary to coronary heart disease. This had no material effect on the associations observed.

Discussion

In this study, we have shown that markers of cardiac injury hs-cTnT, cardiac wall stretch NT-proBNP, and general inflammation hsCRP are substantially elevated among those receiving treatment for alcohol problems at the narcology clinic compared with the general population. Most important, there was a significant linear increasing trend of NT-proBNP across 4 groups of drinkers: nonproblem drinkers, hazardous drinkers, harmful drinkers, and the narcology clinic sample. Similarly, there was a linear increase in hsCRP levels over drinking groups.

NT-proBNP has been developed and primarily used in the clinical contexts of diagnosis and prognosis of heart failure.[5] However, in population-based samples, low-grade elevation in NT-proBNP was shown to be an early marker of cardiac injury that is not yet clinically evident.[6,39] In this study, we showed markedly elevated levels of NT-proBNP in the sample receiving treatment at a narcology clinic and intermediate elevation in the general population sample of harmful drinkers. This is consistent with a previous report from the Izhevsk family study and the Belfast (UK) component of the PRIME (Prospective Epidemiological Study of Myocardial Infarction) study that showed elevated NT-proBNP in hazardous drinkers.[40] This finding is further supported by increased risk of heart failure among heavy drinking men in the prospective BRHS (British Regional Heart Study).[24] However, our study goes further by showing that there is a biomarker dose-response effect across the 4 categories of heavy drinking at levels of NT-proBNP that are below clinical thresholds for heart failure. Other studies of this question that had inconsistent findings were limited by the fact that the populations they studied had much lower levels of alcohol consumption.[18,21,23]

After the adjustment for the possible mediators of the association between alcohol use and NT-proBNP (blood pressure, blood lipid indexes, BMI, and kidney function), the regression coefficients were partly attenuated. This could be explained by an effect of alcohol on kidney function because it was shown that risk of chronic kidney disease is higher in people with alcohol use disorder.[41] Increased blood pressure caused by heavy alcohol use[42,43] may be responsible for a decrease in kidney function and may lead to hypertensive cardiac injury. Low BMI and altered lipid metabolism in the narcology clinic subsample, caused by alcoholic malnutrition, poor diet, and effects of alcohol, may further contribute to the damage of the myocardium.

The direct toxic effect of alcohol on the heart as a result of persistent heavy drinking is an established mechanism of alcoholic cardiomyopathy.[44] The condition can be undiagnosed, interact with the atherosclerotic damage to cardiovascular system, and increase the risk of sudden cardiac death.[1] Estimates of the risk of diagnosed alcoholic cardiomyopathy in people with alcohol use disorders varied between 1% and 40%, depending on the patient population studied.[44] The observed increasing trend in NT-proBNP levels across 4 categories of alcohol exposure in our study gives support to the hypothesis that heavy drinking causes subclinical nonischemic myocardial damage. The association of heavy alcohol use with NT-proBNP in our study is consistent with previous reports of increased NT-proBNP in left ventricular hypertrophy, atrial fibrillation, and stroke.[7–9]

Cardiac troponin T elevation is a biomarker used for the diagnosis of acute myocardial infarction. Development of the testing technology and introduction of hs-cTnT tests led to recognition that low-grade elevations of hs-cTnT are predictive of future cardiovascular events and death in general population.[4,6,45] Detectable hs-cTnT is observed in a sizable proportion of individuals without diagnosis of CVD.[18] In our study, detectable levels of hs-cTnT were observed in 98% of the study sample, which is higher than in some other population-based cohorts,[6,46] but comparable to others.[19] It has been suggested that long-term elevation of hs-cTnT is explained to a greater extent by indexes of heart failure (eg, higher left ventricular mass and lower left ventricular ejection fraction) and increased NT-proBNP levels than indexes of atherosclerosis or ischemia.[46,47] Also, hs-cTnT has been found to be a direct marker of ongoing myocardial fibrosis.[48] Similarly, in our sample of heavy drinkers at the narcology clinic, elevation of hs-cTnT may indicate nonischemic injury to the myocardium that occurred because of exposure to high doses of alcohol or its metabolites, such as acetaldehyde. After adjustment for possible mediators (blood pressure, blood lipid indexes, BMI, and kidney function), the regression coefficients for the relationship between heavy alcohol use and hs-cTnT did not change. Therefore, the effect of heavy alcohol consumption on the myocardium may be explained by the direct injury of myocardium by alcohol or its metabolites that leads to cell death. However, it is unclear why the group of harmful drinkers in the general population have lower levels than nonproblem drinkers,

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although the CIs around the estimates of effect for hs-cTnT are large. A literature search for other studies that looked at association between alcohol consumption and hs-cTnT identified only reports with relatively moderate level of alcohol use, which reported either decreased or the same hs-cTnT levels in some groups of drinkers compared with nondrinkers.[18,21–23] The reasons for this phenomenon may lie in low precision of hs-cTnT at low levels, unknown factors influencing the performance of the test, and selection of the comparison group for analysis by categories of alcohol consumption.

A U-shaped relation between alcohol intake and CRP was previously observed, with heavy drinkers showing higher CRP than moderate drinkers.[49,50] The elevation of CRP in our study in the narcology clinic sample and the trend for elevated hsCRP across harmful and hazardous drinkers in the general population may have several explanations. The low-grade elevation of hsCRP in individuals with no overt disease is nonspecific and may reflect exposure to proinflammatory influences, including smoking, particulate air pollutants, aspects of diet, medications, obesity, and the metabolic syndrome.[13] Although we made efforts to adjust for many of these factors, there is still a strong possibility of residual confounding. Beyond this, there are several explanations for increased hsCRP in the narcology clinic subsample, including, but not limited to, toxic effects of alcohol and its metabolites on the liver, ranging from fatty liver to steatosis, process of detoxification, and exposure to the specific medications during treatment for alcohol problems. Finally, elevated levels of hsCRP in the narcology clinic subsample and the trend across categories of drinkers in the general population may be secondary to an atherosclerotic process facilitated by harmful and hazardous drinking. Thus, it is not possible from our study to determine which of these various explanations may account for the association of hsCRP with harmful and hazardous patterns of drinking.

Strengths and Limitations

Our study has several strengths that make it a significant contribution to the body of evidence on detrimental effects of alcohol on cardiovascular health by relating harmful and hazardous alcohol use to the markers of cardiac damage, cardiac wall stretch, and low-grade inflammation. We were able to recruit a substantial number of participants with substantial variation in the level and intensity of alcohol drinking. In addition, this study has addressed a gap in the research literature about the impact of high levels of exposure on cardiac injury[44] in a country where this exposure is relatively common[25] and rates of CVD mortality are among the highest in the world.[26]

Previous studies of association between alcohol consumption and CVD biomarkers are few and done in populations with relatively moderate quantities of consumed alcohol, whereas this study was able to differentiate between different patterns of heavy alcohol use, including extreme drinking patterns commonly observed in patients treated in Russian state-run narcology services. Although patients there may experience multiple comorbidities, they are most likely the consequence of an extremely heavy pattern of alcohol use. Their admission to the narcology clinic was because of the need for detoxification rather than organic comorbidities. Therefore, it is a good setting to study the mechanisms of cardiac injury caused by heavy alcohol use; and the results are likely to be generalizable to all countries and populations.

A particular strength of our study is that the collection of detailed questionnaire information on patterns and quantities of alcohol consumption during the past year has allowed us to construct a plausible grouping of general population participants according to degree of harmfulness of their alcohol consumption with strong face validity. Moreover, our ability to separate nondrinkers from nonproblem drinkers and the availability of data on confounders beyond age and sex have minimized the bias common to many other studies on alcohol drinking and CVD.[51] We cannot fully exclude the possibility of some residual confounding by smoking that was measured by self-report. Acknowledging the limitation of cross-sectional studies to show the direction of the association, we look forward to the prospective studies that may give better support for our conclusions.

Conclusions

The elevated levels of both NT-proBNP and hs-cTnT in the group of extremely heavy drinkers from the narcology subsample are consistent with heavy alcohol drinking leading to nonischemic damage of the heart. Elevated NT-proBNP in harmful drinkers from the general population provides further evidence for this. Furthermore, exclusion of individuals who had a previous diagnosis of coronary heart disease did not have an impact on the substantive results. However, this study cannot definitively exclude that heavy drinking may also contribute to increased CVD risk through ischemic pathways, as could be indicated by elevated hsCRP. The significance of findings in this study and the relative importance of different pathophysiological processes in harmful and hazardous drinkers should be further investigated using heart imaging methods. Echocardiography and cardiac magnetic resonance imaging can give more information about significance of cardiomyopathy-related indexes in excessive drinkers. Computerized tomography coronary angiogram or carotid ultrasound will allow direct measurement of the extent of

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atherosclerosis and will answer the question about significance of elevated hsCRP levels in harmful and hazardous drinkers.

The results of this study help to explain why heavy alcohol drinking has been related to excess mortality in Russia if considered in the context of previous studies exploring causes of high cardiovascular mortality in Russia.[31] Public health researchers and practitioners need to take account of the cardiotoxic effects of heavy drinking in populations, even when the levels of diagnosed frank alcoholic cardiomyopathy may be relatively low.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

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Table S1. Association of high values of hs-cTrT (above 9.34 ng/L) with levels of alcohol use (logistic regression analysis).

	Model 1 (adjusted for age and sex), OR (95% CI)	Model 2 (additionally adjusted for smoking and education), OR (95% CI)	Model 3 (additionally adjusted for other covariates), OR (95% CI)
hsTrT, N = 2595.			
Alcohol use			
Narcology clinic subsample	1.72 (1.22, 2.44)	2.01 (1.36, 2.97)	2.25 (1.5, 3.39)
General population sample, harmful drinkers	0.38 (0.17, 0.84)	0.46 (0.2, 1.03)	0.48 (0.21, 1.1)
General population sample, hazardous drinkers	0.91 (0.68, 1.23)	0.92 (0.68, 1.25)	0.92 (0.67, 1.26)
General population sample, non-problem drinking	1.0 [reference group]	1.0 [reference group]	1.0 [reference group]
General population sample, non-drinkers	1.03 (0.72, 1.47)	0.99 (0.69, 1.43)	0.89 (0.61, 1.3)
P-value for trend test (among drinkers), df=1	0.132	0.0734	0.032
P-value for heterogeneity test, df=4	< 0.001	< 0.001	< 0.001

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Figure S1. The directed acyclic graph (DAG) depicting the suggested causal relationship between heavy alcohol use and heart damage (hs-cTrT serving as a biomarker).

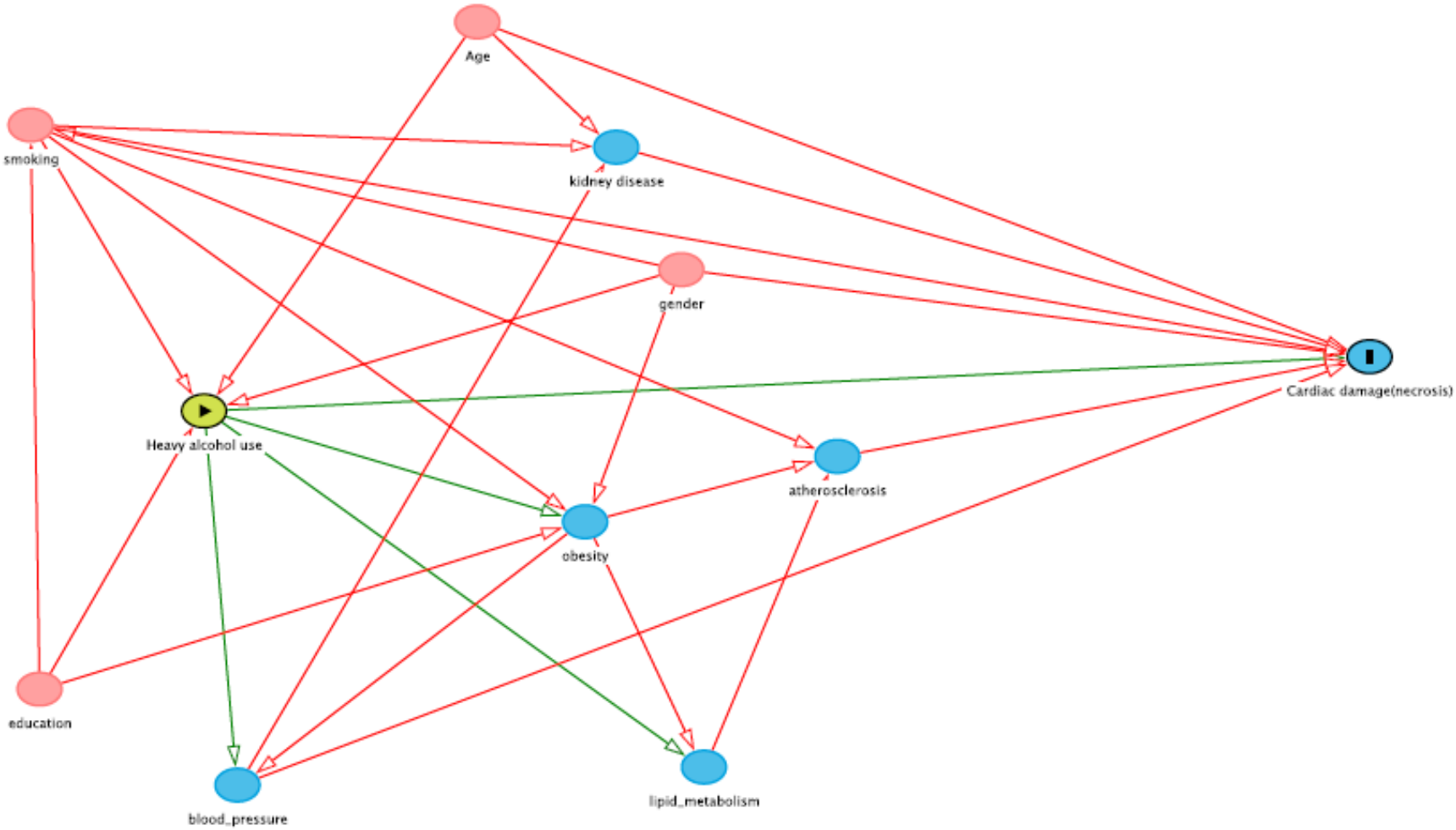

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Figure S2. The directed acyclic graph (DAG) depicting the suggested causal relationship between heavy alcohol use and cardiac wall stretch (NT-proBNP serving as a biomarker).

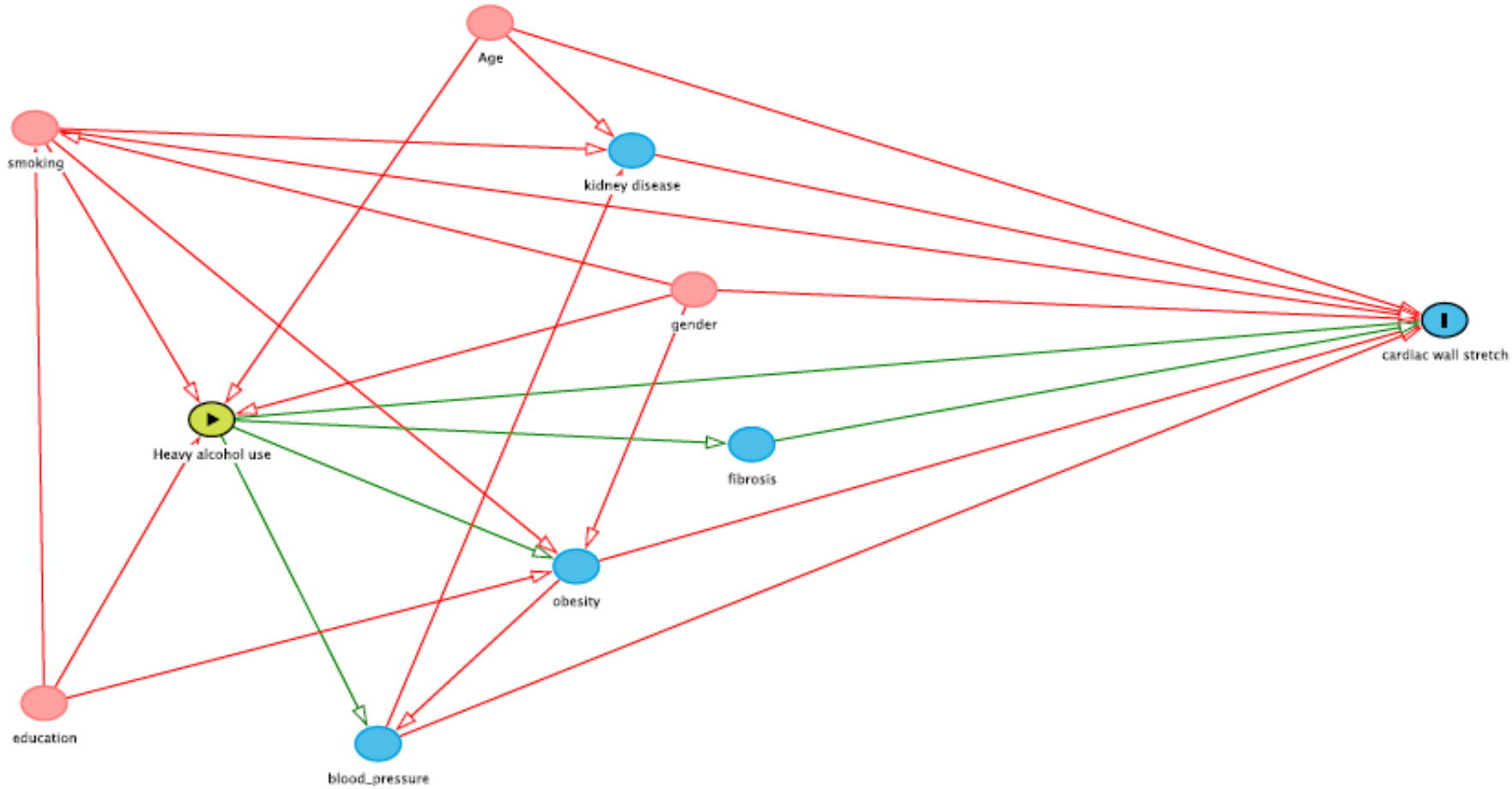

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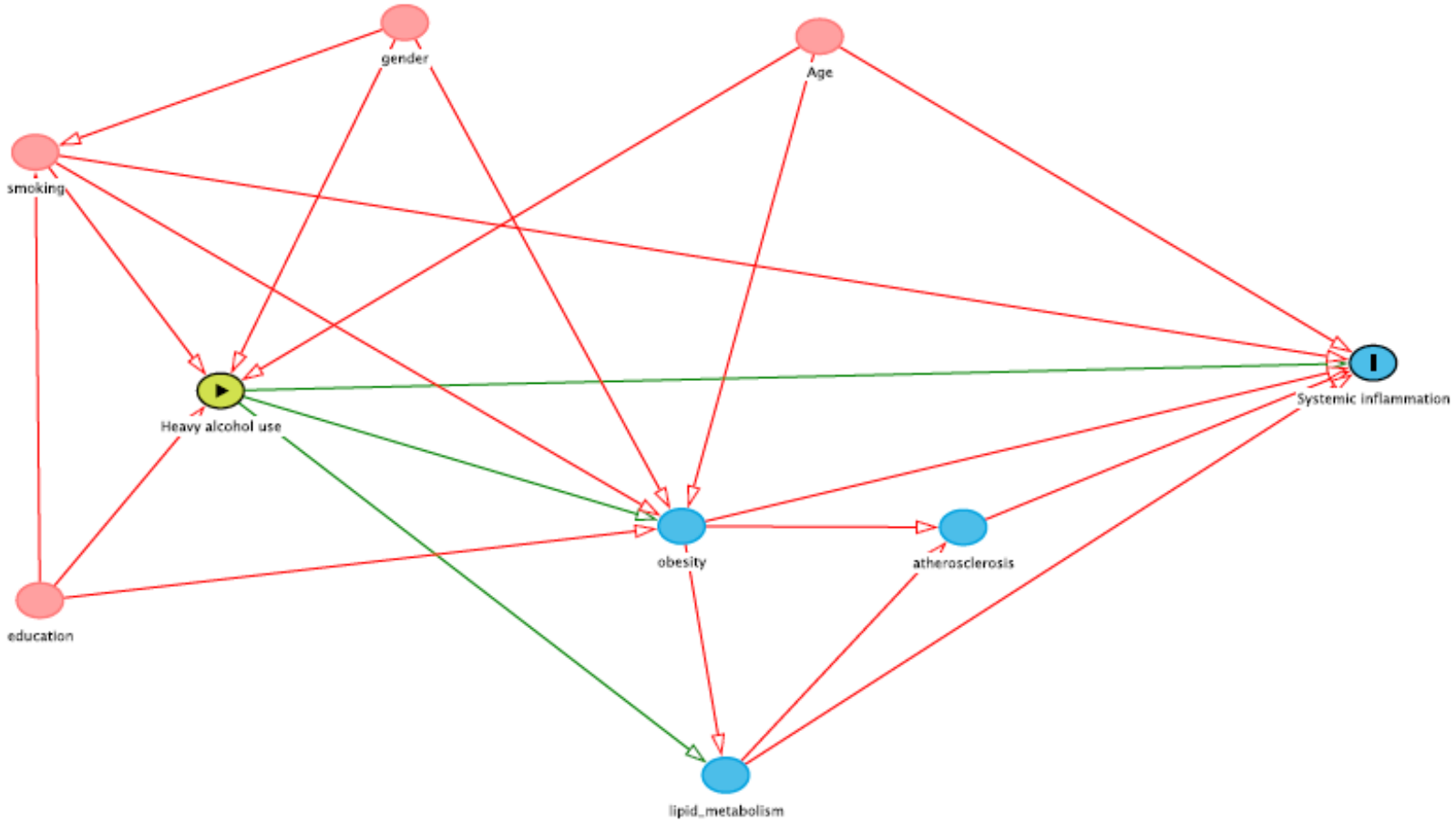
Figure S3. The directed acyclic graph (DAG) depicting the suggested causal relationship between heavy alcohol use and systemic inflammation (hsCRP serving as a biomarker).



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Paper 3

What factors explain the much higher diabetes prevalence in Russia compared to Norway? Major sex-differences in the contribution of adiposity

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Abstract

Background. Compared to many other countries Russia has a high prevalence of diabetes in men and women. However, contrary to what is found in most other populations, risk is greater among women than men. The reasons for this are unclear.

Methods. Prevalence and risk factors for diabetes at ages 40-69 years were compared in two population-based studies: Know Your Heart (KYH) (Russia, 2015-2018, N=4121) and the seventh wave of the Tromsø Study (Tromsø 7) (Norway, 2015-2016, N=17649). Diabetes was defined by level of glycated hemoglobin and/or self-reported diabetes and/or diabetes medication use. Marginal structural models were used to estimate the role of key risk factors for diabetes in differences between the studies.

Results. Age-standardized prevalence of diabetes was higher in KYH compared to Tromsø 7, in men (11.6% vs 6.2%) and in women (13.2% vs 4.3%). Age-adjusted odds ratios for diabetes (KYH/Tromsø 7) were 2.01 (95% CI 1.68, 2.40) for men and 3.66 (3.13, 4.26) for women. Adiposity (body mass index and waist circumference) explained none of this effect for men but explained 46.0% (39.6, 53.8) for women. Addition of smoking and C-reactive protein, as further mediators, slightly increased the percentage explained of the difference between studies to 55.5% (46.5, 66.0) for women but only to 9.9% (-0.6, 20.8) for men.

Conclusions. Adiposity is a key modifiable risk factor that appears to explain half of the almost 3-fold higher female prevalence of diabetes in Russia compared to Norway, but none of the 2-fold male difference.

Key words: Russia, body mass index, obesity, diabetes

Introduction

Diabetes has an independent effect on risk of cardiovascular events (1), and causes long-term microvascular complications (2, 3). The disease is heterogeneous in nature and progression and is broadly classified into Type 1 diabetes and Type 2 diabetes (4). Type 2 diabetes is strongly associated with obesity and related lifestyle factors and is the most common type in adults (4).

Population aging and the world-wide rise of obesity have contributed to the marked rise in Type 2 diabetes prevalence in many countries, although there remains substantial international variation (5). Data on Type 2 diabetes prevalence in Russia have been reported in a few population-based studies based either on glycated haemoglobin (6-8) or fasting glucose (9-11). These studies report relatively high prevalence of Type 2 diabetes in Russia ranging from 7% or 16% with the highest burden being in women compared to men at older ages. The notably higher prevalence in women compared to men is atypical compared to many other countries (4, 5). The high prevalence of diabetes in Russia compared to the neighbouring countries in Western Europe is of particular interest because this may contribute to the very high levels of cardiovascular disease (CVD) in Russia (12). However, no systematic attempt has been made previously to investigate which risk factors may explain the relatively high prevalence of diabetes in Russia compared to elsewhere.

At early stages symptoms of Type 2 diabetes are absent or remain unnoticed, therefore a substantial proportion of Type 2 diabetes remain undiagnosed and not managed (13). Previous studies in Russia reported a very high proportion of undiagnosed diabetes (up to 54%) (8). This can lead to delay in management of the condition and health care interventions directed to reduce cardiometabolic risk factors like hypercholesterolemia, hypertension, hypoglycaemia, obesity.

In this study we aim to investigate whether high diabetes prevalence in Russia compared to Norway is explained by known risk factors of diabetes. We use data collected in two recent cross-sectional population-based studies conducted Russia and Norway that defined diabetes in a comparable manner. These countries share a border and have similar population age structure.

Methods

Study populations

We used data on men and women aged 40-69 years who took part in two population-based studies. The Know Your Heart (KYH) study (14) is a cross-sectional study conducted in Russia in 2015-2017. The seventh wave of Tromsø study (Tromsø 7) (15) was conducted among the residents of the municipality of Tromsø (Norway) in 2015-2016.

Know Your Heart (Russia). A random population-based sample of 35 – 69 year old participants (n=5071) stratified by age, sex and district was recruited in Arkhangelsk and Novosibirsk cities (Russia). Trained interviewers visited the sampled addresses and recorded information about resident's health, socio-demographic characteristics and lifestyle (51% of approached agreed to participate). Participants were then invited to take part in a health check that usually occurred 1-2 weeks later in a research clinic and 4512 (35-69 years old) agreed (89%). Our analysis is based on 4121 participants aged 40-69 years who attended the health check (Supplementary Figure 1). The health check included a medical examination, questionnaire and biological sample collection. The medical examination included blood pressure measurements, and recording of weight and height. The questionnaire collected data on health problems, life style, and medication use. The blood samples were non-fasting, but participants were asked not to eat and drink for 4 hours before the health check. Within two hours after venipuncture, blood samples were centrifuged, and serum was frozen at -80C. Frozen samples were shipped to a laboratory in Moscow and analyzed in a single batch at the end of the fieldwork. Further details of the study design have been published elsewhere (14).

Tromsø 7 (Norway). All inhabitants of the municipality of Tromsø aged 40 years and above were invited to take part in Tromsø 7 and 21083 participated (65%), of whom 17649 aged 40-69 years were included in our analysis (Supplementary Figure 2). All participants completed questionnaires and examinations including biological sampling. The questionnaire included broad set of questions on life style, medication use, and disease. Blood samples (non-fasting) were processed immediately after collection and the laboratory assays of the biomarkers were done the same day at the Department of Laboratory Medicine, University Hospital of Northern Norway (ISO certification NS-EN ISO 15189:2012). Further details of the design of the Tromsø study have been published elsewhere (15).

Outcomes

The outcome of the study is the prevalence of diabetes mellitus defined as HbA1c $\geq$ 6.5% (48 mmol/mol) (16), and/or self-reported diabetes and/or use of medication for diabetes (Supplementary Table 1). Use of medication for diabetes was collected based on answer to the question about diabetes medication use and/or drugs taken during last four weeks coded with code A10 (antidiabetics) of Anatomical Therapeutic Chemical (ATC) classification (17). In KYH, diabetes self-report was determined from the question "Have you ever been told by a doctor or nurse that you have diabetes mellitus?". In Tromsø 7, participants who answered that they currently have diabetes on the question "Have you or have you ever had diabetes?" were recorded as having diabetes. A person with HbA1c $\geq$ 6.5% (48 mmol/mol) was considered undiagnosed if did not report having diabetes or taking diabetes medications. Prediabetes was defined as HbA1C $\geq$ 5.7% (39 mmol/mol) and < 6.5% (48 mmol/mol) among those who did not report that they have diabetes or take diabetes medications (16).

HbA1c was measured in a whole blood by immuno-turbidimetric test on AU 680 Chemistry System (Beckman Coulter) in KYH and by Capillary electrophoresis on Capillarys 3 tera with the MCA laboratory HbA1c calibrator traceable to the International Federation of

Clinical Chemistry (IFCC) Reference Measurement in Tromsø 7. A calibration study between the two laboratories was conducted and HbA1C levels were adjusted appropriately to make them directly comparable (18).

We were not able to distinguish between Type 1 and Type 2 diabetes in this study as consistent and comparable data on age at onset and other distinctive characteristics of these two conditions were not available.

Exposure variables

Adiposity was assessed using body mass index (BMI) and waist circumference (WC). WC was measured at the narrowest part of the trunk (KYH) or at the umbilical level (Tromsø 7). To ensure comparability, WC in Tromsø 7 was converted to the narrowest waist using a conversion equation (19). Mean systolic and diastolic blood pressure was calculated as the mean of the second and third measurements. Smoking was categorized as ex-, never-smokers and current smokers (1–10 cigarettes day, 11–20/day, >20/day). Total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and high sensitivity C-reactive protein (hsCRP) were measured (14, 18). A calibration study of the laboratory biomarkers was conducted to harmonize measurements between studies (18).

Ethical approval

Ethical approval for the study was received from the ethics committees of the London School of Hygiene & Tropical Medicine (approval number 8808, 24.02.2015), Novosibirsk State Medical University (approval number 75, 21/05/2015), the Institute of Preventative Medicine (no approval number; 26/12/2014), Novosibirsk and the Northern State Medical University, Arkhangelsk (approval number 01/01-15, 27/01/2015). The Regional Committee for Research Ethics approved Tromsø 7 (REC North ref. 2014/940). Both studies have

conformed to the principles embodied in the Declaration of Helsinki. All participants gave signed consent.

Statistical analysis

Sex-specific prevalence of diabetes, prediabetes, and undiagnosed diabetes in the two studies were compared after age-standardization to the Standard European Population 2013. We calculated age-adjusted means and prevalences of cardiometabolic characteristics in men and women with diabetes and without diabetes separately in KYH and Tromsø 7.

To examine if the differences in prevalence of diabetes between studies may be explained by different levels of diabetes risk factors we conducted mediation analysis using marginal structural models (20) which allow the decomposition of total effect of exposure into that mediated by specific factors (indirect effect) the remaining (direct) effect. In our analysis, the study (KYH vs Tromsø 7) was considered the exposure, while diabetes risk factors were considered possible mediators. BMI and WC (both in quintiles) were included at the first step, controlling for age (21). A further model introduced smoking and hsCRP as additional mediators, the latter as it reflects proinflammatory status (21, 22).

Statistical analysis was performed using R version 3.6.0 (package medflex 0.6-6) and SAS software 9.4, SAS Institute Inc., Cary, NC, USA.

Sensitivity analysis

Self-report of diabetes diagnosis may be not fully reliable. We therefore conducted a sensitivity analysis for mediation analyses and comparison of CVD risk factor profile where defined diabetes was based on HbA1c values alone.

Results

Age-standardised prevalences of diabetes, prediabetes and undiagnosed diabetes were higher in KYH compared to Tromsø 7 among men and women (Table 1, Supplementary Table 1). The proportion of those with diabetes who were undiagnosed was higher in KYH than in Tromsø 7 both in younger and older age group (Table 2).

Next we compared cardiometabolic risk factor profiles among participants with diabetes and without diabetes in both studies (Table 3). In both studies men and women with diabetes had higher BMI and waist circumference, higher systolic and diastolic blood pressure and higher hsCRP levels than those who don't have diabetes. In KYH levels of total and LDL-cholesterol were similar in participants with and without diabetes, however, in Tromsø 7 total and LDL-cholesterol were lower in participants with diabetes. Smoking prevalence was similar in participants with and without diabetes in both studies. Substantial differences in mean risk factor levels were observed between KYH and Tromsø 7.

The age-adjusted odds of having diabetes in KYH was twice that in Tromsø 7 among men and more than three times higher among women as shown by the size of the total effect in Table 4. Treating BMI and WC and as mediators of the association between study and diabetes prevalence explained 46.0% (39.6, 53.8) of the diabetes differences between KYH and Tromsø 7 among women, but did not appear to explain any of the differences among men. Addition of smoking and hsCRP as mediating factors to the model increased to 55.5% (46.5, 66.0) the fraction of the difference in prevalence between studies in women (Table 4). Among men there was weak evidence of a much smaller percentage being for men: 9.9% (-0.6, 20.8). It was notable that the residual (natural direct effect) effect not mediated by BMI and WC were similar in men and women.

Sensitivity analysis

Sensitivity analysis using a diabetes case definition based solely on HbA1c values did not substantially change the findings of the mediation analysis, or of the comparison of the CVD risk factor profile (data available from authors).

Discussion

In this study we compared the prevalence of diabetes in two population-based studies conducted in Russia and Norway using the same case definitions. We found much higher prevalence of diabetes in KYH (11.6% in men and 13.2% in women) compared to Tromsø 7 (6.2% in men and 4.3% in women). The prevalence of diabetes was higher in women than in men in the Russian sample, which is the opposite of what is observed in Norway and other countries (4, 5). We also found that there is a higher proportion of undiagnosed diabetes in Russia than in Norway with proportions of previously undiagnosed diabetes of 36.9% among men and 26.8% among women in KYH.

We attempted to explain the differences in prevalence of diabetes between the two countries using mediation analysis and found that adiposity measured by BMI and WC could explain up to 46% of the difference in diabetes prevalence between studies in women but did not explain the differences between studies observed in men. Taking further account of smoking and hsCRP as mediation factors increased to 55.5% the percentage differences in diabetes prevalence between studies in women that could be explained.

Our estimates of diabetes prevalence in Russia are in line with previous studies although not all of them are published in the peer review literature or contain sufficient detail on age-specific diabetes prevalence (7, 10, 11, 23). Two recent multi-region studies in Russia reported age-specific prevalence of diabetes and found that women at older ages have higher prevalence of diabetes than men (8, 9). The NATION study (2013-2015) estimated Type 2 diabetes prevalence based on both HbA1c and self-report: 7.0% women versus 7.9% men aged 45–59

years old, and 14.1% women versus 9.9% men aged 60–79 years had diabetes (8) . The ESSE-RF study (10 regions of Russian Federation, 2012-2014) estimated prevalence of diabetes mellitus based on self-report and fasting glucose: 9.4% of men and 7.4% of women aged 45-54 years old, and 13.6% of men and 16.5% of women aged 55-64 years old had diabetes mellitus (9). Similarly to our study, other studies conducted in Russia report that a high proportion of diabetes is undiagnosed: 54% in NATION study (8), 43% in HAPIEE (10), 27% in UEMS (11). Differences between these estimates and estimates from our study can be explained by different age structure of the studied populations, different access to health care services in Russian regions, different methods for diabetes prevalence estimates.

Estimates of the prevalence of diabetes in Norway are available from national registries with prospectively collected data on prescriptions of antidiabetic drugs and diabetes diagnoses from hospitals and primary care visits for all residents in Norway aged from 30 to 89 years. Crude prevalence of Type 2 diabetes increased from 4.9% to 6.1% from 2009 to 2014; and diabetes prevalence was higher in men than women (6.8% vs 5.3% in 2014) (24). However, these estimates do not include undiagnosed diabetes cases that would be detected by screening. Intensive pharmacological and life-style management of diabetes delays onset and slows the progression of diabetes complications (25, 26). Our study has shown that proportion of undiagnosed diabetes is apparently smaller in Norwegian compared to Russian study but is still of significant public health concern given the potential health consequences of unmanaged diabetes (27).

Weight reduction and diet modification interventions in people with impaired glucose tolerance reduced the incidence of diabetes in the randomized controlled trials (28, 29). Therefore, life-style interventions would be beneficial for both persons with clinically defined diabetes and persons with prediabetes (30). In our study prevalence of prediabetes was higher in KYH compared to Tromsø 7 which means there is much potential for diabetes prevention.

Our data do not explain in full why prevalence of diabetes differs in Norway and Russia particularly among men. Our measures of adiposity (BMI and WC) explained a substantial proportion of difference among women (46%), but these factors did not make an important contribution to differences among men. Interestingly, after accounting for adiposity the remaining difference in diabetes prevalence between KYH and Tromsø study was similar for men and women (double the odds of diabetes prevalence). It was previously shown that even among people of European ancestry differences exist in the relationship between body fat and BMI (31). Also, the association of obesity and diabetes was shown to be stronger in low education groups, which suggest that socioeconomic circumstances may influence vulnerability to adiposity (32).

It has been previously demonstrated that smoking is associated with diabetes with a relative risk of 1.4 (adjusted for the baseline BMI) (22, 33). hsCRP reflects the level of general inflammation and is positively associated with obesity and diabetes (21). As prevalence of smoking and hsCRP levels are higher among Russian men compared to men in Norway, we expected them to contribute to some of the difference in diabetes prevalence. However, we did not observe an additional contribution of these factors to explaining the differences in diabetes prevalence when adiposity measures were already included in the model. Among women, smoking and hsCRP made a small additional contribution to difference in diabetes prevalence between studies after accounting for adiposity.

There are other potential explanations of the differences in diabetes prevalence between studies, such as diet (34, 35), levels of physical activity (36), and sedentary behaviour (37). Unfortunately, data on these factors that are comparable between our two studies are currently not available.

Type 2 diabetes is a multifactorial disease and involves genetic, behavioural and environmental factors, and their interaction (38). However, researchers have still a limited

understanding of the genetic and epigenetic contribution to Type 2 diabetes: only 10-15% of heritability can be explained by known genetic variants (39). At the present time we do not have genomic data for both studies in order to investigate any differences between them.

Limitations

The major limitation of the mediation analysis in our study is the cross-sectional nature of the data. People who knew they had diabetes could have attempted to lose weight, increase physical activity, eat a healthier diet, and stop smoking. Beyond this it is likely that our anthropometric measures of adiposity in the two populations failed to adequately capture differences in the extent of visceral abdominal adiposity which is particularly strongly related to diabetes risk (40).

Although we were not able to distinguish between Type 1 diabetes and Type 2 diabetes in our study, our results will be principally driven by Type 2 diabetes because it constitutes between 90%-95% of all diabetes in these populations (4).

Finally, care must be taking before generalizing the study findings to the populations of Russia and Norway as a whole. Firstly, the studies were conducted in three cities whose characteristics will differ in some respects from the national populations. In addition there is the uncertainty about whether the participants we studied were representative of their own cities' populations. The Tromsø 7 study had a good response rate (65%) as did the study in Arkhangelsk (68%), although in Novosibirsk the response rate was low (41%) (14). The participants who did not attend the health check in KYH study were likely to have more adverse risk factor profile than those who did (Supplementary Tables 2 and 3). However it is notable that our estimates of diabetes prevalence from KYH are consistent with those of other population-based studies in Russia. Similarly, prevalence estimates for diabetes in Tromsø 7 are similar to the study reporting diabetes prevalence in the whole of Norway.

Conclusions

The major differences in diabetes prevalence between Russia and Norway have important implications for health services in Russia and could contribute to the differences in CVD mortality between the two countries. Adiposity indices, smoking and CRP only partially explained the differences in diabetes prevalence between studies in women and did not explain differences between diabetes prevalence in men. A substantial proportion of unexplained differences remained and requires further investigation. People with undiagnosed diabetes are not prescribed recommended glucose-, blood pressure- and lipid lowering drugs, as well as anti-smoking counselling that can be expected to reduce risk of CVD and other complications of diabetes. The proportion of undiagnosed diabetes in Russia is alarmingly high given potential health consequences for individuals and subsequent burden from avoidable complications of diabetes on the health care system.

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Data availability. Requests to access the data set of KYH study from bona fide researchers may be sent to the International Project on Cardiovascular Disease in Russia (41). Application for access to Tromsø Study data can be sent to email: tromsous@uit.no

Conflict of interest. The authors declare that there are no conflict of interest that might bias, or be perceived to bias, their work.

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Tables

Table 1. Numbers of people and age-standardized prevalence [a] of diabetes, undiagnosed diabetes, prediabetes in Know Your Heart and Tromsø 7 by sex

	Know Your Heart, % (95% CI) [b]	**Tromsø 7, % (95% CI) [b]**
Men (N)	1732	8349
Diabetes cases (N) [c]	219	514
Number of undiagnosed diabetes cases (N) [d]	76	94
Number of prediabetes cases (N) [e]	612	1875
Diabetes mellitus prevalence [c]	11.6% (10.3, 12.8)	6.2% (5.6, 6.7)
Undiagnosed diabetes [d]	4.04% (3.4, 4.7)	1.15% (0.9, 1.4)
Prevalence of prediabetes [e, f]	35.3% (33.2, 37.4)	22.7% (21.8, 23.6)
Women (N)	2389	9300
Diabetes cases (N) [c]	361	395
Number of undiagnosed diabetes cases (N) [d]	91	48
Number of prediabetes cases (N) [e]	824	2055
Diabetes mellitus prevalence [c]	13.3% (12.3, 14.2)	4.3% (3.8, 4.8)
Undiagnosed diabetes [d]	3.5% (3.1, 3.9)	0.5% (0.3, 0.8)
Prevalence of prediabetes [e, f]	35.1% (33.4, 36.9)	22.6% (21.7, 23.4)

[a] Age-standardized to the Standard European Population 2013, $p<0.001$ for all comparisons.

[b] All percentages are based on total number of participants in KYH and Tromsø 7

[c] Diabetes defined as HbA1C ≥ 6.5% (48 mmol/mol) and/or self-reported diabetes and/or use of medication with ATC-code A10 (antidiabetics) according to the Anatomical Therapeutic Chemical (ATC) classification

[d] Undiagnosed diabetes defined as HbA1c ≥ 6.5% (48 mmol/mol), no self-reported diabetes and no diabetes medication use

[e] Prediabetes defined as HbA1C ≥ 5.7% (39 mmol/mol) and <6.5% (48 mmol/mol), no self-reported diabetes and no diabetes medication use

[f] missing data KYH: HbA1c – 124; diabetes medication: 423; Tromsø 7: self-report of diabetes - 413; HbA1c – 212;

Table 2. Proportion of undiagnosed diabetes [a] among participants with measured HbA1c by sex and 15-year age-groups in Tromsø 7 and Know Your Heart

	Know Your Heart		**Tromsø 7**	
	Diabetes [a,b] (N)	Undiagnosed diabetes [a,c] (N, % of diabetes cases)	Diabetes (N)	Undiagnosed diabetes (N, % of diabetes cases)
Men				
Total sample	207	76 (36.9 %)	507	94 (18.5 %)
40-54 years old	55	23 (38.3 %)	191	38 (19.9 %)
55-69 years old	152	58 (37.2 %)	316	56 (17.7 %)
Women				
Total sample	342	91 (26.8 %)	389	48 (12.3 %)
40-54 years old	55	26 (45.6 %)	144	12 (8.3 %)
55-69 years old	287	68 (23.7 %)	245	36 (14.7 %)

[a] Number of diabetes and undiagnosed diabetes cases presented only for participants with complete data on HbA1c

[b] Diabetes among participants with measured HbA1c was defined as HbA1C ≥ 6.5% (48 mmol/mol) and/or self-reported diabetes and/or use of medication with ATC-code A10 (antidiabetics) according to the Anatomical Therapeutic Chemical (ATC) classification

[c] Undiagnosed diabetes among participants with measured HbA1c was defined as HbA1c ≥ 6.5% (48 mmol/mol), no self-reported diabetes and no diabetes medication use

Table 3. Cardiometabolic risk factors and smoking [a] in Know Your Heart and Tromsø 7 stratified by diabetes status and sex.

	KYH		Tromsø 7	
	With diabetes [b]	Without diabetes [b]	With diabetes [b]	Without diabetes [b]
Men (N)	219	1513	514	7835
BMI (kg/m2)	32.1 (31.3, 32.9)	27.2 (27.0, 27.4)	30.4 (29.9, 30.9)	27.8 (27.7, 27.8)
Waist (cm)	109.0 (107.1, 111.0)	95.4 (94.9, 96.0)	105.7 (104.4, 106.9)	97.9 (97.7, 98.1)
Total cholesterol (mean, mmol/L)	5.34 (5.17, 5.52)	5.27 (5.22, 5.33)	4.97 (4.85, 5.08)	5.49 (5.47, 5.51)
HDL-cholesterol (mean, mmol/L)	1.17 (1.11, 1.23)	1.35 (1.33, 1.37)	1.23 (1.2, 1.27)	1.38 (1.38, 1.39)
LDL- cholesterol (mean, mmol/L)	3.50 (3.34, 3.66)	3.46 (3.41, 3.51)	3.16 (3.06, 3.26)	3.73 (3.7, 3.75)
Ln-transformed triglycerides, (mean, mmol/L)	0.69 (0.6, 0.78)	0.34 (0.32, 0.37)	0.65 (0.59, 0.71)	0.41 (0.4, 0.42)
Ln-transformed CRP, (mean, mmol/L)	1.00 (0.84, 1.16)	0.29 (0.24, 0.34)	0.48 (0.38, 0.58)	0.04 (0.02, 0.06)
SBP (mean, mmHg)	142.1 (139.1, 145.1)	137.3 (136.4, 138.2)	135.7 (133.9, 137.5)	130.7 (130.3, 131.0)
DBP (mean, mmHg)	87.1 (85.4, 88.8)	86.4 (85.8, 86.9)	78.9 (77.9, 79.9)	78.8 (78.6, 79.0)
Current smoker (proportion)	0.33 (0.26, 0.4)	0.38 (0.36, 0.41)	0.19 (0.16, 0.23)	0.20 (0.19, 0.21)
Women (N)	361	2028	395	8905
BMI (kg/m2)	33.1 (32.3, 33.9)	28.2 (28.0, 28.4)	30.8 (30.0, 31.6)	26.6 (26.5, 26.7)
Waist (cm)	102.1 (100.3, 103.9)	88.5 (88.0, 89.0)	93.2 (91.5, 94.9)	81.7 (81.5, 82.0)
Total cholesterol (mean, mmol/L)	5.56 (5.39, 5.73)	5.51 (5.46, 5.55)	5.34 (5.18, 5.5)	5.55 (5.52, 5.57)
HDL-cholesterol (mean, mmol/L)	1.41 (1.35, 1.46)	1.63 (1.61, 1.65)	1.42 (1.37, 1.47)	1.74 (1.73, 1.75)
LDL- cholesterol (mean, mmol/L)	3.55 (3.4, 3.7)	3.57 (3.53, 3.61)	3.43 (3.29, 3.57)	3.57 (3.55, 3.59)
Ln-transformed triglycerides, (mean, mmol/L)	0.65 (0.58, 0.73)	0.22 (0.2, 0.24)	0.63 (0.56, 0.7)	0.14 (0.13, 0.15)
Ln-transformed CRP, (mean, mmol/L)	1.00 (0.86, 1.14)	0.26 (0.21, 0.3)	0.79 (0.66, 0.91)	0.00 (-0.02, 0.02)
SBP (mean, mmHg)	137.7 (134.8, 140.5)	127.6 (126.8, 128.4)	135.6 (133.0, 138.2)	123.2 (122.8, 123.5)
DBP (mean, mmHg)	83.2 (81.8, 84.6)	80.9 (80.5, 81.4)	74.3 (73.1, 75.6)	72.6 (72.4, 72.8)
Current smoker (proportion)	0.13 (0.09, 0.18)	0.17 (0.15, 0.19)	0.19 (0.15, 0.25)	0.19 (0.18, 0.2)

[a] Adjusted for age

[b] Diabetes defined as HbA1C ≥ 6.5% (48 mmol/mol) and/or self-reported diabetes and/or use of medication with ATC-code A10 (antidiabetics) according to the Anatomical Therapeutic Chemical (ATC) classification

Table 4. Odds ratios [a] showing natural direct and indirect effects [c] of study (KYH vs Tromsø 7) on diabetes [b] prevalence assessed from mediation analyses and mediated percentage for different sets of risk factors (BMI, waist circumference, smoking, hsCRP) by sex

	Model 1 BMI and waist circumference included as mediators	Model 2 BMI, waist circumference, smoking, hsCRP included as mediators
Men		
Natural direct effect	2.02 (1.70, 2.40)	1.87 (1.57, 2.24)
Natural indirect effect	0.99 (0.95, 1.04)	1.07 (0.99, 1.16)
Total effect	2.01 (1.68, 2.40)	2.01 (1.69, 2.38)
Percentage mediated	-1.1% (-8.9, 5.5)	9.9% (-0.6, 20.8)
Women		
Natural direct effect	1.99 (1.70, 2.35)	1.77 (1.49, 2.11)
Natural indirect effect	1.81 (1.68, 1.94)	2.04 (1.85, 2.26)
Total effect	3.66 (3.13, 4.26)	3.62 (3.10, 4.21)
Percentage mediated	46.0% (39.6, 53.8)	55.5% (46.5, 66.0)

[a] Adjusted for age

[b] Diabetes defined as HbA1C ≥ 6.5% (48 mmol/mol) and/or self-reported diabetes and/or use of medication with ATC-code A10 (antidiabetics) according to the Anatomical Therapeutic Chemical (ATC) classification

[c] Total effect of exposure is decomposed into natural direct and indirect effect. Natural indirect effect means effect of exposure that is mediated by specific set of risk factors. Natural direct effect is the remaining effect of an exposure after quantifying the natural indirect effect. In our analysis, the study (KYH vs Tromsø 7) was considered the exposure, while diabetes risk factors were considered possible mediators.

Supplementary Table 1. Number of cases of diabetes mellitus in KYH and Tromsø 7 according to different criteria of case definition.

	KYH (N, %)[a]	Tromsø 7 (N)[a]
Men		
HbA1c ≥ 6.5% (48 mmol/mol)	168 (76.7)	404 (78.6)
Self-report of diabetes	141 (64.4)	393 (76.5)
Use of medication for diabetes	91 (41.6)	352 (68.5)
Any of the above (total diabetes cases)	219	514
Women		
HbA1c ≥ 6.5% (48 mmol/mol)	229 (63.4)	264 (66.8)
Self-report of diabetes	255 (70.6)	311 (78.7)
Use of medication for diabetes	189 (52.3)	272 (68.9)
Any of the above (total diabetes cases)	361	395

[a] Percent of total diabetes cases

Supplementary Table 2. Differences in main study variables for KYH participants (general population sample) who attended the health check and who did not, women* (N=2619).

	Attended the health check (N=2385)	Did not attend the health check (N=234)	P-value
Age (years), mean (sd), min-max	55.9 (8.7) 40-69	54.7 (9.0) 40-69	0.047
City			<0.0001
Arkhangelsk, % (N)	96.3 (1237)	3.7 (48)	
Novosibirsk, % (N)	86.1 (1148)	13.9 (186)	
Married, % (N)	51.1 (1218)	41.9 (99)	0.007
Education less than college level, % (N)	21.3 (519)	31.2 (72)	0.001
In regular paid work, % (N)	58.5 (1325)	49.6 (124)	0.028
Not enough money for food or clothes, % (N)	22.2 (534)	26.4 (59)	0.154
Depression severity (PHQ-9)† ≥ 5, % (N)	41.3 (986)	37.4 (87)	0.251
Anxiety severity (GAD-7)† ≥ 5, % (N)	26.8 (640)	23.0 (54)	0.21
Drinker, % (N)	81.9 (1936)	72.4 (170)	0.001
CAGE† score total ≥ 2, % (N)	3.7 (101)	7.1 (20)	0.008
Current smoker, % (N)	15.1 (383)	24.7 (63)	<0.0001
Blood pressure medication, % (N)	36.1 (917)	31.9 (76)	0.239
Lipid lowering medication, % (N)	14.7 (433)	10.7 (30)	0.092
Self-reported hypertension, % (N)	53.4 (1269)	52.9 (117)	0.904
Self-reported myocardial infarction, % (N)	3.2 (113)	2.7 (9)	0.682
Self-reported heart failure, % (N)	15.0 (395)	10.9 (27)	0.087
Self-reported stroke, % (N)	2.8 (81)	4.5 (12)	0.116
Self-reported diabetes, % (N)	7.6 (244)	8.7 (25)	0.547
Visited general practitioner more than once in the last 12 month, % (N)	39.1 (942)	28.2 (65)	0.001
Was hospitalized at least once in the last 12 month, % (N)	15.1 (366)	14.6 (34)	0.848

*adjusted for age

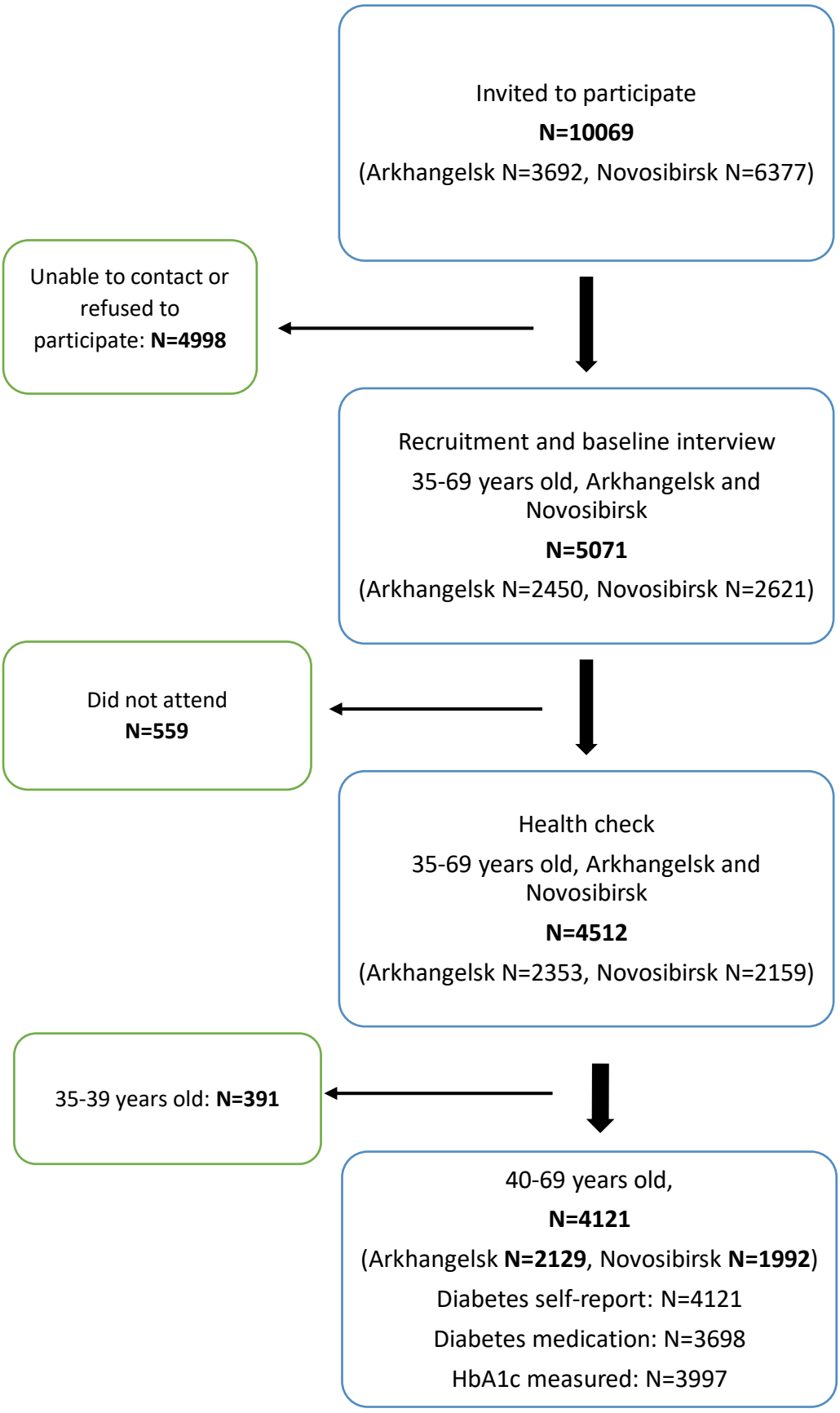
†PHQ-9: Patient Health Questionnaire – 9; GAD-7: General Anxiety Disorder – 7; CAGE: "cut-annoyed-guilty-eye", screening tool for alcohol-related problems.

Supplementary Table 3. Differences in main study variables for KYH participants who attended the health check and who did not, men* (N=1982).

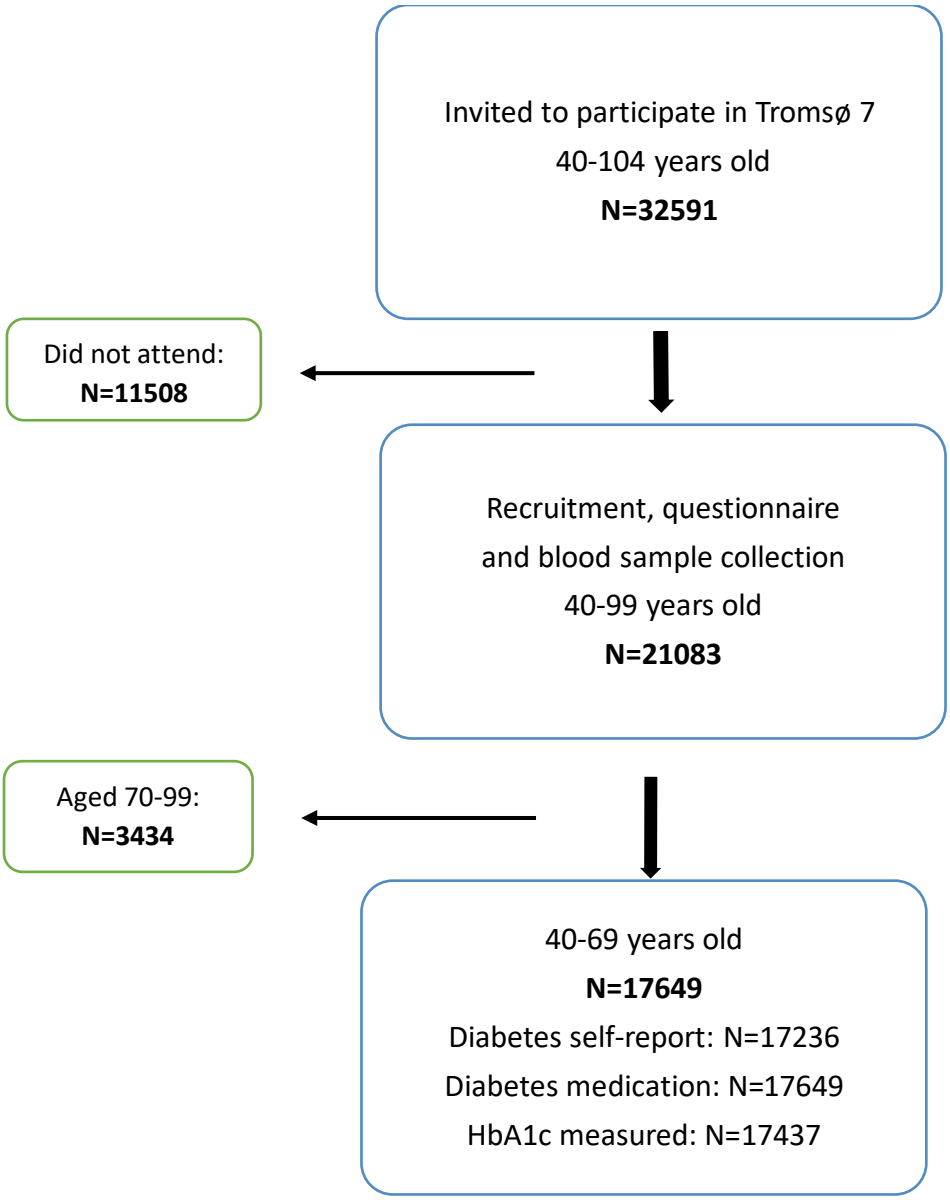
	Attended the health check (N=1731)	Did not attend the health check (N=251)	P-value
Age (years), mean (sd), min-max	56.1 (8.5) 40-69	56.5 (8.7) 40-69	0.513
City			<0.0001
Arkhangelsk, % (N)	95.9 (888)	4.1 (38)	
Novosibirsk, % (N)	79.8 (843)	20.2 (213)	
Married, % (N)	78.3 (1351)	67.7 (170)	<0.0001
Education less than college level, % (N)	29.8 (519)	47.9 (121)	<0.0001
In regular paid work, % (N)	61.6 (1027)	49.7 (122)	0.001
Not enough money for food or clothes, % (N)	17.4 (301)	26.2 (66)	0.001
Depression severity (PHQ-9*)>=10, % (N)	26.0 (451)	28.6 (72)	0.387
Anxiety severity (GAD-7)>=5, % (N)	14.8 (257)	15.2 (38)	0.893
Drinker, % (N)	84.6 (1459)	79.5 (198)	0.037
CAGE score total>=2, % (N)	20.1 (352)	31.9 (80)	<0.0001
Current smoker, % (N)	36.3 (631)	62.0 (155)	<0.0001
Blood pressure medication, % (N)	31.4 (567)	18.3 (51)	<0.0001
Lipid lowering medication, % (N)	11.6 (218)	6.3 (18)	0.01
Self-reported Hypertension, % (N)	49.7 (860)	35.6 (92)	<0.0001
Self-reported Myocardial Infarction, % (N)	7.7 (156)	6.6 (20)	0.493
Self-reported heart failure, % (N)	9.9 (205)	11.0 (34)	0.549
Self-reported stroke (self-report), % (N)	3.7 (83)	5.1 (17)	0.231
Self-reported diabetes, % (N)	7.2 (134)	5.1 (14)	0.196
Visited general practitioner more than once in the last 12 month, % (N)	26.4 (465)	16.4 (43)	0.001
Was hospitalized at least once in the last 12 month, % (N)	16.1 (289)	14.8 (39)	0.577

*adjusted for age

†PHQ-9: Patient Health Questionnaire – 9; GAD-7: General Anxiety Disorder – 7; CAGE: "cut-annoyed-guilty-eye", screening tool for alcohol-related problems.



Supplementary Figure 1: Flow chart for Know Your Heart study



Supplementary Figure 2. Flow chart for Tromsø 7

Appendix

Supplementary Table 1. Differences in main study variables for Tromsø 6 participants who participated in Tromsø 7 and those who did not (among invited), women* (N=6880).

	Tromsø 7 participants (N=4776)	Tromsø 7 non-participants (N=2104)	P-value
Age (years), mean (sd), min-max	61.9 (14.5) 32-87	55.6 (11.3) 32-87	<0.0001
Total cholesterol (mmol/L), mean (sd)	5.74 (1.08)	5.63 (1.18)	<0.0001
HDL-cholesterol (mmol/L), mean (sd)	1.66 (0.42)	1.62 (0.46)	<0.0001
LDL-cholesterol (mmol/L), mean (sd)	3.59 (0.94)	3.48 (1.02)	<0.0001
Triglyserids, (mmol/L), GM	1.22 (0.48)	1.26 (0.48)	0.016
CRP, (mmol/L), GM	1.32 (0.98)	1.48 (1.03)	<0.0001
HbA1c (%), GM	5.56 (0.09)	5.61 (0.11)	<0.0001
Estimated glomerular filtration rate (ml/min/1.73m2), mean	92.0 (13.7)	91.6 (18.1)	0.264
BMI, mean (sd)	26.5 (4.56)	26.7 (4.93)	0.46
SBP, mean (sd)	134 (23.3)	135 (27.5)	0.009
DBP, mean (sd)	74.9 (9.95)	75.1 (10.8)	0.451
Drinker, % (N)	89.8 (4218)	87.2 (1550)	<0.0001
Binging at least once a month, % (N)	3.2 (195)	3.9 (97)	0.001
Education less than college level, % (N)	64.5 (2841)	67.6 (1496)	<0.0001
Current daily smoker, % (N)	17.9 (897)	22.7 (545)	<0.0001
Married, % (N)	58.2 (2786)	51.8 (947)	<0.0001
Blood pressure medication, % (N)	18.2 (906)	18.9 (648)	0.21
Lipid lowering medication, % (N)	9.7 (506)	9.6 (353)	0.662
Diabetes, % (N)	5.0 (238)	6.3 (227)	<0.0001
Angina (self-report), % (N)	1.8 (118)	2.1 (144)	0.049
Stroke (self-report), % (N)	1.3 (67)	1.7 (83)	0.002
Heart attack (self-report), % (N)	1.4 (83)	1.7 (116)	0.005

*adjusted for age

Supplementary Table 2. Differences in main study variables for Tromsø 6 participants who participated in Tromsø 7 and those who did not (among invited), men* (N=6016).

	Tromsø 7 participants (N=4130)	Tromsø 7 non-participants (N=1886)	P-value
Age (years), mean (sd), min-max	60.1 (13.6), 32-87	56.1 (11.1) 32-87	<0.0001
Total cholesterol (mmol/L), mean (sd)	5.54 (1.05)	5.44 (1.09)	0.001
HDL-cholesterol (mmol/L), mean (sd)	1.37 (0.37)	1.33 (0.40)	0.001
LDL-cholesterol (mmol/L), mean (sd)	3.59 (0.92)	3.49 (0.95)	<0.0001
Triglyserids, (mmol/L), GM	1.44 (0.53)	1.48 (0.51)	0.064
CRP, (mmol/L), GM	1.38 (0.92)	1.53 (1.01)	<0.0001
HbA1c (%), GM	5.64 (0.10)	5.71 (0.12)	<0.0001
Estimated glomerular filtration rate (ml/min/1.73m2), mean	92.8 (13.4)	92.4 (17.2)	0.218
BMI, mean (sd)	27.3 (3.63)	27.2 (4.02)	0.271
SBP, mean (sd)	138 (19.4)	138 (22.1)	0.085
DBP, mean (sd)	81.1 (9.99)	81.0 (10.6)	0.579
Drinker, % (N)	94.3 (3859)	92.8 (1637)	<0.0001
Binging at least once a month, % (N)	14.6 (676)	15.5 (294)	0.091
Education less than college level, % (N)	59.4 (2377)	62.1 (1223)	<0.0001
Current daily smoker, % (N)	16.1 (686)	20.7 (468)	<0.0001
Married, % (N)	69.5 (2792)	63.4 (1122)	<0.0001
Blood pressure medication, % (N)	19.0 (808)	20.2 (529)	0.024
Lipid lowering medication, % (N)	13.7 (605)	14.3 (401)	0.192
Diabetes, % (N)	6.6 (281)	7.7 (217)	0.001
Angina (self-report), % (N)	3.5 (182)	4.2 (184)	0.003
Stroke (self-report), % (N)	2.4 (109)	3.0 (103)	0.005
Heart attack (self-report), % (N)	5.0 (249)	5.7 (234)	0.003

*adjusted for age

Supplementary Table 3. Differences in main study variables for KYH participants (general population sample) who attended the health check and who did not, women* (N=2901).

	Attended the health check (N=2621)	Did not attend the health check (N=280)	P-value
Age (years), mean (sd), min-max	54.3 (9.7) 35-69	51.9 (10.5) 35-69	<0.0001
City			<0.0001
Arkhangelsk, % (N)	96.1 (1373)	3.9 (56)	
Novosibirsk, % (N)	84.8 (1248)	15.2 (224)	
Married, % (N)	52.1 (1362)	40.7 (117)	<0.0001
Education less than college level, % (N)	20.5 (551)	31.0 (84)	<0.0001
In regular paid work, % (N)	62.1 (1521)	51.4 (158)	0.004
Not enough money for food or clothes, % (N)	21.1 (563)	26.1 (68)	0.064
Depression severity (PHQ-9)† ≥ 5, % (N)	41.0 (1076)	35.5 (98)	0.078
Anxiety severity (GAD-7)† ≥ 5, % (N)	27.3 (714)	23.4 (66)	0.163
Drinker, % (N)	82.2 (2139)	71.2 (202)	<0.0001
CAGE† score total ≥ 2, % (N)	4.1 (117)	7.3 (25)	0.008
Current smoker, % (N)	15.7 (430)	25.8 (80)	<0.0001
Blood pressure medication, % (N)	31.8 (932)	29.0 (81)	0.38
Lipid lowering medication, % (N)	12.5 (438)	9.2 (31)	0.097
Self-reported hypertension, % (N)	49.6 (1318)	52.3 (133)	0.434
Self-reported myocardial infarction, % (N)	2.6 (113)	2.5 (10)	0.902
Self-reported heart failure, % (N)	13.7 (410)	9.6 (28)	0.056
Self-reported stroke, % (N)	2.4 (82)	3.7 (12)	0.141
Self-reported diabetes, % (N)	7.0 (252)	7.8 (26)	0.578
Visited general practitioner more than once in the last 12 month, % (N)	37.8 (1005)	29.0 (78)	0.005
Was hospitalized at least once in the last 12 month, % (N)	14.7 (392)	14.7 (40)	0.992

*adjusted for age

†PHQ-9: Patient Health Questionnaire – 9; GAD-7: General Anxiety Disorder – 7; CAGE: "cut-annoyed-guilty-eye", screening tool for alcohol-related problems.

Supplementary Table 4. Differences in main study variables for KYH participants who attended the health check and who did not, men* (N=2170).

	Attended the health check (N=1890)	Did not attend the health check (N=280)	P-value
Age (years), mean (sd), min-max	54.6 (9.6) 35-69	54.5 (10.1) 35-69	0.909
City			<0.0001
Arkhangelsk, % (N)	96 (980)	4 (41)	
Novosibirsk, % (N)	79.2 (910)	20.8 (239)	
Married, % (N)	77.9 (1467)	66.7 (186)	<0.0001
Education less than college level, % (N)	28.9 (552)	48.9 (137)	<0.0001
In regular paid work, % (N)	64.4 (1161)	52.2 (144)	<0.0001
Not enough money for food or clothes, % (N)	16.9 (320)	25.1 (70)	0.001
Depression severity (PHQ-9*)>=10, % (N)	26.4 (499)	28.9 (81)	0.372
Anxiety severity (GAD-7)>=5, % (N)	14.8 (280)	17.1 (48)	0.313
Drinker, % (N)	85.0 (1600)	80.1 (223)	0.033
CAGE score total>=2, % (N)	20.7 (396)	32.3 (91)	<0.0001
Current smoker, % (N)	36.8 (697)	63.3 (177)	<0.0001
Blood pressure medication, % (N)	28.5 (579)	16.2 (52)	<0.0001
Lipid lowering medication, % (N)	10.5 (224)	5.5 (18)	0.007
Self-reported Hypertension, % (N)	47.1 (895)	34.4 (99)	<0.0001
Self-reported Myocardial Infarction, % (N)	6.5 (156)	5.5 (20)	0.476
Self-reported heart failure, % (N)	9.5 (215)	10.4 (35)	0.618
Self-reported stroke (self-report), % (N)	3.0 (83)	4.1 (17)	0.236
Self-reported diabetes, % (N)	6.3 (134)	4.7 (15)	0.27
Visited general practitioner more than once in the last 12 month, % (N)	25.4 (492)	15.1 (44)	<0.0001
Was hospitalized at least once in the last 12 month, % (N)	15.8 (308)	14.8 (43)	0.682

*adjusted for age

†PHQ-9: Patient Health Questionnaire – 9; GAD-7: General Anxiety Disorder – 7; CAGE: “cut-annoyed-guilty-eye”, screening tool for alcohol-related problems.

Links to the Tromsø Study invitation letters, questionnaires and informed consent forms

Information brochure: https://uit.no/Content/467891/brosjyre.troms%C3%B87.pdf

Questionnaire 1: https://uit.no/Content/507611/Q1%20Troms%C3%B8%207.pdf

Questionnaire 2:

https://uit.no/Content/701797/cache=20202909120726/Q2.troms%C3%B87.webside.oppdatert.sept2020.pdf

Informed consent form:

https://uit.no/Content/575211/cache=20180805144729/Samtykke.den7.Tromsoundersokelsen.pdf

Links to Know Your Heart invitation letters, questionnaires and informed consent forms

https://wellcomeopenresearch.org/articles/3-67 Supplementary material.