UNIVERSITY OF COPENHAGEN
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LIPIDS AND LIPOPROTEINS IN MORBIDITY AND MORTALITY

- with special emphasis on the nonfasting state, lipoprotein(a), and cardiovascular disease

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Assessment committee:
Professor Gunnar Gislason, University of Copenhagen (chair)
Professor Kjetil Retterstøl, University of Oslo (1st opponent)
Professor Henrik Kjærulf Jensen, Aarhus University (2nd opponent)

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Langsted A, Nordestgaard BG. Nonfasting lipids, lipoproteins, and apolipoproteins in individuals with and without diabetes: 58 434 individuals from the Copenhagen General Population Study. *Clin Chem.* 2011;57:482-9.(2)

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Preface

I started at the Department of Clinical Biochemistry, Herlev and Gentofte Hospital as a medical student back in 2007 with a part-time job as an examiner at the Copenhagen General Population Study. After a while Børge Nordestgaard came to me and asked if I had ever considered doing research and he convinced me to take ½ year off from my studies to do research under the supervision of him and Jacob Freiberg. This was the start of my passion for research. After finishing medical school, I continued as a PhD-student at the department and was later employed as a registrar, partly interrupted by employment at (many) different clinical departments.

I owe all my academic advancement to Børge Nordestgaard who keeps pushing my limits of achievement and expects nothing less than my absolute best. Without his constant encouragements I would not have written this thesis and I would never have reached this stage of my research career. Also, I wish to thank the Heads of the Department Niels Fogh-Andersen, Agnete Møller Sørensen, Pia R. Kamstrup, and Kristina Rasmussen for creating a work-environment open for research opportunities. I also learned a lot from Anne Tybjærg-Hansen and the group at Rigshospitalet and I am grateful to Stig E. Bojesen and Shoaib Afzal for their always helpful attitudes and patience. I would like to thank the laboratory technicians at the DNA research team, who have done a lot of the hard work for me. Last, but not least I am very grateful to Signe Vedel-Krogh and Camilla Kobylecki for their friendship and support, they make clinical biochemistry so much more fun.

Finally, I wish to thank my family for their support over the years, most importantly my partner Jacob and our fantastic children Storm, Vilde, Bjørk, and Sif.

New Zealand, March 2022

Scope

The overall scope of the present thesis was to gain better insight into how lipids and lipoproteins influence risk of morbidity and mortality with a special emphasis on the nonfasting state, lipoprotein(a), and cardiovascular disease. All studies included were carried out using large prospective cohorts of the Danish general population.

Although highly connected, two main areas of lipid research are presented separately in the present thesis, that is, the nonfasting state and lipoprotein(a) - both related to cardiovascular disease. The former has influenced lipid profile testing for patients worldwide, while the latter has helped improve understanding of lipoprotein(a) as a causal risk factor for cardiovascular disease. The "nonfasting" part of the thesis is focused on lipids, lipoproteins, and apolipoproteins and how they respond to normal food intake and if a nonfasting lipid profile can be used for cardiovascular risk prediction (Papers I, II, and III). The "lipoprotein(a)" part focuses on morbidity and mortality associated with high or low lipoprotein(a) levels (Papers IV, V, VI, VII, and VIII). The eight included studies have different designs where the three early papers related to nonfasting lipid profiling for cardiovascular disease risk all were observational epidemiological studies (Papers I, II, and III). In Paper IV we included both observational and genetic analyses to examine the role of lipoprotein(a) in familial hypercholesterolemia and in Paper V the role of lipoprotein(a) in a *PCKS9* loss of function mutation. In Papers VI, VII, and VIII we included both observational and genetic, Mendelian randomization designs to investigate the causal associations of lipoprotein(a) levels with major bleeding, all-cause mortality, and overall morbidity, respectively.

In the present thesis, the first part describes the methods used in the papers: the study populations, the registries used to ascertain endpoints, the Mendelian randomization design, and potential biases. The second part gives a brief overview of the basis of Papers I, II, and III regarding fasting versus

nonfasting lipid and lipoprotein levels for cardiovascular risk prediction, pathophysiology of lipids and lipoproteins, changes in lipids and lipoproteins after food intake, nonfasting samples and predictive value, and history of implementing nonfasting lipid profiling. In the third part, the focus is on lipoprotein(a) in atherosclerosis, familial hypercholesterolemia, a proprotein convertase subtilisin/kexin type 9 (*PCSK9)* loss of function mutation, and in mortality and morbidity, with some final comments on potential therapies. Finally, I end the thesis with perspectives, conclusions, and future research areas. I hope you will enjoy reading my thesis.

Introduction

15 years ago most guidelines on cardiovascular risk prediction from societies throughout the world recommended to measure lipids and lipoproteins in individuals fasting (9-11) as this was seen as the gold standard in clinical practice. At that time, this seemed reasonable as little data was available to contradict this procedure. The reasoning was that this had been the standard for many years due to less variation in lipids and lipoproteins in the fasting versus nonfasting state. Other arguments for having individuals fast included that clinical cut-off levels were determined based on fasting samples and as a result a set of nonfasting cut-off levels might confuse clinicians, and another argument was the effects of fat-tolerance tests on plasma triglycerides resulting in misleading calculations of low-density lipoprotein (LDL) cholesterol (as in most laboratories LDL cholesterol is calculated by the Friedewald formula that includes triglycerides) (Figure 1).

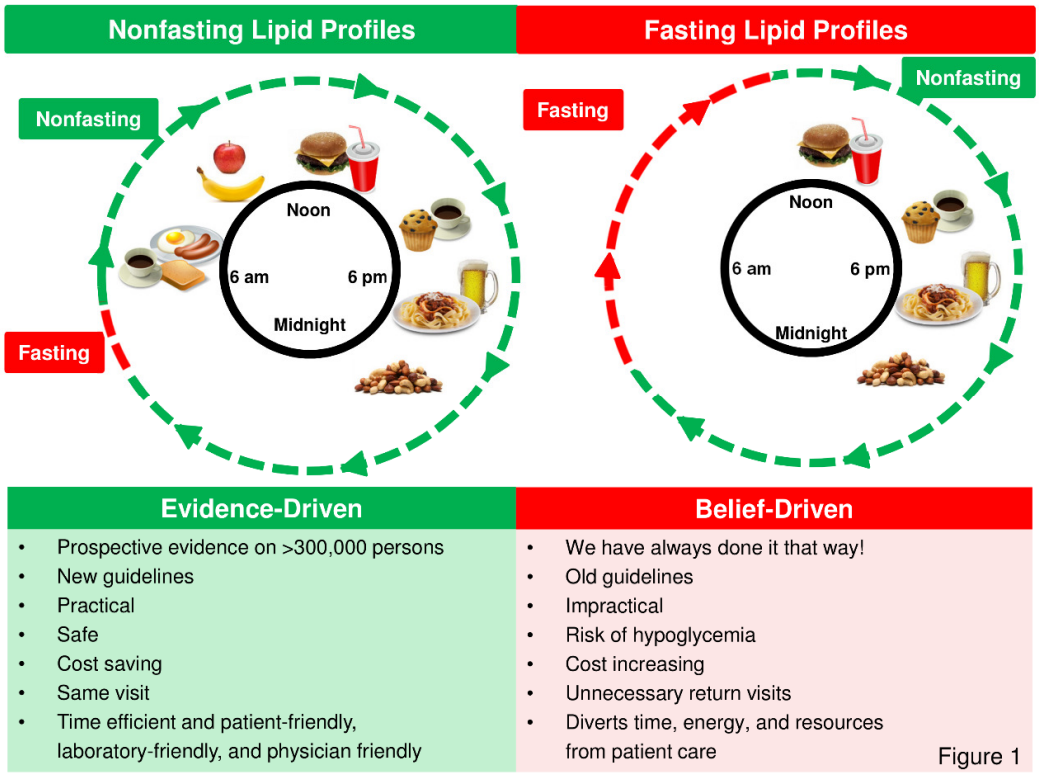

Figure 1. *Arguments for and against the use of fasting or nonfasting lipid profiles. From: Langsted A, Nordestgaard BG. Pathology. 2019;51(2):131-41.(11)*

Today, numerous medical societies all over the world recommend a nonfasting lipid profile for cardiovascular risk prediction because nonfasting measurements have several advantages. First, it would simplify blood sampling for the individual patient by not experiencing the inconvenience of fasting. Second, it would be convenient for the laboratory and the general practitioner by not having to reschedule appointments if the patient is nonfasting. Third, as most people eat regularly during the day they are mainly in the non-fasting state and a lipid profile in this state will more accurately reflect the lipids that the arterial wall is exposed to. Forth, importantly elimination of the risk of hypoglycemia during fasting for individuals with diabetes will make blood sampling safer.

When taking a lipid blood sample for cardiovascular risk prediction, clinicians have mainly focused on total and LDL cholesterol; however, studies have shown that for some patients with low levels of cholesterol, residual risk remains and other lipoproteins have recently brought on attention. Lipoprotein(a) was first discovered more than 50 years ago in 1963 by the Norwegian Kåre Berg(12). Lipoprotein(a) is an LDL-like particle with an apolipoprotein(a) part attached by a disulfide bond(13, 14). Apolipoprotein(a) resembles plasminogen due to its kringle structures and whereas plasminogen contains kringles I, II, III, IV, and V, apolipoprotein(a) in lipoprotein(a) only contains kringles IV and V. The kringle of main interest is kringle IV (KIV) as this varies greatly in number because of the variation of the subtype 2 (KIV2) domain that can have from 2 and up to more than 40 copies. The number of repeats is strongly and inversely correlated with plasma lipoprotein(a) levels. Several relatively recent developments have resulted in an enormous increase of interest in lipoprotein(a) spanning from the discovery of the *LPA* gene coding for apolipoprotein(a) in the late 1980ies(15, 16), over large studies showing that increased levels of lipoprotein(a) were both observationally and genetically associated with high risk of cardiovascular disease from 2009(17-19) including causal associations with myocardial infarction, aortic valve stenosis, and atherosclerotic stenosis(17-23), to consideration of the clinical importance of

lipoprotein(a) in cardiovascular disease in major guidelines and medical societies starting in 2010(24). Additionally, promising lipoprotein(a) lowering drugs that lowers apolipoprotein(a) by antisense oligonucleotides or small interfering RNA technology have been tested and seems to lower lipoprotein(a) by up to 95%(25, 26) and have also led to even greater interest than before.

Part I: Methods

In the following, the study populations used in all papers from the present thesis are described and the ascertainment of endpoints with its limitations, the Mendelian Randomization concept and finally, potential biases and limitations are discussed. For a detailed description on statistical analyses and biochemical and DNA analyses please read the individual Papers I through VIII.

Study populations

In all eight papers included in this thesis we studied individuals from the Copenhagen City Heart Study, the Copenhagen General Population Study, or a combination of the two. The two cohorts are similar in the methods used to invite individuals, gathering of personal information, examining participants, and ascertainment of follow-up and endpoints as described later.

The Copenhagen City Heart Study is a prospective cardiovascular cohort study including approximately 24,000 individuals aged 20-100 and includes 4 different examinations. All invitations were sent to individuals living in the greater Copenhagen area determined by their postal address. For the first examination 19,698 individuals were invited in 1976-78 and stratified into 5-years age groups with an emphasis on individuals aged 35 to 70 years. At this first examination there was a response rate of 74%. At the second examination undertaken in 1981-83 all individuals who attended the first examination wer invited and supplemented by 500 women and men at the age of 20 to 24 years. The second examination had a total response rate of 70%. At the third examination undertaken in 1991-94 all individuals from the previous examinations were invited, supplemented by 3000 women and men at the age of 20 to 49 years. The total response rate at the third examination was 61%. Finally, at the fourth examination carried out in 2001-03 all individuals who attended previous examinations were invited, supplemented by an additional 1040 young

individuals aged 20 to 34 years with a total response rate of 50%. At any examination all individuals from the examinations before were invited if they were alive and resided in Denmark regardless of their postal address.

The Copenhagen General Population Study is a cohort study including approximately 110,000 individuals aged 20-100 and was carried out in 2003-2015. The study consisted of white individuals of Danish descent from different municipalities of the great Copenhagen area: 25% of all women and men aged 20-39 were invited and 100% of individuals aged ≥40 were invited with a total response rate of 46%.

Both studies included white Danish individuals of Danish descent, meaning that every invited participant had been born in Denmark, possessed Danish citizenship, and that both their parents had been born in Denmark and possessed Danish citizenship according to the national Danish Central Person Registry; this is true for the Copenhagen General Population Study and the two first examinations of the Copenhagen City Heart Study, while a small number of individuals from other countries living in Denmark also participated in the 3rd and 4th examination of the Copenhagen City Heart Study. Nevertheless, when relevant in genetic studies, individuals not being white of Danish descent were excluded from analyses. In Denmark every citizen is given a unique ten-digit number (the civil registration number) when born or immigrating and by this number individuals aged 20 years or above were randomly invited to participate in the two cohorts. The municipalities included in the cohorts had individuals from both rural and city areas, individuals from poor and rich areas, non-educated and well-educated individuals, individuals with high and low income, among others. Individuals were invited by postal mail and received in the mail a comprehensive questionnaire with questions on medical history, family situation, smoking and alcohol habits, education, work, income, mental health, and physical activity. The examination of participants was performed at a hospital and included among others, measurement of height and weight, blood pressure, pulse,

information on time since last meal, a lung function test, and blood sampling for biochemical and genetic analyses. A trained examiner checked the questionnaire on the day of attendance and asked clarifying questions if needed.

Endpoints

In all studies included in the present thesis endpoint data was derived from different nationwide registries. By using the ten-digit civil registration number, it is possible to identify all individuals living in Denmark in various registries. When admitted to a hospital or in the event of death it is mandatory for the responsible doctor to register the correct diagnosis/es, and these are then automatically collected and stored in the national registries. The registries used in the papers are described below.

The Danish Civil Registration System is the backbone of all registries in Denmark. It was established in 1968, originally because of introducing withholding tax to register every person living in Denmark. Persons living in Denmark are assigned a unique personal number when born or immigrating. The number is made from 10 digits, position 1-2 is the individual's birthday, position 3-4 is the individual's birth month, position 5-6 is the birthyear (without century), and position 7-10 is a serial number, where position 10 if an even number indicates a woman and an uneven number indicates a man. All civil registration numbers are personal and are never re-used. The civil registration number is the key that makes it possible to connect and merge information about an individual across a wide variety of both public and private registries. This registry is updated on a 24-hour basis and is considered 100% correct.

The national Danish Patient Registry was established in 1977 and is a nation-wide registry containing information on activities in Danish hospitals. The registry includes a description of

which diseases a patient suffered from when in contact with the hospital and how these diseases were treated. Further, it contains information on residency, sex, age, date and time of admission, hospital code, nature of the visit, among much more. The registry contains information from both public and private somatic and psychiatric admissions. This registry has changed over time and some of the major changes include: i) from the start of the registry in 1977 only patients who were admitted to hospitals were registered, ii) in 1994 the registration of diseases by the World Health Organization (WHO) International Classification of Diseases (ICD) changed from the 8th edition to the 10th edition in Denmark, and iii) in 1995 outpatients, psychiatric hospital contacts, and emergency room contacts were included. The Patient Registry is primarily used for statistics concerning planning of health resources, monitoring of patient rights, surveillance of incidences of diseases and treatments, and as in this case for health research.

The national Danish Causes of Death Registry gathers information on all deaths in Denmark. When a person dies in Denmark, a medical doctor performs an inquest and fills out a death certificate on causes (by WHO ICD codes), place, time, and other information concerning the death. Since 1871 it has been mandatory to fill out a death certificate in Denmark and in 1970 all deaths from 1943 and onwards were registered electronically in the national Danish Causes of Death Registry.

Validity of registries

A major problem using register diagnoses is the potential bias of misclassification of endpoints, which also applies to our studies. First, a problem can occur if the diagnosis is not registered correct questioning the validity. For the diagnoses of cardiovascular disease used numerous times in the present thesis, several studies have previously been done to investigate the robustness of the registers in Denmark. A study including 148 primary diagnoses of myocardial infarction from ICD-10 code I21 carried out in Aarhus, Denmark found a positive predictive value of 100%(27) and

another study reviewing 950 hospital records from Northern Denmark from 1998 to 2007 found a positive predictive value of 98% for myocardial infarction ICD-10 codes I21-I23(28). Further, a study from 2009 included 1577 individuals from the Danish Diet, Cancer, and Health Study and reviewed all hospital records from patients with registered acute coronary syndrome and found a 66% positive predictive value for acute coronary syndrome, 82% for myocardial infarction, 92% for myocardial infarction if the patient was discharged from a hospital ward, and 28% for unstable angina pectoris(29). Another study examined individuals registered in the Danish sub-study of the WHO MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Project and found the predictive value and sensitivity of myocardial infarction in the national Patient Registry compared to DANMONICA to be 78% and 97%, respectively with corresponding values in the Danish Registry of Causes of Death of 62% and 89%, respectively(30). As for other endpoints used in this thesis such as bleedings, the validity of these diagnoses has not been examined in the Danish registers. Another problem with endpoint ascertainment from registers is if there is different misclassification between controls and cases, as this could also lead to bias. This seems unlikely as we recruited irrespectively of previous history of disease and prospective events are registered independently of participation in our study.

Mendelian randomization

The concept of Mendelian randomization relies on Mendel's Law of Segregation that is, allele-pairs being separated and allocated randomly during meiosis (Figure 2). The Mendelian randomization approach is a form of instrumental variable analysis where the instruments are genetic variants, and the thought is to circumvent confounding without the inclusion of adjustment for confounders, since the allocation of alleles is presumed to be independent of external factors such as lifestyle and environment. The other main problem of conventional observational epidemiology apart from

confounding is reverse causation, which is also automatically circumvented in Mendelian randomization studies as the genome of an individual is stable from meiosis and onwards, meaning that diseases later in life cannot possible influence a person's genome.

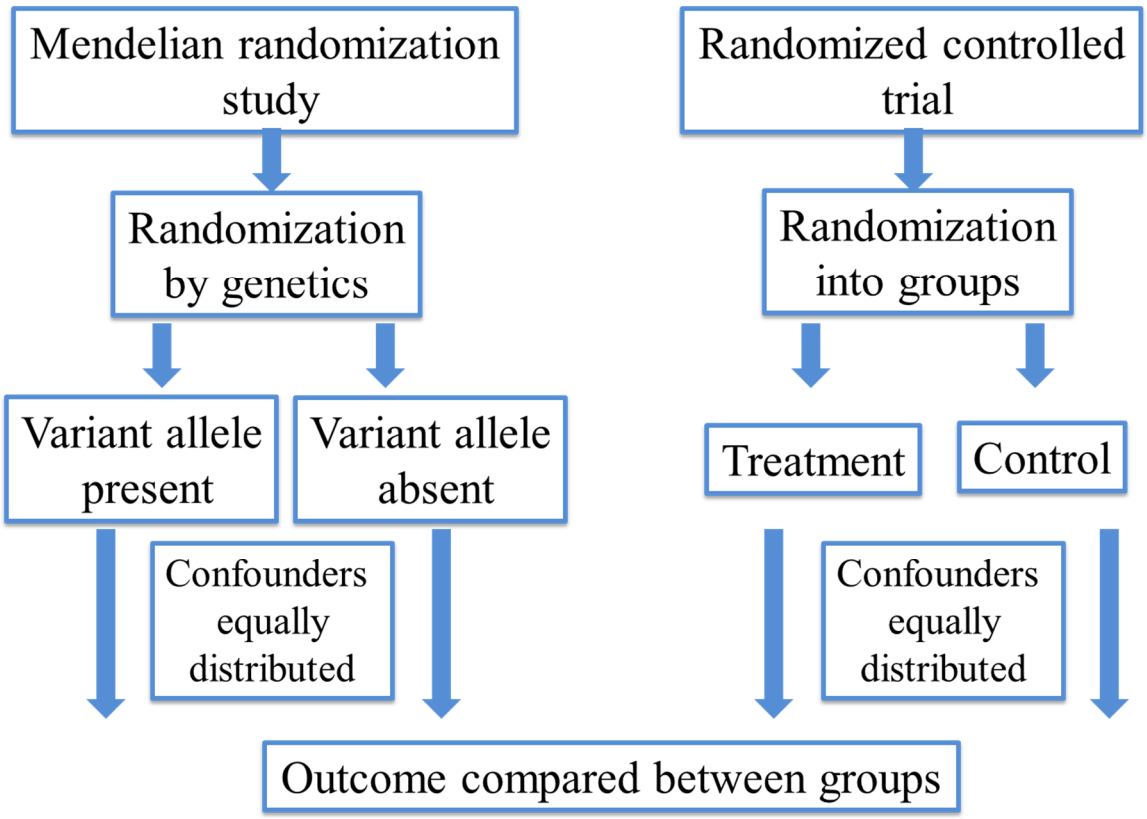

Figure 2. *Flowchart to compare Mendelian randomization studies and randomized controlled trials.*

There are 3 main assumptions when using instrumental variables. First the instrument/genetic variant must be associated with the exposure (=the relevance assumption), second, the instrument/genetic variant must be independent of confounders of the exposure and outcome relation (=the exclusion assumption), and third, the instrument/genetic variant must be associated with the outcome only through the exposure (=the independence assumption). There are several potential possibilities to violate the assumptions in Mendelian randomization studies.

It is necessary to include a strong and reliable genetic instrument to get unbiased estimates, and this can be evaluated by the F-value and R^2 statistics(31). The genetic variants used in the papers of the present thesis have extremely high F value and the variations in lipoprotein(a) levels explained by our instruments are very high, making these excellent instruments for Mendelian randomization studies (Figure 3), whereas weak instruments can result in bias resulting in estimates closer to that of observational studies(32, 33). In fact, the instruments used in the studies in the present papers are among the strongest of any genetic instrument described anywhere in the human genome(34).

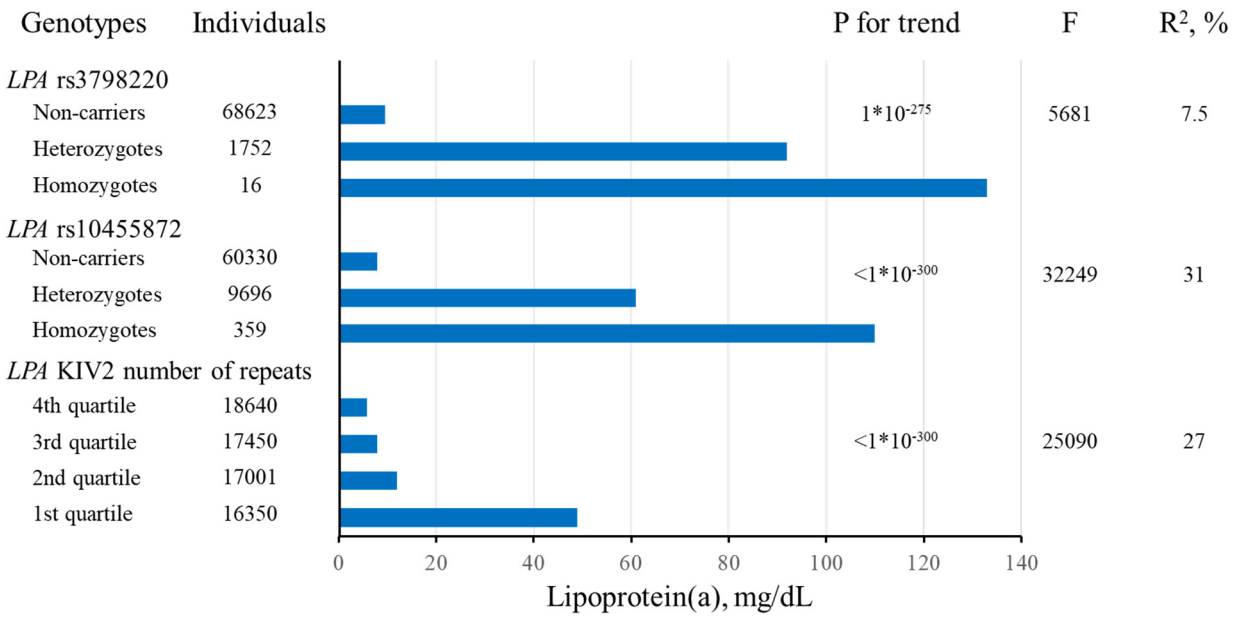

Figure 3. *Plasma lipoprotein(a) according to genotype used as instrumental variables in genetic analyses in this thesis. Blue bars are median levels, P values are for trend across genotypes and quartiles, F values are for the statistical strength of the instrument, and R^2 values are a measure of explained variation. Data from the Copenhagen General Population Study including 109,830 individuals.*

Another way to violate the first assumption is by choosing incorrect genotypes, for example choosing variants from literature where the association of the variant with the exposure may be

inadequately documented. Horizontal pleiotropy can violate the second and the third assumption, and is when the genetic variant affects two or more not related phenotypes, and in Mendelian randomization studies the problem occurs when the chosen genetic variant is associated both with the phenotype in question, but also with another phenotype associated with the outcome(35-37). For example, in our studies if we wanted to examine in a Mendelian randomization study the association of lipoprotein(a) and risk of myocardial infarction and we choose the *LPA* rs10455782 SNP that we know is highly associated with lipoprotein(a) levels. Let's say this genetic variant was also associated with LDL cholesterol levels that we know are causally associated with myocardial infarction, then it would result in pleiotropy. The assumptions could also be violated by population stratification which is the case if the frequencies of the genetic variant and the outcome are both affected by a subgroup of individuals, for example due to ethnicity, leading to random associations(38). In our studies in this thesis we included a homogenous ethnic population of only white Danish individuals to account for population stratification bias.

Causality and potential biases

To establish causality in human medicine, randomized intervention trials are regarded as the "gold standard"; however, Mendelian randomization studies can be used to establish causality by using a genetic variant that influences an exposure rather than an intervention. One advantage of Mendelian randomization compared to randomized trials is the lifelong exposures due to genetics, rather than short term exposures due to medicine. The weakest design for inferring causality is observational studies as these have major problems (beside confounding and reverse causation as described above) with biases such as selection bias due to selection for inclusion or follow-up and information or bias due to errors or differences in measurements between cases and controls. In our studies we invited randomly from the general population, exposures were measured on all randomly selected

individuals, and the outcomes were ascertained from registries on all participants. Exposures and endpoints were unknown to participants, to the medical doctors that recorded the various diagnoses in the nationwide health registries, and to the examiners that recorded subjective and objective data. These facts help minimize the potential biases mentioned above. Another problem is healthy-sampling bias because those not attending the studies relative to the ones that do attend in general would be more sick or unhealthy and have poorer socioeconomic background; however, results of our studies will only be affected if participation would lead to effect modification between the exposure and the outcome. In our study such a scenario does not seem likely as the effect of genetically elevated lipoprotein(a) or fasting status should be substantially different between the attenders and the non-attenders to influence our results. Thus, although this can never be fully excluded, selection and information bias likely were not major problems in our studies.

As mentioned above, other problems in observational studies that are more relevant for the studies included in this thesis include confounding, that is, factors that modify the association between an exposure and an outcome due to the confounder being independently associated with both the exposure and the outcome. Further, reverse causation, that is, the outcome is affecting the exposure and not the other way around is another problem. Importantly, confounding and reverse causation will at most influence our genetic studies and the observational studies on lipoprotein(a) minimally, as the plasma level of this lipoprotein is about 90% genetically determined(14).

Part II: Nonfasting lipid profile

Lipid profile for cardiovascular risk prediction

Elevated plasma cholesterol has for many years been a well-established risk factor for cardiovascular disease (CVD) and several drugs that lower total and LDL cholesterol have been found to lower the risk of CVD. Over time, different lipoproteins have been the focus of clinical research (Figure 4). In the early 1980ies total cholesterol was regarded as the main lipid risk factor for CVD and since no treatment was yet available the first advice was to stick to a diet low on fat and cholesterol. In the late 1980ies plasma triglycerides began receiving attention(39-41). Starting in the mid-1990ies several randomized clinical trials showed that lowering of total or LDL cholesterol indeed lowered the risk of CVD(42-45) and consequently leading to a tremendous clinical interest in lowering of LDL cholesterol, the main fraction of total cholesterol. Furthermore, low HDL cholesterol gained prominence as a CVD risk factor in epidemiological studies in the 1990ies and beginning of 2000(46, 47) leading to development of treatments to increase HDL cholesterol. However, none of these resulted in reduced risk of CVD(48-53), and genetic studies found that HDL cholesterol was not a causal cardiovascular risk factor(54-56) and therefore clinical interest in HDL cholesterol declined. Triglycerides are inversely correlated with HDL cholesterol(57, 58) and recently, there has been renewed interest in triglycerides and remnant cholesterol as risk factors for CVD(59-65). As reviewed later in this thesis, interest in high lipoprotein(a) levels as a risk factor for CVD has increased steadily during the past decade.

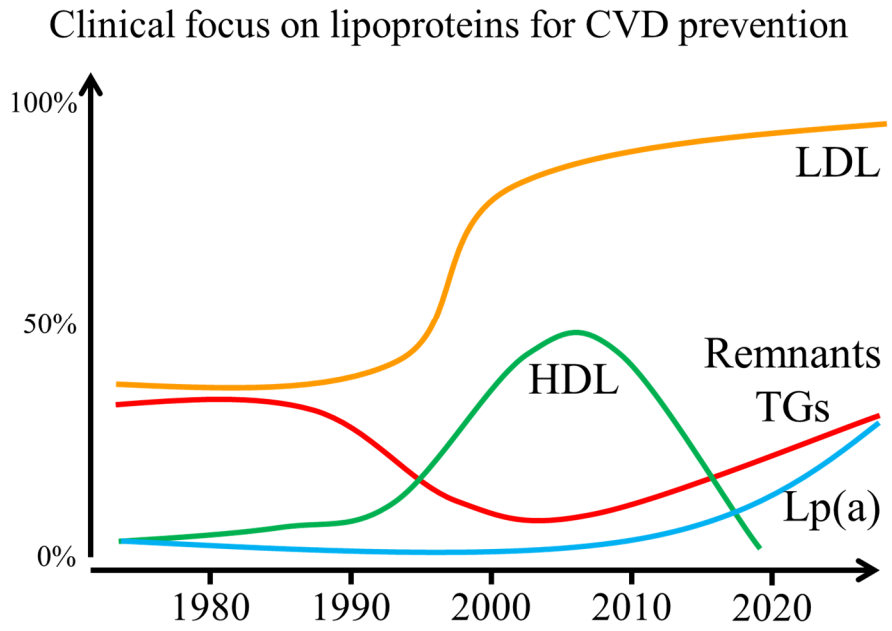

***Figure 4**. A historical perspective of the clinical interest and focus of cardiovascular research on lipids and lipoproteins. CVD = cardiovascular disease. LDL = low-density lipoprotein. HDL = high-density lipoprotein. TGs = Triglycerides. Lp(a) = lipoprotein(a). Adapted from Nordestgaard BG. Circ Res. 2016;118(4):547-63. (66)*

When measuring a lipid profile in the clinical setting used to assess the risk of cardiovascular disease it would normally include total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. More analytes could be added to expand the profile. Remnant cholesterol could be added and is calculated as total plasma cholesterol subtracted LDL cholesterol subtracted HDL cholesterol and it has been found to be an independent additional risk factor(57, 67-70) and can be added without any extra cost. Non-HDL cholesterol could also be added to the standard lipid profile and is calculated as total cholesterol subtracted HDL cholesterol, and thereby includes the cholesterol in all atherogenic lipoproteins. Non-HDL cholesterol has been associated with risk of cardiovascular disease and reducing non-HDL cholesterol has been shown to lower the risk of cardiovascular disease(71-74). Lipoprotein(a) is also an independent risk factor and it has been recommended to measure it once if a person is at intermediate or high risk for CVD, had premature

CVD, suffers from familial hypercholesterolemia, had recurrent CVD or high LDL cholesterol despite high-dose statin treatment(24). Recently, it has also been recommended in Europe, Canada, and India that any person should have lipoprotein(a) measured once in a lifetime, ideally together with the first lipid profile for CVD risk assessment(75-77). Finally, apolipoprotein B is used as a risk marker in some laboratories; however, more standardization is needed(78). Apolipoprotein B is present as a single molecule in all lipoproteins except HDL, and controversies exist on the added value of measuring apolipoprotein B(79-83). Smaller fractions of lipoproteins such as small dense LDL cholesterol have also been associated with risk of ischemic heart disease and myocardial infarction, but these results were often attenuated when adjusting for other lipids, lipoproteins, or triglycerides(84-88) and are not measured in most standard laboratories. The standard classification method of lipoproteins is determination by ultracentrifugation and is based on density, while another method, nuclear magnetic resonance spectroscopy, can provide a more detailed measure of the distribution of cholesterol in the different subclasses of lipoprotein, possibly providing insight into which lipoproteins are most atherogenic(89); however, this is both technically complicated and is most likely not available for standard laboratories in the near future. Other analytes such as apolipoproteins other than apolipoprotein B and yet other fractions of lipoproteins have also over time been suggested as valid risk factors for CVD; however, their clinical use is still scarce(10, 78).

Pathophysiology of lipids and lipoproteins

For decades it has been known that high levels of lipids and lipoproteins cause atherosclerotic cardiovascular disease, but cholesterol and triglycerides also have vital functions in the human body. The vital functions include triglycerides as an energy source for cells in numerous organs, including muscles, they can be stored in fat cells as extra energy, and they are used as insulation of the skin. Cholesterol is used to produce bile acids in the liver to allow intestinal absorption of

dietary lipids and the fat-soluble vitamins A, D, E, and K. Cholesterol is also a precursor for steroid hormones, including sex hormones, and the active form of vitamin D, which in turn regulate the reproductive system and bone homeostasis.

Lipids are insoluble in water and are therefore transported in the blood bound in lipoproteins. Lipoproteins consist of a core containing triglycerides and cholesteryl esters (insoluble) covered by a membrane of apolipoproteins, phospholipids, and cholesterol in the free form(90). Lipoproteins are divided into six main types: chylomicrons, very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), LDL, lipoprotein(a), and HDL and as implied the lipoproteins are separated due to their density. Lipids from the diet are transported from the intestine to the bloodstream as chylomicrons and thereby delivers triglycerides to peripheral tissues (fat and muscle tissue) and delivers a small amount of cholesterol to the liver. The enzyme lipoprotein lipase is present in capillary endothelial cells and metabolizes triglycerides in chylomicrons and then chylomicron remnants can be taken up by the liver. In the liver lipoprotein synthesis results in release of VLDLs that have their triglyceride content hydrolyzed by lipoprotein lipase to form IDLs, then the enzyme hepatic lipase through further triglyceride hydrolysis will convert IDLs to LDLs. The LDL receptor present on liver and other cells then takes up LDL. Hydrolyzed triglycerides can be deposited in adipose tissue, be used in the muscle's energy metabolism, or be transported to other tissues. Outside meals during low availability of glucose, triglycerides are mobilized in adipose tissue and free fatty acids are released for energy.

The development of atherosclerosis begins by injury or disease of the artery, hereby resulting in a fragile endothelium prone to entry of lipoproteins. Beneath the endothelium monocytes are then activated and later transformed into foam cells after uptake of intimal lipoproteins penetrating from the circulating blood in the artery lumen into the intima(91-95). LDL and remnant particles can enter the arterial intima and once trapped in the intima the LDL particles can only be engulfed by

foam cells after modification, whereas modification is not needed for remnant particles(96, 97). The much larger chylomicrons and VLDL particles are not able to enter the intima due to their much larger sizes (Figure 5). Over the years a consistent elevated level of LDL cholesterol and remnant cholesterol will likely result in atherosclerotic progression. As more cholesterol is taken up by foam cells in the intima, the arterial wall will thicken and create an obstruction in the lumen eventually reducing the blood flow. If these plaques rupture and subsequently blood clots formed, they can completely block smaller (or larger) arteries causing no oxygen delivery to the affected areas, resulting in acute conditions such as myocardial infarction or ischemic stroke.

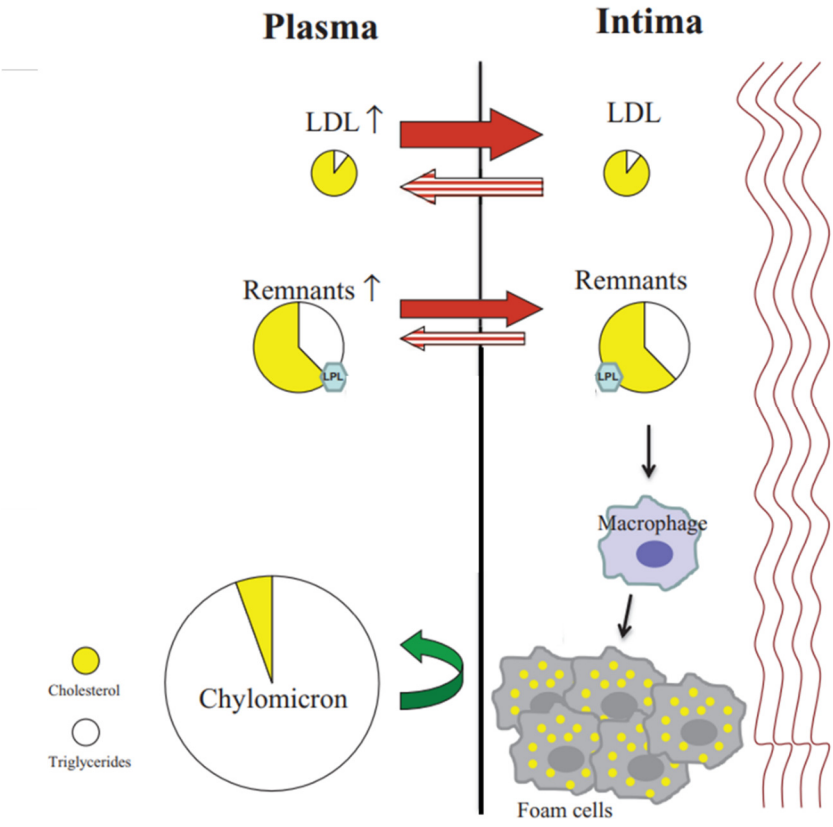

Figure 5. *Overview of the endothelial and how high LDL cholesterol and remnant cholesterol can play a role in the development of atherosclerotic plaques through triglyceride hydrolysis and accumulation of cholesterol in foam cells. LDL = low-density lipoprotein. LPL = lipoprotein lipase. Adapted from Nordestgaard BG. Circ Res. 2016;118(4):547-63. (66)*

In individuals with elevated plasma cholesterol but with triglycerides below 2 mmol/L (<176 mg/dL), called isolated hypercholesterolemia, LDL is the main carrier of atherogenic cholesterol. When both plasma cholesterol and triglycerides are elevated (triglycerides of 2 - 10 mmol/L (176 - 880 mg/dL)), LDL, VLDL, IDL, and chylomicron remnants all carry the atherogenic cholesterol. And finally, when triglycerides are substantially elevated (≥10 mmol/L (≥889 mg/dL)) the atherosclerotic risk is most likely not extreme because much of the cholesterol is carried by chylomicrons and large VLDL unable to enter the intima (Figure 5). However, at high levels of triglycerides there is a high risk of acute pancreatitis(98).

Changes in lipids and lipoproteins after normal food intake

Previously, a lipid profile was recommended to be done in the fasting state and one reason was due to changes seen in fat tolerance tests(99, 100). These tests were done by having participants ingest 1 gram of fat per kilogram body weight and subsequently measure plasma cholesterol and triglycerides at different times after the ingestion. After such a fat load, triglyceride levels increased both in healthy individuals and in patients with previous cardiovascular disease; however, the increases vary greatly among individuals, but peaks for most individuals at three to four hours after fat ingestion and returns to normal after six to eight hours. For obese individuals and/or individuals with diabetes the increase is higher and more prolonged(101, 102). However, in these studies, total cholesterol and LDL cholesterol do not increase significantly after a fat load and importantly, most individuals do not eat such large amounts of fat in a single meal.

In Paper I we included 33,391 individuals from the Copenhagen General Population Study to investigate if levels of lipids, lipoproteins, and apolipoproteins varied significantly after normal food intake(1). The participants showed up for a general health examination and were asked when they had their last meal. Then a blood sample was collected for biochemical analyses. In our study

total cholesterol, LDL cholesterol, and HDL cholesterol were surprisingly all reduced in individuals at zero to five hours following the last food intake when compared to fasting individuals (Figure 6). In contrast, triglycerides did increase up to six hours after last food intake. Non-HDL cholesterol, apolipoprotein A1, and apolipoprotein B did not change significantly after normal food intake.

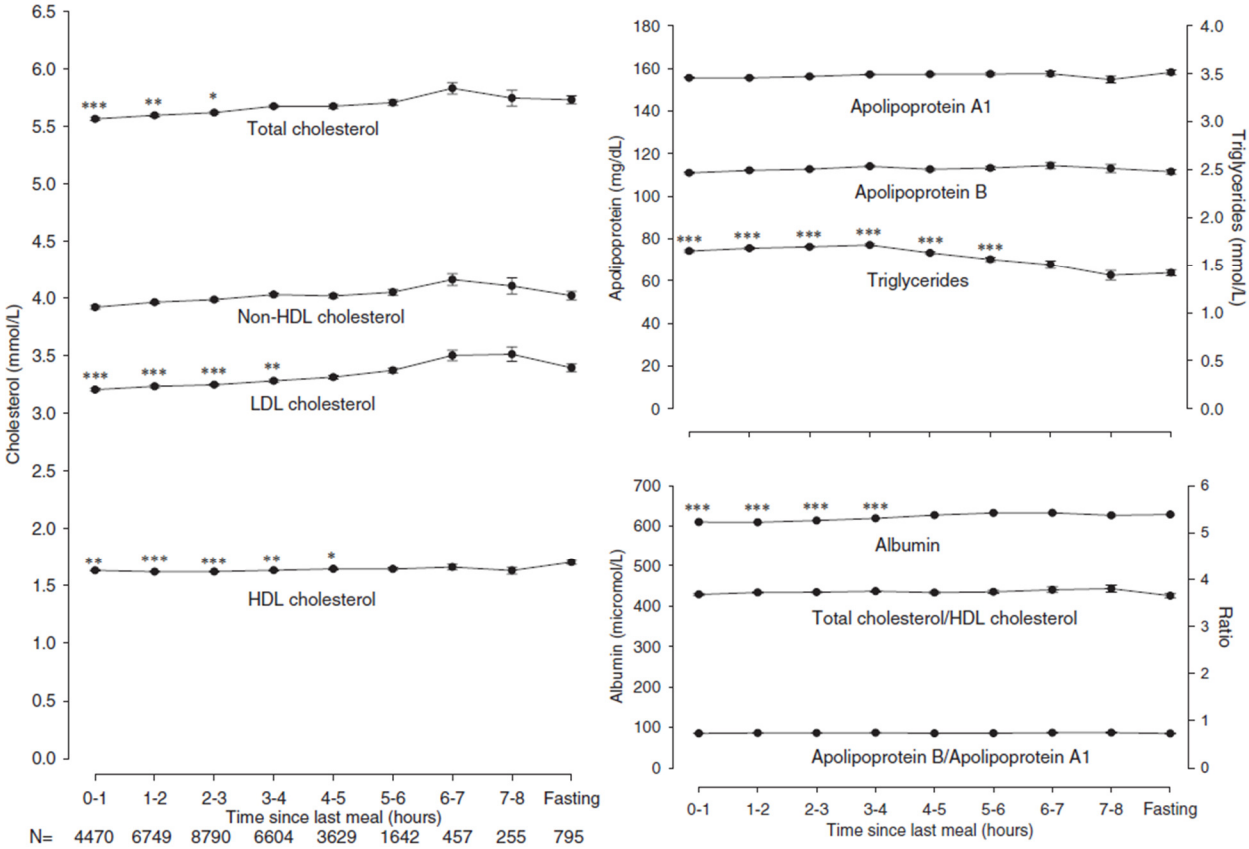

Figure 6. *Levels of lipids, lipoproteins, apolipoproteins, and albumin at hours since the last food intake. All levels were adjusted for sex and age and the unpaired p values were Bonferroni-corrected. * = p < 0.05. ** = p < 0.01. *** = p < 0.001. LDL = low-density lipoprotein. HDL = high-density lipoprotein. From Langsted A et al. Circulation. 2008;118:2047-56.(1)*

To examine if the observed lower levels of total cholesterol and LDL cholesterol could be because of the fact that most individuals drink water or other fluids together with their meal and thereby causing hemodilution, we adjusted cholesterol and triglycerides levels for albumin as this is a good

indicator of hydration status. In our study after adjusting results for albumin levels, total cholesterol and LDL cholesterol did not change after normal food intake(1).

In summary, we only observed small changes in lipids, lipoproteins, and apolipoproteins after normal food intake. Without adjustment for albumin the maximum change for total cholesterol was -0.2 mmol/L (-8 mg/dl) at 0-2 hours since food intake, for LDL cholesterol -0.2 mmol/L (-8mg/dL) at 0-2 hours, for HDL cholesterol -0.1 mmol/L (-4 mg/dL) at 0-5 hours, and for plasma triglycerides +0.3 mmol/L (+26 mg/dL) at 1-4 hours since food intake(9) and these results were similar when adjusting for age and sex or multivariable (Figure 7).

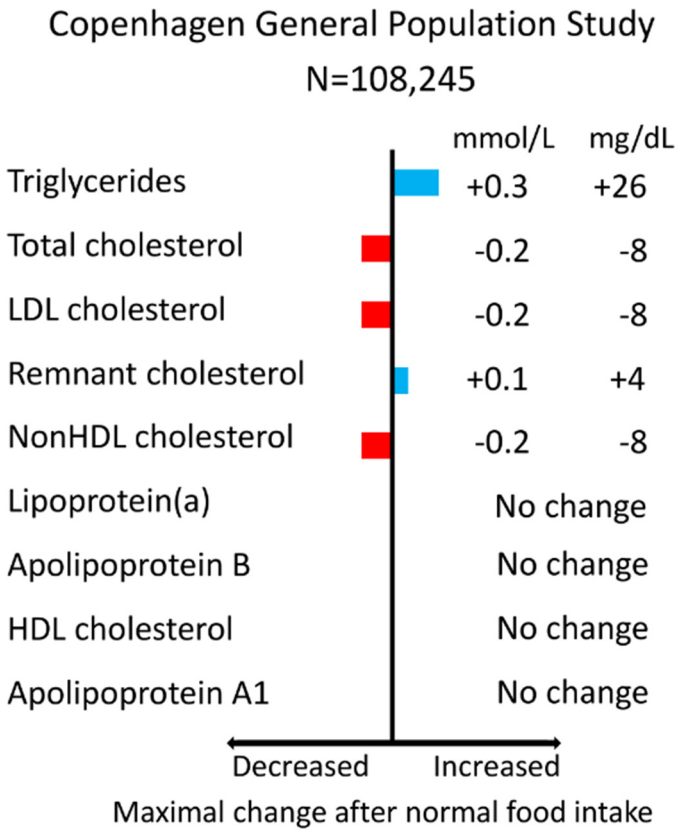

Figure 7*. Maximal changes of lipids, lipoproteins, and apolipoproteins after normal food intake. Based on 92,285 individuals from the Copenhagen General Population Study. Adapted from Nordestgaard, Langsted et al. Eur Heart J. 2016;37(25):1944-58.(9)*

Another large study including 209,180 individuals from the Calgary Laboratory Services in Canada also examined the effect of normal food intake on plasma triglycerides and lipids. They included information on all individuals with a lipid profile in a 6-month period registered in the laboratory

system in Canada including 99% community-based individuals and 1% hospital-based individuals. The maximum change for total cholesterol no change, for LDL cholesterol -0.1 mmol/L (4 mg/dL), for triglycerides was +0.3 mmol/L (+26 mg/dL), and for HDL cholesterol no change(103). In that study when stratifying for sex, still only minimal changes were seen for plasma triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol after normal food intake in both women and men. Further, in another study from the Woman's Health Study including 26,330 female healthcare professionals aged above 44 years and free of cardiovascular disease and cancer, had blood drawn and reported at what time they last ate(104). The maximum change for plasma triglycerides was +0.2 mmol/L (+18 mg/dL), for total cholesterol -0.1 mmol/L (4 mg/dL), for LDL cholesterol -0.2 mmol/L (-8 mg/dL), and for HDL cholesterol no change. Also, in the National Health and Nutrition Survey from the US including 12,744 children aged 3 to 17 years they examined the fluctuations of plasma triglycerides and lipids after normal food intake(105). The maximum change for plasma triglycerides was +0.1 mmol/L (+9 mg/dL), for total cholesterol -0.1 mmol/L (-4 mg/dL), for LDL cholesterol -0.1 mmol/L (-4 mg/dL), and for HDL cholesterol no change.

Summarizing the above 4 large studies with a total of 356,499 individuals from the different general populations, the levels of total cholesterol, LDL cholesterol, and possibly HDL cholesterol were lower up to 4 hours after the last food intake. One likely explanation is, as examined in our study, that the blood is simply diluted due to joint fluid intake and therefore measurements of cholesterol fractions are lower due to hemodilution. All 4 studies found a modest increase in plasma triglycerides for up to 7 hours since the last meal. The increase in plasma triglycerides is partly due to triglyceride-rich chylomicrons containing apoB-48 taken up from the intestine and partly due to VLDL containing apoB-100 synthesized in the liver and continuously being secreted from the liver in response to the delivery of dietary fat from chylomicron remnants.

It is well known that individuals suffering from diabetes are at higher risk of cardiovascular disease than individuals without diabetes, where a factor in the increased risk is due to higher plasma triglyceride levels(106-108). There has been controversies regarding whether individuals with diabetes should have a nonfasting lipid profile for cardiovascular risk prediction, as triglycerides are known to be higher in these individuals and as triglycerides postprandially could be the main culprit in development of atherosclerosis(109-111). In Paper II we included 58.434 individuals in the Copenhagen General Population Study to examine if levels of lipids, lipoproteins, and apolipoproteins were altered differently following normal food intake in individuals with diabetes and without(2). All individuals had a full lipid profile measured at the day of examination and 3.9% (n=2270) of the population had diabetes. Individuals with diabetes compared to those without had higher levels of plasma triglycerides (median levels 1.8 mmol/L versus 1.4 mmol/L), lower levels of HDL cholesterol (1.4 mmol/L versus 1.6 mmol/L), and lower levels of LDL cholesterol (2.4 mmol/L versus 3.2 mmol/L) (Table 1). The lower levels of LDL cholesterol were due to the very high proportion of individuals with versus without diabetes being on statins (52% versus 8%) due to the fact that patients with diabetes in Denmark are closely followed by their General Practitioner and preventive measures are of great attention.

Characteristics	With diabetes	Without diabetes
n	2270	56 164
Men, %	45	44
Age, years	66 (59–73)	57 (47–66)
Total cholesterol, mmol/L	4.9 (4.1–5.6)	5.6 (4.9–6.3)
Non-HDL cholesterol, mmol/L	3.3 (2.6–4.2)	3.9 (3.2–4.7)
LDL cholesterol, mmol/L	2.4 (1.8–3.2)	3.2 (2.6–3.9)
HDL cholesterol, mmol/L	1.4 (1.1–1.7)	1.6 (1.3–2.0)
Triglycerides, mmol/L	1.8 (1.2–2.8)	1.4 (1.0–2.1)
Albumin, μmol/L	595 (561–629)	600 (566–636)
Lipid-lowering therapy, %	52	8

***Table 1**. Characteristics of 2270 individuals with diabetes and 56,164 without in the Copenhagen General Population Study at baseline examination. Adapted from Langsted A et al. Clin Chem. 2011;57:482-9.(2)*

For individuals with diabetes and without diabetes the levels of total, non-HDL, LDL, and HDL cholesterol only changed minimally after normal food intake and in both groups the patterns were similar (Figure 8)(2). Levels of total, non-HDL, and LDL cholesterol were lower up to five hours since the last meal; however, only statistically significant for individuals without diabetes and for LDL cholesterol in individuals with diabetes. For plasma triglycerides the results were similar in the two groups; however, the increase seen up to 6-7 hours following the last meal was significant for individuals without diabetes but not with diabetes, probably due to more statistical power in the first group (56,164 versus 2270 individuals, respectively).

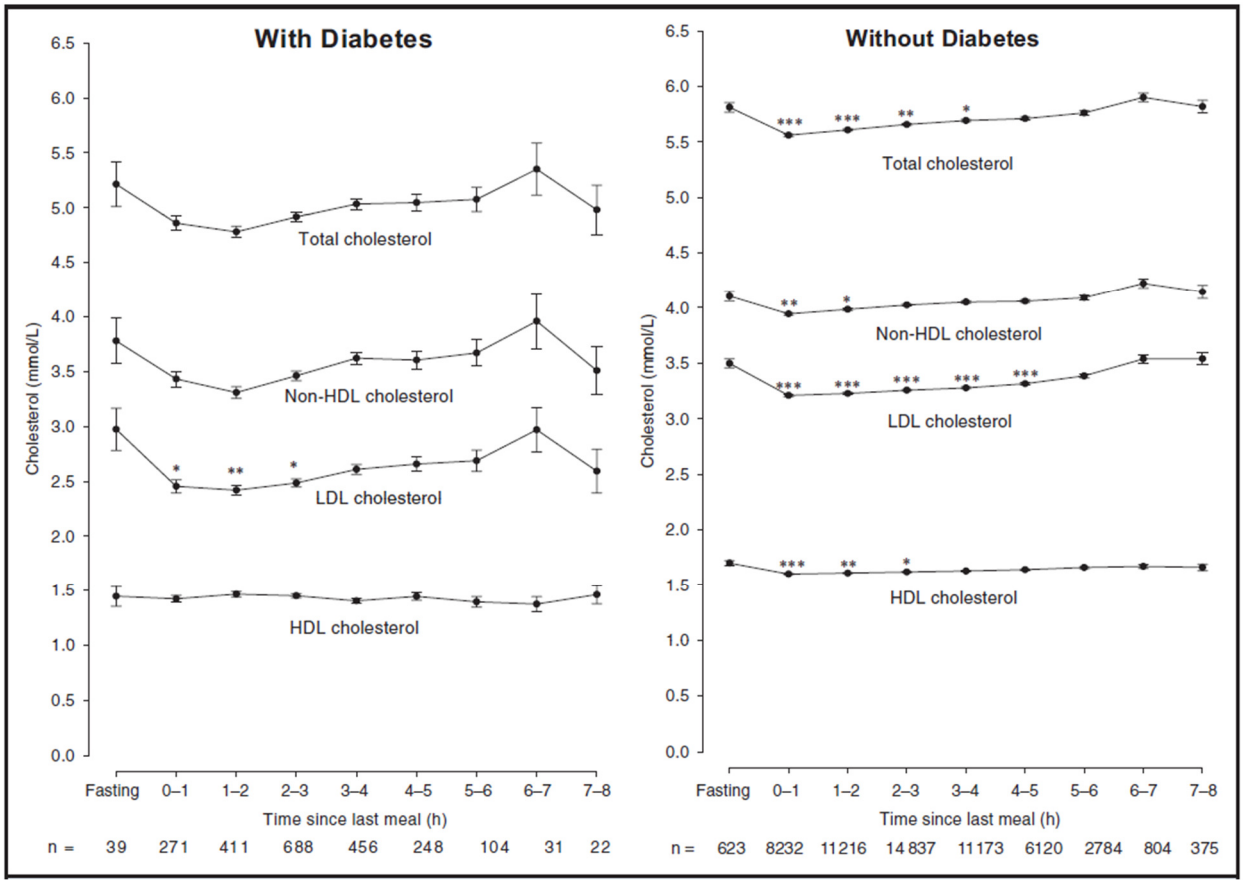

Figure 8. *Levels of lipids and lipoproteins following time since the last meal for 2270 individuals with diabetes and 56,164 individuals without diabetes in the Copenhagen General Population Study. All levels were adjusted for sex and age and p values were Bonferroni-corrected for multiple comparison. * = p < 0.05. ** = p < 0.01. *** = p < 0.001. LDL = low-density lipoprotein. HDL = high-density lipoprotein. From Langsted A et al. Clin Chem. 2011;57:482-9.(2)*

Plasma albumin levels were reduced 5 hours postprandially in all individuals and when adjusting results in Figure 8 for albumin levels there were no longer any differences in levels as a response to normal food intake. This was most likely due to hemodilution as mentioned before as most individuals drink fluids along their meals. However, plasma triglycerides were still modestly increased following normal food intake. The maximum absolute changes were -0.6 mmol/L (-23 mg/dL) and -0.3 mmol/L (-12 mg/dL) for LDL cholesterol and +0.2 mmol/L (+18 mg/dL) and +0.2

mmol/L (+18 mg/dL) for plasma triglycerides for individuals with and without diabetes, respectively.

Predictive value of nonfasting samples

Previous guidelines including cardiovascular risk prediction have recommended measuring lipid profiles when individuals were fasting(10, 44). To be fasting requires not eating or drinking for more than 8 hours, and as most individuals eat regular meals every 4-6 hours with snacks in-between, they are in the non-fasting state in the vast majority of a 24-hour cycle (Figure 1). The arguments as to why testing should be done in the fasting state include the increases in triglycerides seen in historic studies after a fat-tolerance test(112, 113); however, most individuals rarely eat the amount of fat included in a fat tolerance test at a single meal and consequently the increase in plasma triglycerides will in reality be much lower. In a consensus statement from 2016 we included 5538 patients who had both fasting and nonfasting triglycerides and LDL cholesterol measured at Herlev and Gentofte Hospital in the period 2011-2015 and found levels to be similar both overall, stratified by concentrations, and in those with or without diabetes (Figure 9)(9). The median triglyceride level for all 5538 nonfasting samples was 1.41 mmol/L (interquartile range(IQR): 0.96–2.06) (125 mg/dL (85–183)) and for all fasting samples 1.37 mmol/L (0.97–2.04) (121 mg/dL (86–181)). Triglyceride levels in fasting samples were slightly lower than in nonfasting samples when triglycerides were ≤4 mmol/L (≤354 mg/dL) but higher when triglycerides were >4.0 mmol/L (>354 mg/ dL). The median LDL cholesterol level for all 4141 nonfasting samples was 2.6 mmol/L (IQR: 2.0–3.5) (101 mg/dL (77–135)) and for all fasting samples 2.5 mmol/L (1.9–3.3) (97 mg/dL (73–128)).

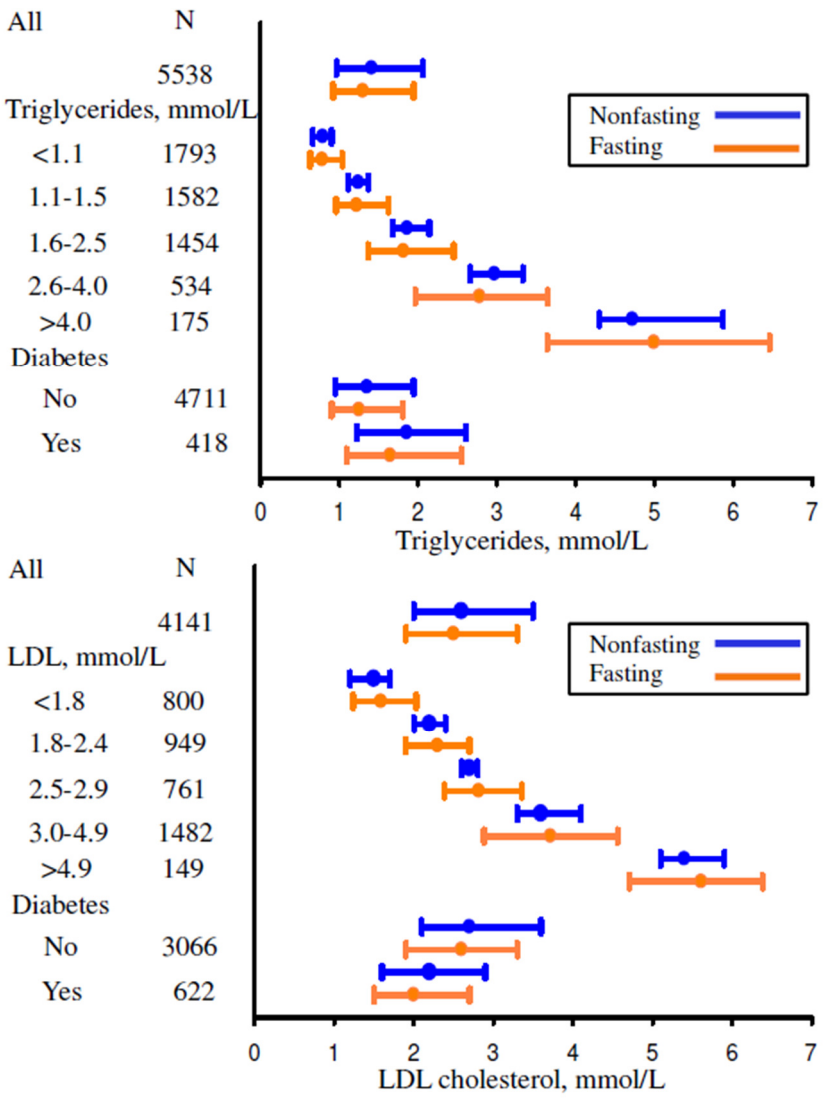

***Figure 9**. Levels of plasma triglycerides and LDL cholesterol measured in the non-fasting and fasting states in the same individuals. All values are medians with interquartile ranges. Based on laboratory data from Herlev and Gentofte Hospital in the period 2011 through 2015. From Nordestgaard, Langsted et al. Eur Heart J. 2016;37(25):1944-58.(9)*

Another argument for using fasting samples has been the use of the Friedewald equation to calculate LDL cholesterol, as this equation includes levels of plasma triglycerides. However, studies have shown that LDL cholesterol calculated and directly measured are highly comparable both using fasting and nonfasting profiles(9, 114, 115). Additionally, an argument has been that in many randomized trials that show an effect of lipid-lowering therapy on cardiovascular disease risk they used fasting measurements; however, several large trials such as the Heart Protection Study with statins used lipid measurements in the nonfasting state and many population based studies such as the Tromsø Heart Study, the Women's Health Study, and the Copenhagen General Population

Study used random nonfasting samples. Furthermore, as reviewed in the section above four large-scale cohorts found levels of triglycerides on average to increase only up to 0.3 mmol/L (27 mg/dL) after normal food intake. One argument could be that a fasting sample is necessary at nonfasting triglyceride levels above 5 mmol/L (441 mg/dL); however, this most likely would be a high measurement due to high intake of fat-containing food within 3-5 hours before blood sampling and another subsequent random nonfasting blood sample would then be considerably lower(9). In the laboratory, flagging of abnormal results to alert patients and doctors is at most times based on age-specific reference intervals. However, in many Western populations, sedentary lifestyles and high fat diets result in very high upper reference limits with extensive cardiovascular disease risk in the normal area and therefore it is reasonable to use cut-off values instead. As mentioned before, normal eating habits only has a modest influence on lipids and lipoproteins; however, it is of course crucial that nonfasting results are as good as fasting when the risk of cardiovascular disease and mortality is evaluated.

In Paper III we examined the association of nonfasting cholesterol and triglycerides on risk of ischemic heart disease, myocardial infarction, and all-cause mortality from a general population(3). We included a total of 13,972 women and men from the first examination of the Copenhagen City Heart Study with up to 31 years of follow-up. In this general population cohort, at baseline none were taking lipid-lowering medication as statins were not even marketed at that time. We found that at nonfasting plasma cholesterol levels from <5 mmol/L to ≥9 mmol/L, mean LDL cholesterol levels increased by 4.2 mmol/L and mean remnant cholesterol levels increased by 0.8 mmol/L (Figure 10, upper panel). At nonfasting triglycerides from <1 mmol/L to ≥5 mmol/L, the corresponding levels were 0.1 mmol/L and 1.6 mmol/L, respectively (Figure 10, lower panel).

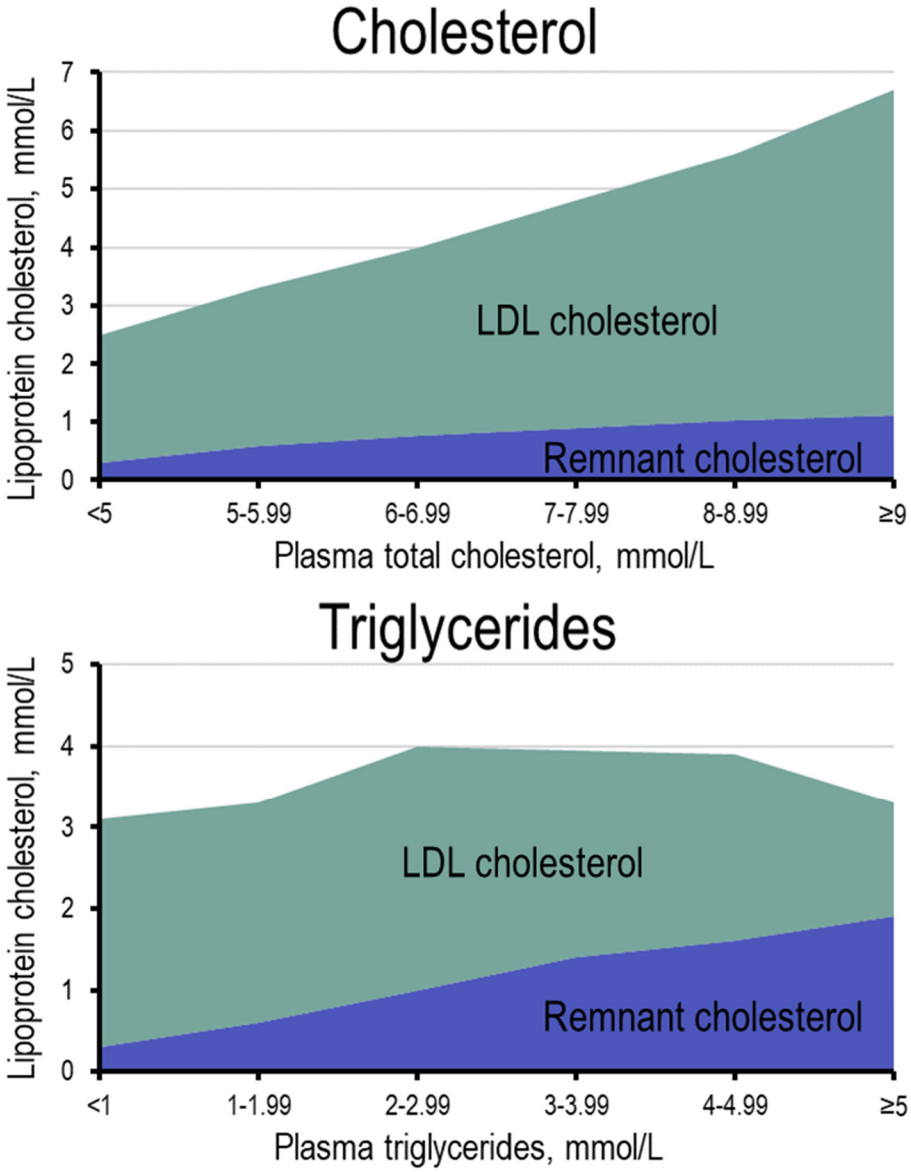

Figure 10. *Mean levels of lipoprotein cholesterol in categories of nonfasting plasma total cholesterol and nonfasting plasma triglycerides in 9798 individuals in the Copenhagen City Heart Study. Adapted from Langsted A et al. J Intern Med. 2011;270:65-75.(3)*

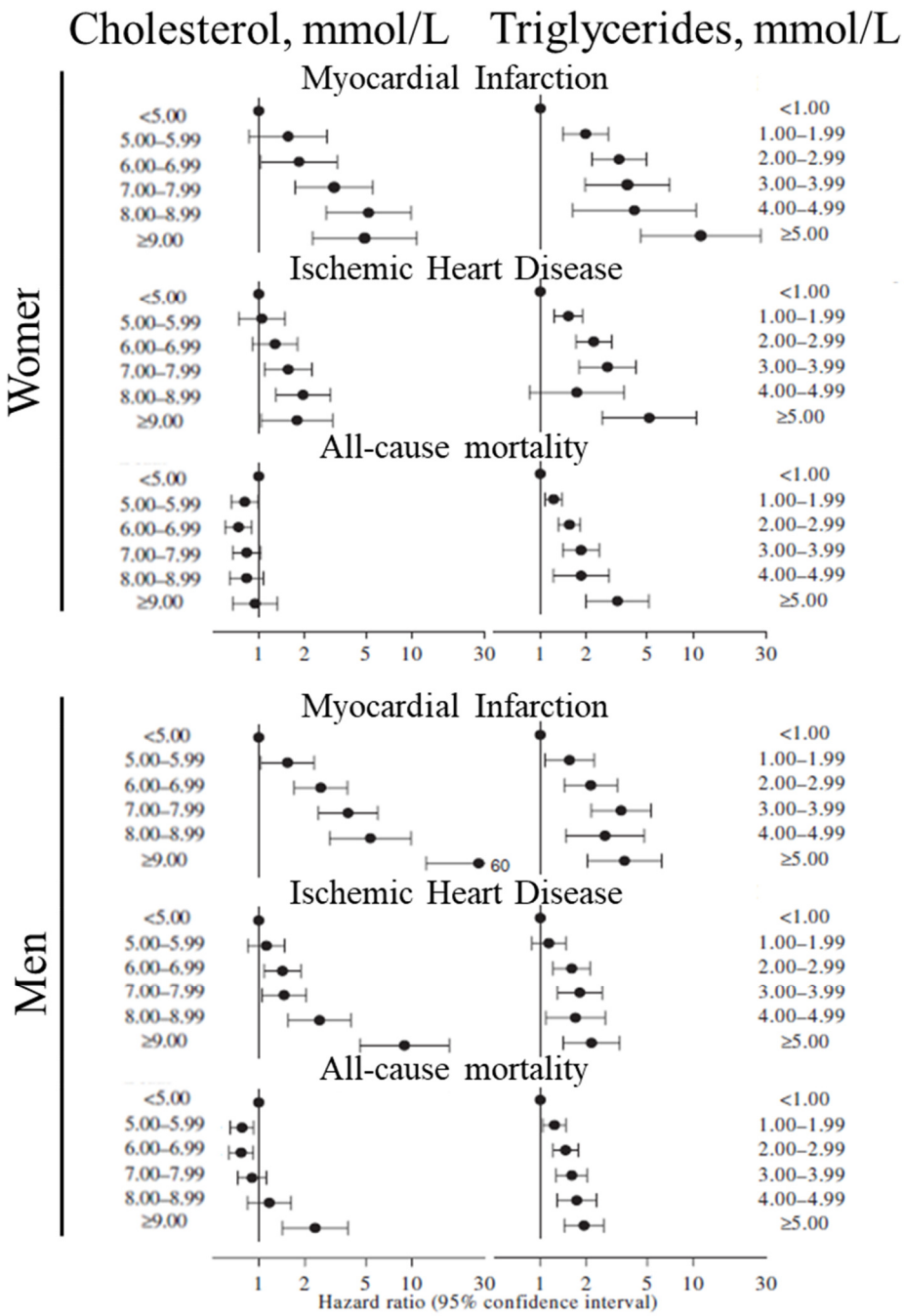

Figure 11. *Nonfasting total cholesterol and triglycerides and risk of ischemic heart disease, myocardial infarction, and all-cause mortality. Analyses were adjusted for age, hypertension, smoking, alcohol intake, and statin use and for menopausal status and hormone replacement therapy in women. Including 7581 women and 6391 men in the Copenhagen City Heart Study in the 1976-78 examination. Adapted from Langsted A et al. J Intern Med. 2011;270:65-75.(3)*

For 7581 women included 768 suffered from myocardial infarction, 1737 from ischemic heart disease, and 4398 died after enrolment(3). Comparing women with total cholesterol ≥9 mmol/L (≥347 mg/dL) to those <5 mmol/L (<193 mg/dL) yielded a hazard ratio of 2.5 (95% confidence interval (CI): 1.6-4.0) for myocardial infarction, 1.4 (1.0-1.9) for ischemic heart disease, and 1.0 (0.8-1.2) for all-cause mortality (Figure 11). Comparing women with nonfasting triglycerides ≥5 mmol/L (≥440 mg/dL) to those with levels <1 mmol/L (<88 mg/dL) yielded a hazard ratio of 4.2 (95% CI: 2.5-7.2) for myocardial infarction, 2.7 (1.7-4.1) for ischemic heart disease, and 2.0 (1.5-2.7) for all-cause mortality.

For the 6391 men, 1151 suffered from myocardial infarction, 2019 from ischemic heart disease, and 4416 died and when comparing men with total cholesterol ≥9 mmol/L (347 mg/dL) with those <5 mmol/L (193 mg/dL) yielded a hazard ratio of 5.3 (95% CI: 3.6-8.0) for myocardial infarction, 3.0 (2.2-4.3) for ischemic heart disease, and 1.5 (1.2-2.0) for all-cause mortality(3). Comparing men with nonfasting triglycerides ≥5 mmol/L (≥440 mg/dL) to those with levels <1 mmol/L (<88 mg/dL) yielded a hazard ratio of 2.1 (95% CI: 1.5–2.8) for myocardial infarction, 1.5 (1.2–2.0) for ischemic heart disease, and 1.5 (1.2–1.7) for all-cause mortality.

We further estimated the absolute risk of ischemic heart disease over 10 years stratified for the known risk factors sex, age, smoking, hypertension, nonfasting cholesterol, and nonfasting triglycerides(3). The highest absolute risk of 60% was observed in men who smoked, had hypertension, were older than 60 years, and had total nonfasting cholesterol >9 mmol/L (Figure 12). We also found similar higher risk of ischemic heart disease and myocardial infarction with stepwise higher nonfasting total cholesterol and higher nonfasting triglycerides, but surprisingly for the association to risk of all-cause mortality it was only found for high levels of nonfasting triglycerides but not for high levels of nonfasting total cholesterol. We later investigated this in more detail in a study of 108,243 individuals in the Copenhagen General Population Study and found that both low

and high levels of LDL cholesterol were associated with high risk of all-cause mortality in a co-called U-shaped association(116), which could be why when comparing low with high levels of cholesterol we found no risk increases in our first study(3). A possible explanation for this U-shaped finding could be reverse causation, that is, that individuals with serious illness as for example cancer have low levels of LDL cholesterol leading to high risk of mortality; however, in the study when excluding individuals with known disease, the U-shaped association persisted(116).

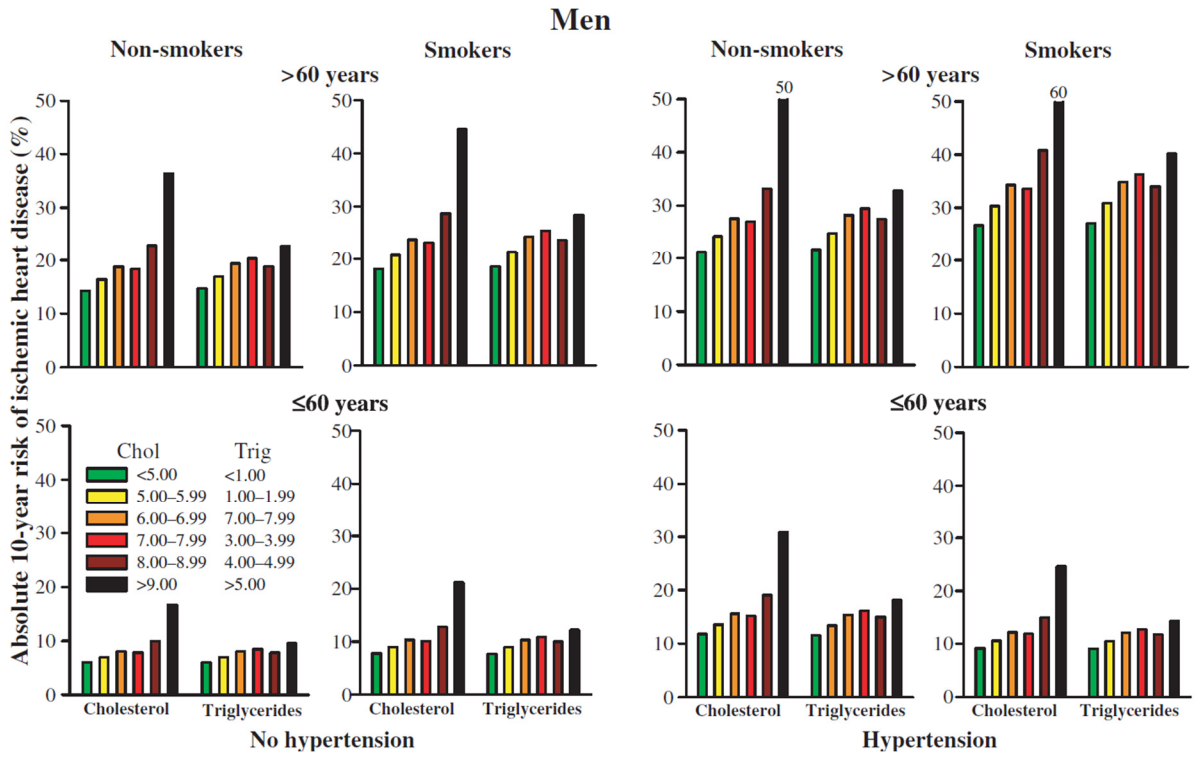

Figure 12. *For ischemic heart disease the absolute ten-year risk in men by nonfasting total cholesterol/ nonfasting triglycerides, smoking, hypertension, and age. Including 6391 men in the Copenhagen City Heart Study. Adapted from Langsted A et al. J Intern Med. 2011;270:65-75.(3)*

Previously, other studies have also examined the association of fasting versus nonfasting blood samples for risk prediction of atherosclerotic cardiovascular disease. In a meta-analysis published in 2007 including 29 prospective studies for risk of coronary heart disease found the predictive value

of triglycerides to be similar in 22 studies with 7484 events where individuals had samples taken when fasting and in 7 studies with 2674 events where individuals had samples taken when not fasting(117). In 2009 another meta-analysis published by the Emerging Risk Factor Collaboration included 68 prospective studies and found that for highest versus lowest quintile of non-HDL cholesterol the hazard ratio for coronary heart disease was 1.4 (95% CI: 1.3-1.5) for 199,076 individuals in 48 studies using fasting samples and 1.7 (1.5-2.0) for 103,354 individuals in 20 studies using nonfasting samples, and further, the predictive values for triglycerides and HDL cholesterol were similar in the 48 studies using fasting samples compared to the 20 studies using nonfasting samples(47). Furthermore, in 2008 a paper including 26,330 women from the Women's Health Study found the predictive value of triglycerides for cardiovascular disease to be similar in fasting and nonfasting women with hazard ratios for highest versus lowest quintile of 2.6 (95% CI: 1.9-3.7) for 19,983 fasting individuals and 2.5 (1.4-4.6) for 6347 nonfasting individuals(104). In addition, in 2014 a paper was published from the National Health and Nutrition Examination Survey III including 16,161 individuals and they found that for risk of cardiovascular mortality the hazard ratio for the top versus the bottom tertile of LDL cholesterol was 3.0 (95% CI: 2.0-4.6) for 10,023 fasting individuals and 4.0 (2.6-6.2) for 6138 nonfasting individuals(118). Finally, in 2019 a study of 8270 participants from the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm with individuals having fasting and nonfasting lipid levels measured one month apart, found that per 40 mg/dL higher LDL cholesterol the risk of myocardial infarction and fatal coronary heart disease combined were 1.3 (95% CI: 1.1-1.6) when individuals were fasting and 1.3 (1.1-1.6) when individuals were nonfasting(119). Taken together, elevated levels of nonfasting lipids and lipoproteins are solid risk predictors of cardiovascular disease just like fasting levels. In a review article from 2018, we reported that when including 108,602 randomly nonfasting individuals in the Copenhagen General Population Study the highest versus the lowest quintiles of plasma

triglycerides, total cholesterol, LDL cholesterol, remnant cholesterol, non-HDL cholesterol, lipoprotein(a), and apolipoprotein B were all associated with increased risk of myocardial infarction and ischemic heart disease and for HDL cholesterol and apolipoprotein A1 with decrease risk (Figure 13)(11).

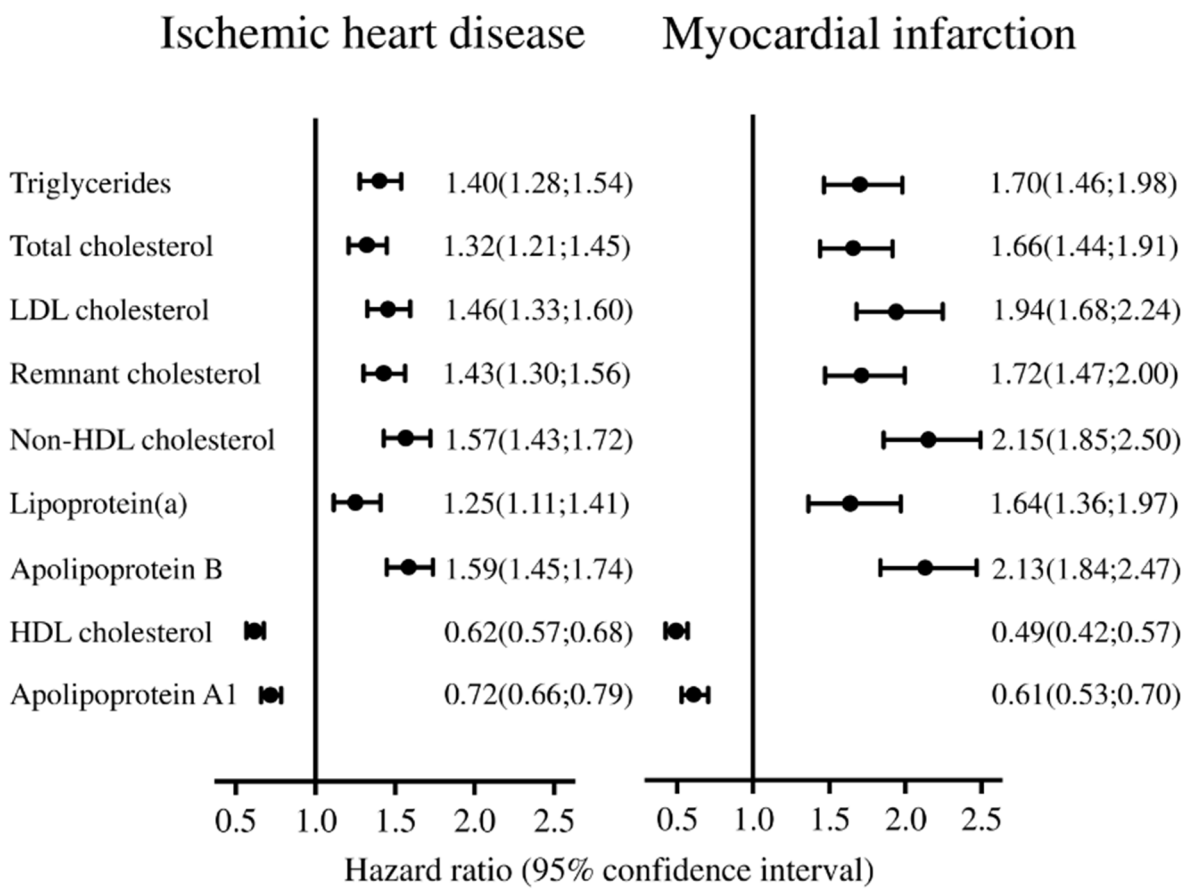

***Figure 13**. Hazard ratios for myocardial infarction and ischemic heart disease of the highest versus lowest quintile of different nonfasting lipids, lipoproteins, and apolipoproteins. Based on 108,602 individuals in the Copenhagen General Population Study. From Langsted A, Nordestgaard BG. Pathology. 2019;51(2):131-41.(11)*

For plasma triglycerides it is even possible that nonfasting versus fasting levels is better at predicting atherosclerotic cardiovascular disease, as it better reflects the pathogenic effects of postprandial levels(120). In two studies published in 2007 including individuals in the Copenhagen

City Heart Study and the Woman's Health Study, high nonfasting triglycerides were associated with increased risk of myocardial infarction, ischemic heart disease, and mortality in both men and women(121, 122). Furthermore, high nonfasting triglycerides from individuals in the Copenhagen City Heart Study has also been associated with increased risk of ischemic stroke(123), and with high risk of acute pancreatitis in individuals from the Copenhagen General Population Study(98). Lastly, several randomized clinical lipid-lowering trials used nonfasting lipid measurements and found lowering of nonfasting lipid levels to be associated with lower risk of cardiovascular disease; these include the Heart Protection Study testing simvastatin(124), the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine(125), and the Anglo-Scandinavian Cardiac Outcomes Trial testing atorvastatin(126).

As evidence from both observational prospective studies and randomized clinical trials support using nonfasting lipid profiles for cardiovascular risk prediction, the arguments against it seems without scientific grounds. Nonfasting versus fasting measurements have numerous advantages: it gives a more accurate measure of the lipid load present in the blood at the majority a 24-hour cycle as most individuals eat regular meals and are only fasting in the early morning hours before breakfast, it simplifies blood sampling for the individual patient not needing the inconvenience of fasting for many hours prior to attendance at the laboratory and thereby being more flexible as to what time of day the blood sampling can take place, for the laboratory or the clinician it would be convenient not to have to reschedule appointments if the patient is not fasting, and a serious concern is problems with fasting-induced hypoglycemia in individuals with diabetes, where a study has shown that 25% of individuals with diabetes have had an event of hypoglycemia due to fasting for routine blood sampling(127).

History of implementing nonfasting lipid profiling

Since the first studies of nonfasting versus fasting lipid profiles to be used for cardiovascular risk prediction were published more than 10 years ago, many subsequent studies have confirmed these initial findings on the utility of nonfasting samples. Therefore, expert panels and guidelines throughout the world have adopted nonfasting lipid profiles for cardiovascular risk prediction (Figure 14). The first country to recommend this was Denmark, where an expert panel from the Danish Society of Clinical Biochemistry made an official recommendation in 2009 in the nationwide Danish Medical Journal to measure lipids in nonfasting individuals(128). The American Heart Association published a similar statement in 2011 on cardiovascular disease risk recommending measurement of nonfasting plasma triglycerides when screening for risk(72). The Veterans Affairs and US Department of Defense made a joint clinical practice guideline in 2014 for managing dyslipidemias for risk reduction of cardiovascular disease in adults and recommended to measure lipid profiles nonfasting for risk assessment(129). Further, in United Kingdom a clinical guideline from the National Institute for Health and Care Excellence was published on risk stratification and lowering of cardiovascular disease risk including recommendations about lipid levels and in this it was proposed that when measuring lipid profiles to assess the need for lipid-lowering therapy, it is not needed to be fasting(130). In addition in a joint statement from the European Atherosclerosis Society/the European Federation of Clinical Chemistry and Laboratory Medicine published in 2016 it was recommended to be nonfasting when measuring lipid profiles for prediction of cardiovascular risk(9, 131). Another European joint guideline for the managing dyslipidemias published in 2016 from the European Society of Cardiology/ the European Atherosclerosis Society likewise recommend using nonfasting samples(45). Furthermore, in Canada a dyslipidemia guideline from the Canadian Cardiovascular Society was published in 2016 stating

that a nonfasting sample is considered as an acceptable alternative to a fasting sample for cardiovascular risk prediction(132) and also in Canada guidelines from the Canadian Hypertension Education Program stated that a fasting sample is no longer required and that nonfasting samples are equally appropriate(133). Furthermore, in Brazil a joint consensus statement from members of the Brazilian Society of Cardiology, the Brazilian Society of Clinical Analyses, the Brazilian Society of Endocrinology and Metabolism, the Brazilian Society of Clinical Pathology/ Laboratory Medicine, and the Brazilian Society of Diabetes recommended that a nonfasting sample for lipid profiling may be used by the laboratory if including the information about fasting/nonfasting when collecting the sample(134). In the US in 2017 from the American Association of Clinical Endocrinologists/ the American College of Endocrinology a guideline on managing dyslipidemias and on preventing cardiovascular disease likewise stated that using nonfasting lipid profiles are an acceptable alternative if fasting lipid profiles are impractical(135). Finally, the latest task force on clinical practice guidelines from the American Heart Association/ the American College of Cardiology published in 2019 endorsed that using nonfasting lipid profiles can be an alternative to fasting lipid profiles for cardiovascular primary prevention risk assessment and for assessment for individuals not yet on statins(136, 137).

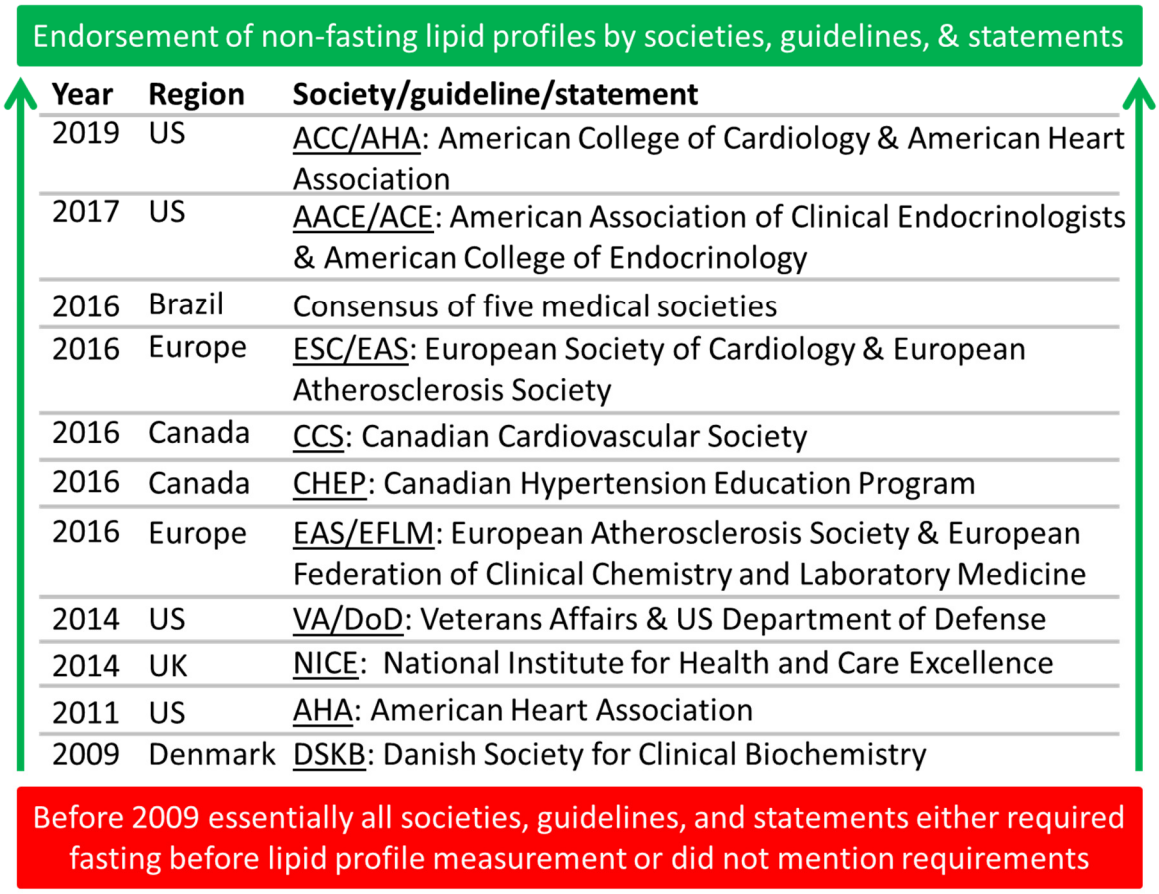

Figure 14. *The historical development of implementing random, nonfasting lipid profiles recommended by societies, guidelines, and statements. Adapted from: Langsted A, Nordestgaard BG. Pathology. 2019;51(2):131-41.(11)*

Recommendations for using lipid profiles in the nonfasting state for risk prediction of cardiovascular disease are spreading widely throughout the world this will not only make blood sampling more simple for patients, laboratories, and clinicians alike, but also lead to better risk prediction and thereby hopefully better treatment and reduction of cardiovascular disease burden.

Part III: Lipoprotein(a)

Plasma concentrations and genetics

Lipoprotein(a) has been a focus of cardiovascular research on and off for more than 50 years and currently, more attention than ever is given to this unique LDL-like particle due to evolving evidence of its pathophysiological effects and the promising new therapies to lower plasma levels. In the general population there is high intra- and inter-ethnical variation in lipoprotein(a) plasma levels. In white European populations, most individuals have levels around 1 to 30 mg/dL; however, levels can be higher than 300 mg/dL(13, 14, 23). In Figure 15 we show the lipoprotein(a) distribution of 70,639 individuals in the Copenhagen General Population Study and in this cohort levels range from the lowest 0.1 mg/dL to the highest measurement of 689 mg/dL, with a median of 8 mg/dL and a mean of 24 mg/dL.

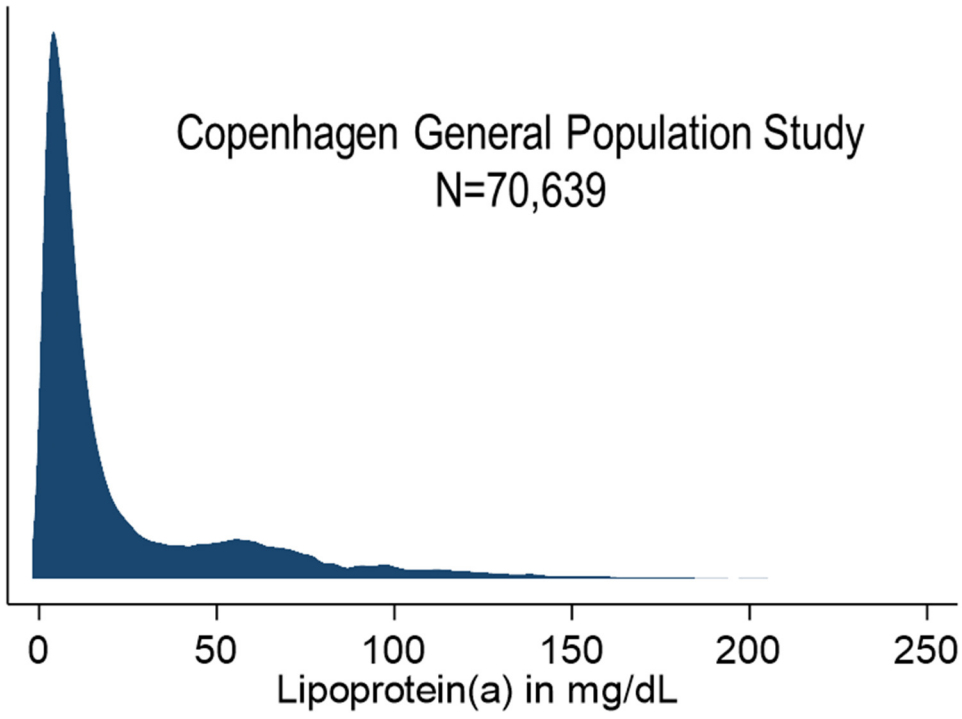

***Figure 15**. Distribution of lipoprotein(a) including 70,639 individuals in the Copenhagen General Population Study. In this figure, lipoprotein(a) levels were truncated at 250 mg/dL, as only 19 individuals had higher levels. The highest concentration observed was 689 mg/dL.*

As for interethnic variety in lipoprotein(a) plasma levels the highest levels are observed in Africans with reported median levels of 27 mg/dL, and the second highest in Arabs with 15 mg/dL, then South Asians with 14 mg/dL, Latin Americans with 12 mg/dL, Europeans with 10 mg/dL, South East Asians with 10 mg/dL, and finally Chinese have the lowest median levels of 8 mg/dL(138, 139).

For other lipoproteins such as LDL, the plasma levels can vary dependent on lifestyle and they can also vary dependent on physiological factors. For lipoprotein(a) these factors have hardly any influence on plasma levels as the levels are mainly determined by genetics. Sex might play a minimal role with lipoprotein(a) levels being higher in women than in men particularly after menopause(140-142). A minimal increase in lipoprotein(a) with age has been observed in some smaller studies(138, 142); however, not much research has been done on this topic. Two factors that do influence lipoprotein(a) levels are liver and renal function. As the apolipoprotein(a) part of lipoprotein(a) is produced in the liver, individuals with reduced liver function such as those suffering from non-alcoholic fatty liver disease (NAFLD) do indeed have lower lipoprotein(a) plasma levels compared to individuals without the disease(143), and others with reduced liver function like in liver cirrhosis, hepatitis C, and liver cancer have lower lipoprotein(a) levels compared to individuals without liver disease(144, 145). As for renal function, lipoprotein(a) levels increase with renal function impairment and are elevated in patients with chronic kidney disease and individuals with low glomerular filtration rate (GFR)(146, 147). Furthermore, high lipoprotein(a) levels are observed in individuals with the nephrotic syndrome(148) and in individuals receiving peritoneal dialysis(149), while lipoprotein(a) levels are decreased after kidney transplantation(146). The lipoprotein(a) elevations seen with renal function impairment are most likely due to reduced clearance related to decreased GFR and protein loss via urine.

The modest association of lipoprotein(a) levels with physiological and lifestyle factors is compatible with the notion that levels of lipoprotein(a) are primarily genetically determined. In the first genetic studies of lipoprotein(a) levels it was thought of as an autosomal dominant trait, while other studies suggested it was a polygenic trait(12, 150-152). Lipoprotein(a) consists of an LDL-like particle and has an apolipoprotein(a) attached that consist of two different kringle domains, number IV and V (the corresponding kringle I, II, and III are only present in plasminogen and not in apolipoprotein(a)). The kringle IV can further be divided into 10 different subtypes, where type 2 can be present in a large variety of repeats. In the late 1980ies it was discovered that the size of apolipoprotein(a) was determined by the copy number of the KIV2 and that these number of repeats were inversely correlated to lipoprotein(a) levels(153-156). We found that in 69,441 individuals in the Copenhagen General Population Study the combined number of repeats of KIV2 on both alleles in the *LPA* gene explained 27% of the lipoprotein(a) variation (Figure 3). Like described above the lipoprotein(a) plasma levels vary to a large extend inter-ethnically and this is also the case for the KIV2 number of repeats and its relation to lipoprotein(a) levels in plasma. Early on, it was shown that KIV2 explained the variation in lipoprotein(a) by 77% for Malays, 70% for Chinese, 37% for Icelanders, 35% for Austrians, 33% for Indians, 25% for Hungarians, and 19% for Sudanese(157).

Later studies revealed other genetic variants to be associated with lipoprotein(a) levels in plasma, including a large gene-chip study from 2009 examining 48,742 single nucleotide polymorphisms (SNPs) from 2100 candidate genes comparing individuals suffering from coronary heart disease to healthy controls(17). That study found two SNPs (rs10455872, rs3798220) in the *LPA* gene encoding the apolipoprotein(a) to be associated with very high coronary heart disease risk and the rs10455872 SNP explained 25% and the rs3798220 SNP 8% of the plasma lipoprotein(a) variation(17). We found in 70,388 individuals in the Copenhagen General Population Study that the rs10455872 explained 31% of lipoprotein(a) levels in plasma and the rs3798220 7% of the plasma

lipoprotein(a) levels (Figure 3). Other studies, both genome-wide linkage studies and association studies, have found multiple other genetic variants in or around the *LPA* gene on chromosome 6q27 as determinants of plasma lipoprotein(a) levels(158-162).

The high genetic influence on lipoprotein(a) levels in plasma makes the *LPA* gene an excellent instrument when studying causality of elevated lipoprotein(a) in relation to morbidity and mortality. Compared to other cardiovascular risk factors the genetic evidence for causality is by far the strongest for lipoprotein(a). Indeed, across the field of Mendelian randomization studies KIV2 and the rs10455872 SNP are among the strongest instruments known for any phenotype.

Lipoprotein(a) in cardiovascular disease

The first studies of the association of elevated lipoprotein(a) with atherosclerotic cardiovascular disease were case-control studies from the 1970ies(163-165). In the following decades, prospective studies were published examining risk of cardiovascular disease for individuals with high lipoprotein(a) levels. In 18 population-based studies published from 1988 to 1998 all but one study had a risk ratio for cardiovascular disease above 1.0 when examining risk in the top versus the bottom tertile of lipoprotein(a) levels and the risk for all 18 studies combined was 1.7 (95%CI: 1.4-1.9)(166). Another 9 prospective studies including studies of individuals with preexisting coronary heart disease, diabetes, or renal disease yielded a corresponding risk ratio of 1.3 (1.1-1.6) when combined. In both analyses, the meta-analyses results were mainly driven by one study due to size: for prospective studies a study published in 1997 using electrophoretically detected lipoprotein(a) found a hazard ratio for top versus bottom lipoprotein(a) level was 1.6 (95% CI: 1.0-2.6) for men and 1.9 (1.3-2.9) for women(167) and for the preexisting disease studies it was a study from the Scandinavian Simvastatin Survival Study from 1997 including 1042 events(168). Similar results were found in an earlier meta-analysis from 1998(169). The Emerging Risk Factor Collaboration

later published a meta-analysis on lipoprotein(a) levels and cardiovascular risk including 36 prospective studies with a total of 126,634 individuals and found a risk ratio for a one standard deviation or 3.5-fold higher level of lipoprotein(a) of 1.12 (95% CI: 1.07-1.18) for myocardial infarction, 1.10 (1.02-1.18) for ischemic stroke, and 1.14 (1.07-1.22) for coronary death(18). In these individual early prospective studies, the association of high lipoprotein(a) levels and increased risk of cardiovascular disease was found in most but not all individual studies. This could be due to numerous factors such as assays not being able to detect correct lipoprotein(a) levels due to the complex structure of the protein, having stored samples in freezers for many years before measuring lipoprotein(a), not enough statistical power in the individual study, or not examining risk at extremely high lipoprotein(a) levels.

The finding of an association of lipoprotein(a) levels with KIV2 size polymorphisms of apolipoprotein(a) was the first discovery of a genetic association of high lipoprotein(a) levels and increased risk of cardiovascular disease(156, 170). Further, strong genetic associations were established much later in two large studies published in 2009. First, a study using a gene chip with 48,742 SNPs in 2100 candidate genes testing for the associations of coronary disease in 3145 cases and in 3352 controls found that the *LPA* locus encoding for lipoprotein(a) had the strongest association. The study further identified the variant rs10455872 at the *LPA* locus with an odds ratio of 1.7 (95% CI: 1.5-2.0) for coronary disease and the variant rs3798220 with a corresponding odds ratio of 1.9 (95% CI: 1.5-2.5). Both of these newly discovered variants were found to be highly associated to high lipoprotein(a) levels and low KIV2 copy number in *LPA* (which determines the number of KIV2 repeats in apolipoprotein(a))(17). Second, a Mendelian randomization study in the Copenhagen City Heart Study found that the causal risk ratio of myocardial infarction for a doubling in levels of lipoprotein(a) was 1.22 (95% CI: 1.09-1.37) for KIV2, compared to a hazard ratio of 1.08 (1.03-1.12) for the observation analysis(19). In 2016 we updated these Mendelian

randomization studies based on the Copenhagen City Heart Study and the Copenhagen General Population Study combined and found for a doubling in plasma lipoprotein(a) levels the causal risk ratio was 1.15 (95% CI: 1.11–1.20) for risk of myocardial infarction using the *LPA* KIV2 number of repeats and 1.10 (1.06–1.13) using the *LPA* rs10455872 SNP compared to the observational hazard ratio of 1.09 (1.07–1.12) (Figure 16, upper part).

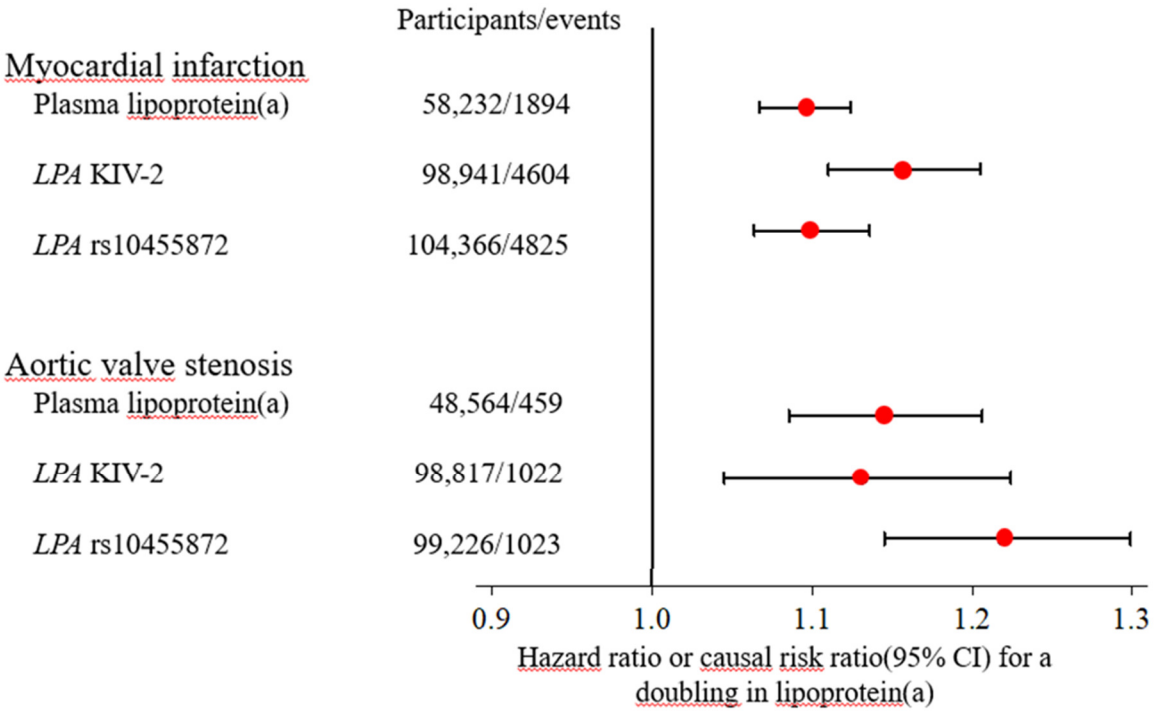

***Figure 16**. Observational and genetic associations for high levels of lipoprotein(a) and risk of myocardial infarction and aortic valve stenosis in the Copenhagen City Heart Study and the Copenhagen General Population Study combined. Observational analyses of plasma lipoprotein(a) levels were by Cox regression models. Causal risk ratios for the genetic analyses were done by instrumental variable analyses adjusted for age and sex. Adapted from Nordestgaard BG, Langsted A. J Lipid Res. 2016;57(11):1953-75.(23)*

Confirmation of genetic evidence emerged with the rs41272114 loss-of-function mutation in the *LPA* gene that associated with low lipoprotein(a) levels and a low risk of cardiovascular disease with an odds ratio of 0.79 (0.66-0.97)(171). Genetic studies from the UK Biobank demonstrated that genetic risk scores including *LPA* variants for high lipoprotein(a) were associated to increased

risk of cardiovascular disease, aortic valve stenosis, peripheral vascular disease, stroke, and heart failure(172-174).

High levels of lipoprotein(a) are also associated observationally and genetically to increased risk of aortic valve stenosis. In a large genome-wide association study the presence of aortic valve and mitral annular calcification by computed tomographic scanning was registered, and the study found the *LPA* rs10455872 SNP was associated with aortic valve calcification and in prospective studies carriers had high risk of aortic valve stenosis(20). In a subsequent prospective study of the Copenhagen General Population Study, both high plasma and genetically lipoprotein(a) levels from Mendelian randomization analyses yielded high risk of incident aortic valve stenosis(22). In a large meta-analysis with both previous studies and a new study from the UK Biobank the relative risk of aortic valve stenosis was 1.7 (95% CI: 1.5-1.9) per allele carrier of *LPA* rs10455872 and 1.4 (1.0-2.0) per allele carrier of *LPA* rs3798220(175). In 2016, using the Copenhagen City Heart Study and the Copenhagen General Population Study combined, we found a causal risk ratio of 1.13 (1.04–1.22) for risk of aortic valve stenosis using the *LPA* KIV2 number of repeats and 1.21 (1.14–1.29) using the *LPA* rs10455872 SNP with a doubling in plasma lipoprotein(a) levels compared to a hazard ratio in observational analysis of 1.14 (1.08–1.20) (Figure 16, lower part).

Familial hypercholesterolemia

Familial hypercholesterolemia is known as a genetic disease most caused by a loss-of-function mutation leading to less LDL-receptors and thereby less removal of LDL cholesterol from the circulation. Other familial hypercholesterolemia causing mutations include loss-of-function mutations in the gene encoding apolipoprotein B-100, and gain-of-function mutations leading to increases in PCSK9. Not all individuals with clinical familial hypercholesterolemia have a known mutation; however, all these mutations cause elevations in LDL cholesterol that are lifelong and

result in extremely high risk of cardiovascular disease. Familial hypercholesterolemia is a more common disease than previously presumed with a prevalence as high as 1 in every 200 to 313 individuals in the general population(176-179).

In Paper IV we included 46,200 individuals in the Copenhagen General Population Study who had measurements of lipoprotein(a) in plasma, *LPA* KIV2 number of repeats, the *LPA* SNP most highly associated with lipoprotein(a) levels (rs10455872), and the most common familial hypercholesterolemia causing mutations in Denmark (*LDLR* Trp23X, *LDLR* Trp66Gly, *LDLR* Trp556Ser, and *APOB* Arg3500Gln). Several different familial hypercholesterolemia clinical diagnoses criteria exist, the major ones being the Dutch Lipid Clinic Network (DLCN) criteria(180), the Simon Broome criteria(181), and the Make Early Diagnosis to Prevent Early Death (MEDPED) criteria(182). When using the DLCN diagnosis criteria we found that for individuals not likely to have a familial hypercholesterolemia diagnosis the mean lipoprotein(a) level was 23 mg/dL, for individuals with a possible familial hypercholesterolemia diagnosis 32 mg/dL, and for individuals with a probable/ definite familial hypercholesterolemia diagnosis 35 mg/dL with a p for trend <0.0001 (Figure 17, blue columns). Importantly, the cholesterol content of lipoprotein(a) is co-measured in a standard measurement of LDL cholesterol and when diagnosing familial hypercholesterolemia by the DLCN criteria the LDL cholesterol level is included. In this study we next used lipoprotein(a) corrected LDL cholesterol levels and found that for individuals not likely to have a familial hypercholesterolemia diagnosis the mean lipoprotein(a) level was 24 mg/dL, for individuals with a possible familial hypercholesterolemia diagnosis 22 mg/dL, and for individuals with a probable/ definite familial hypercholesterolemia diagnosis 21 mg/dL with p for trend=0.46 (Figure 17, red columns). In total numbers, when subtracting the cholesterol in lipoprotein(a) from measurements of LDL cholesterol, 43 individuals or 23% did not meet the probable or definite criteria and 722 individuals or 23% did not meet the possible criteria for

clinical familial hypercholesterolemia when using the DLCN criteria compared to if lipoprotein(a) cholesterol was included in the measurements or calculations of total and LDL cholesterol (Figure 16). When using Simon Broome or MEDPED criteria to clinically diagnose familial hypercholesterolemia, we came to essentially the same conclusion, that is, 25% of those diagnosed clinically with familial hypercholesterolemia get the diagnosis due to elevated lipoprotein(a). *LPA* could therefore be considered a 4th gene for familial hypercholesterolemia.

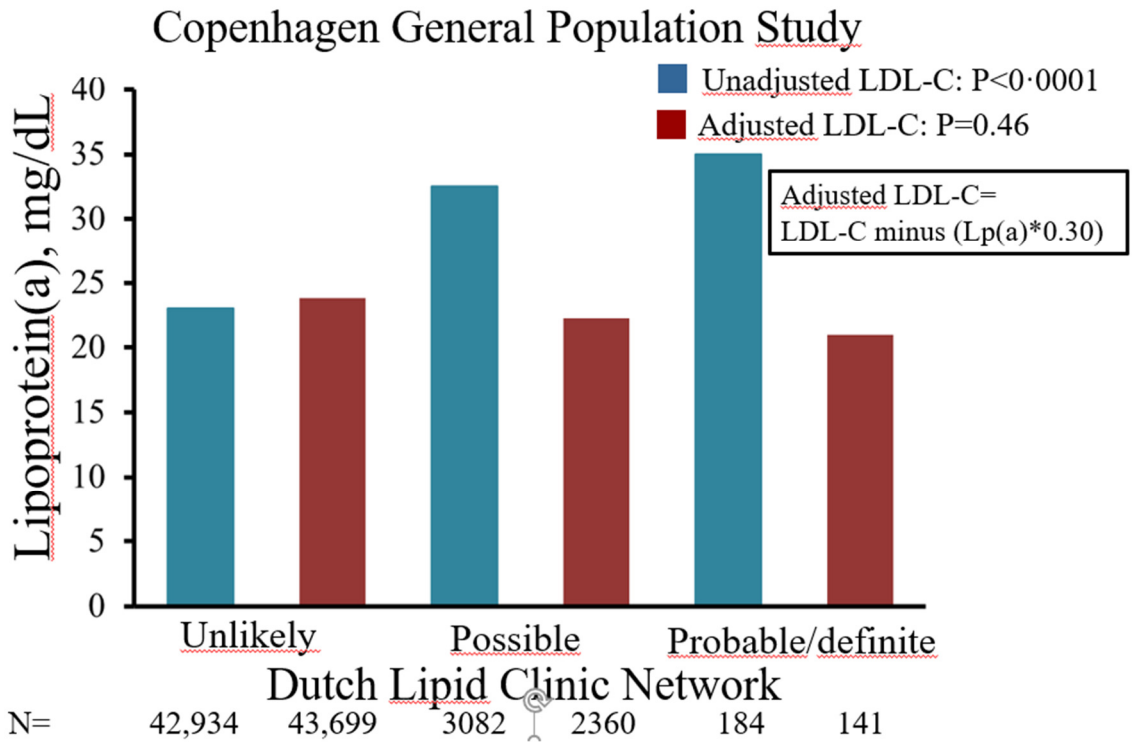

Figure 17*. Levels of lipoprotein(a) by clinical familial hypercholesterolemia diagnosis status from the Dutch Lipid Clinic Network (DLCN) criteria. Based on data from 46,200 individuals from the Copenhagen General Population Study. Adapted from Langsted et al. Lancet Diabetes Endocrinol. 2016;4(7):577-87.(4)*

Due to the very high cardiovascular disease risk for individuals with untreated familial hypercholesterolemia and the risk high of cardiovascular disease for individuals with high lipoprotein(a) levels, we further tested the hypothesis that individuals with clinical familial hypercholesterolemia combined with high lipoprotein(a) levels have the very highest risk

myocardial infarction. Compared to individuals unlikely to have a diagnosis of familial hypercholesterolemia according to the DLCN criteria and having lipoprotein(a) levels <50 mg/dL, we found a hazard ratio for myocardial infarction of 1.4 (95% CI: 1.1-1.7) for individuals unlikely to have a diagnosis of familial hypercholesterolemia and lipoprotein(a) levels ≥50 mg/dL, 3.2 (2.5-4.1) for individuals with a possible, probable, or definite diagnosis of familial hypercholesterolemia and lipoprotein(a) levels <50 mg/dL, and 5.3 (3.6-7.6) for individuals with possible, probable, and definite diagnosis of familial hypercholesterolemia and lipoprotein(a) levels ≥50 mg/dL (Figure 18). For the genetic estimates using KIV2 number of repeats (where low number of repeats are associated with high plasma lipoprotein(a) levels) the corresponding hazard ratios were 1.1 (0.9-1.4), 3.1 (2.4-4.0), and 4.9 (3.4-7.1), respectively. For these analyses results were also similar if we used the MEDPED or Simon Broome criteria.

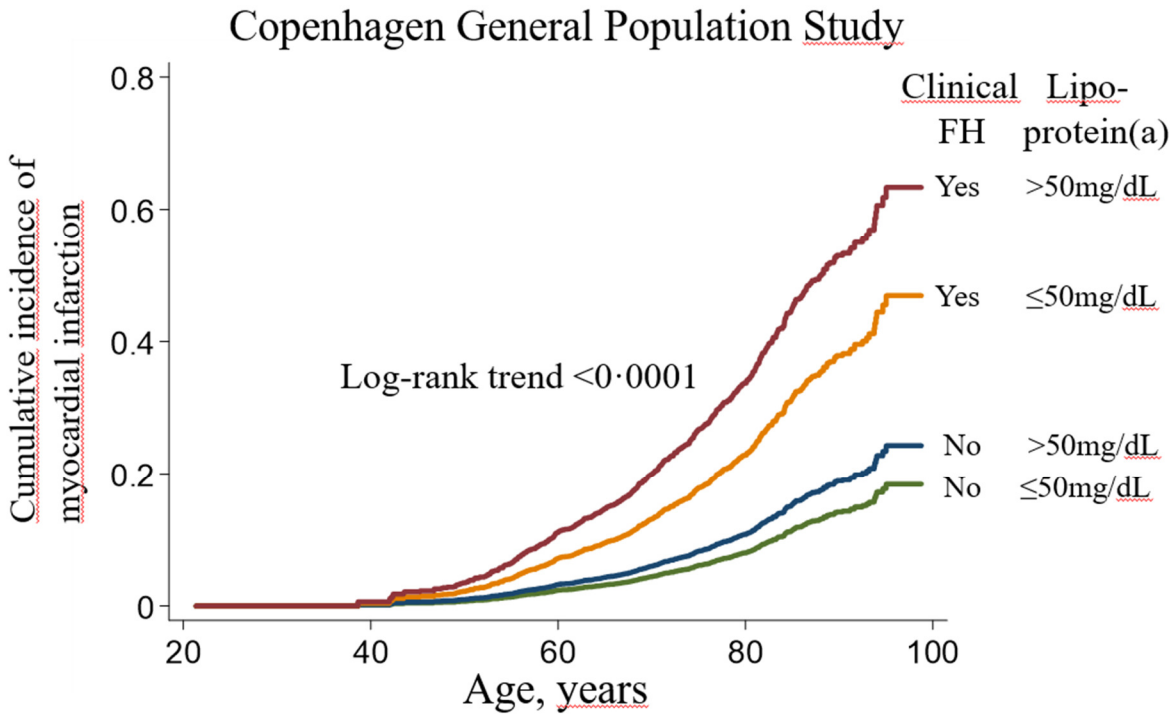

***Figure 18**. Cumulative incidences of myocardial infarction as by familial hypercholesterolemia diagnosis status from the Dutch Lipid Clinic Network (DLCN) criteria and levels of lipoprotein(a) under and above 50 mg/dL. Based on data from 46,200 individuals from the Copenhagen General Population Study. Adapted from Langsted et al. Lancet Diabetes Endocrinol. 2016;4(7):577-87.(4)*

Other studies have examined lipoprotein(a) and its role in individuals with familial hypercholesterolemia, including many early studies(23). A study of 8050 individuals from China published after our study found, similar to our findings, that individuals with a diagnosis of familial hypercholesterolemia and also high lipoprotein(a) levels had the highest early onset coronary artery disease risk(183). In addition, two studies from a large familial hypercholesterolemia cohort from Spain (the Spanish Familial Hypercholesterolemia Cohort Study) found that in familial hypercholesterolemia individuals there was an increased risk of cardiovascular disease for high versus low lipoprotein(a) levels in both sexes(184, 185). Also, a study from Canada found that high levels of lipoprotein(a) compared to low levels were associated to increased cardiovascular disease risk in 388 individuals with heterozygote familial hypercholesterolemia(186). Finally, in a similar sized study from Australia including 390 individuals with mutation-positive familial hypercholesterolemia, high versus low levels of lipoprotein(a) were also associated to increased risk of coronary artery disease(187).

The studies mentioned above supports that having high levels of lipoprotein(a) are adding to the already increased risk of cardiovascular disease seen in familial hypercholesterolemia individuals. When directly measuring total cholesterol and when measuring or calculating LDL cholesterol by the Friedewald equation, the Martin Hopkins equation, or the Sampson equation the cholesterol present in lipoprotein(a), estimated to around 30% of total lipoprotein(a) mass, is co-measured leading to elevated total and LDL cholesterol levels not only caused by familial hypercholesterolemia mutations. Around 90% of familial hypercholesterolemia causing mutations are found in *LDLR* gene, 5% are in the *APOB* gene, and 1% are in the *PCSK9* gene in Europeans, but as shown in our study 25% of clinical familial hypercholesterolemia diagnoses could be due to elevations in levels of lipoprotein(a), thereby making the *LPA* gene the second most important gene for this genetic condition(4).

The importance of high lipoprotein(a) in familial hypercholesterolemia is starting to make its way into guidelines and consensus statements all over the world. For instance the International Familial Hypercholesterolemia Foundation(188), the Canadian Cardiovascular Society(189), and an expert panel statement from Hong Kong(190) all includes lipoprotein(a), whereas the National Institute of Health and Care Excellence (NICE) guidelines from the UK, the American Heart Association(191), and the Japanese Atherosclerosis Society(192) do not mention lipoprotein(a) as of 2022.

It could be beneficial to include lipoprotein(a) as causing familial hypercholesterolemia and measure plasma lipoprotein(a) and perhaps even do gene testing for mutations in the *LPA* gene associated with high lipoprotein(a) levels in individuals referred to gene testing for familial hypercholesterolemia. That said, it could be argued that mutations in the *LPA* gene does not cause monogenic familial hypercholesterolemia; however, not even the known mutations in the *LDLR*, *APOB*, and *PCSK9* gene do always cause a clinical familial hypercholesterolemia diagnosis. Nevertheless, it should be recommended in all guidelines and consensus statements to measure lipoprotein(a) in individuals with familial hypercholesterolemia as a risk factor and to recommend an optimal treatment goal for LDL cholesterol levels and when new treatments become available possibly also for lipoprotein(a) levels.

PCSK9 loss of function mutation and lipoprotein(a)

Lipoprotein(a) levels are mainly genetically determined and the plasma levels are most likely regulated more by synthesis than catabolism(193). Statins are highly effective in lowering LDL cholesterol by increasing LDL receptor expression in the liver, hereby removing LDL cholesterol from the circulation; however, it has been shown that statins have no effect in lowering lipoprotein(a) levels(194). However, lipid-lowering therapy that reduces the synthesis of

apolipoprotein B or the assembly of the LDL particle lowers lipoprotein(a) levels such as seen with mipomersen and lomitapide(195).

PCSK9 is a serine protease that leads to LDL receptor degradation and consequently less removal of LDL cholesterol from the circulation(196, 197). Therefore gain-of-function mutations in the *PCSK9* gene lead to high LDL cholesterol levels and to high risk of cardiovascular disease, while conversely loss-of-function mutations in the *PCSK9* gene lead to increased LDL cholesterol uptake in the liver due to more availability of the LDL receptor and consequently low LDL cholesterol levels in the circulation and low risk of atherosclerotic cardiovascular disease. PCSK9-inhibitors in the form of specific monoclonal antibodies have indeed been shown to lower LDL cholesterol when administered to statin treated individuals, and also to reduce atherosclerotic cardiovascular events in post hoc analyses of clinical randomized trials(198, 199) as well as in proper randomized clinical trials(53, 200). The *PCSK9* R46L loss-of-function mutation has in particular been linked to low LDL cholesterol levels and to decreased risk of cardiovascular disease such as ischemic heart disease and myocardial infarction(196, 201, 202).

In Paper V we studied 103,083 individuals in the Copenhagen General Population Study, the Copenhagen City Heart Study, and the Copenhagen Ischemic Heart Disease Study and tested the hypothesis that the R46L loss-of-function mutation in the *PCSK9* gene associated with low levels of lipoprotein(a) and low aortic valve stenosis and myocardial infarction risk(5). For the *PCSK9* R46L mutation we found median lipoprotein(a) levels of 10 mg/dl for noncarriers, 9 mg/dl for heterozygotes, and 8 mg/dl for homozygotes with a p for trend = 0.02. Compared to *PCSK9* R46L noncarriers the odds ratio of aortic valve stenosis for *PCSK9* R46L carriers was 0.64 (95% CI: 0.44–0.95) and the corresponding odds ratio for myocardial infarction was 0.77 (0.65– 0.92) (Figure 19).

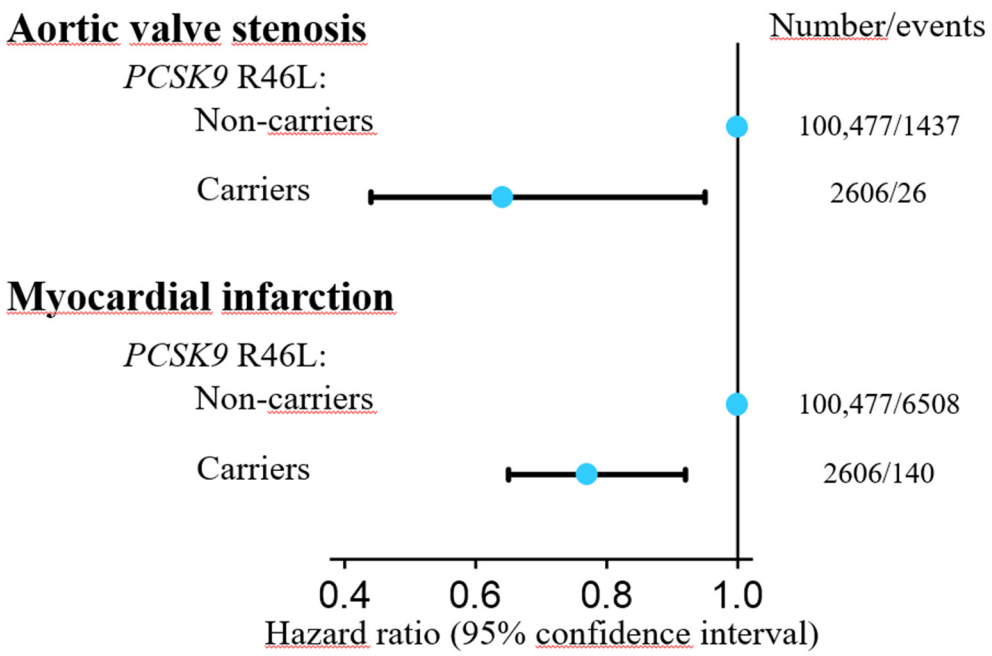

Figure 19. *Risk of aortic valve stenosis (top) and myocardial infarction (bottom) by PCSK9 R46L carriers versus noncarriers. Based on 103,083 individuals in the Copenhagen General Population Study, the Copenhagen City Heart Study, and the Copenhagen Ischemic Heart Disease Study combined. Adapted from Langsted A. J Clin Endocrinol Metab. 2016;101(9):3281-7.(5)*

Since our study was published in 2016 several large clinical randomized trials have published data using *PSCK9* monoclonal antibodies in lowering of lipoprotein(a) to achieve reductions in cardiovascular disease events. One is the FOURIER trial (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) where the PCSK9 inhibitor, evolocumab lowered levels of lipoprotein(a) by 27% and individuals with baseline levels of lipoprotein(a) above and below the median had respectively 23% and 7% risk reduction in major adverse cardiovascular events in post-hoc analyses(203). Another randomized trial is the ODYSSEY Outcomes trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) where treatment with the PCSK9 inhibitor alirocumab lowered lipoprotein(a) levels by 23% and in post-hoc analyses the lipoprotein(a)

reduction appeared to reduce risk of major atherosclerotic cardiovascular events independently in individuals with high baseline lipoprotein(a) levels, but not in individuals with low baseline levels(204). In the LAPLACE-TIMI trial it was found that individuals with high baseline lipoprotein(a) levels when treated with PCSK9 antibodies achieved less percent reduction than individuals with normal levels(205). Therefore, whether lipoprotein(a)-lowering through PCSK9 inhibition reduces risk of atherosclerotic cardiovascular disease needs to be tested directly in the future in individuals recruited selectively due to high lipoprotein(a) levels.

It is interesting that PCSK9 inhibitors were found to significantly lower lipoprotein(a) levels by up to 30% (198, 199), as these inhibitors act by increasing the expression of the LDL receptor and the LDL receptor not likely is associated with clearance of lipoprotein(a) to a major extend. It might be that the lowering of lipoprotein(a) by PCSK9 inhibitors is not sufficient to have a substantial effect on cardiovascular disease risk, it is however possible that it has some added value on top of LDL cholesterol lowering in high risk individuals.

Physiology and pathophysiology of lipoprotein(a) and risk of bleeding

The physiology and pathophysiology of lipoprotein(a) remains somewhat of a mystery. Two times during evolution lipoprotein(a) has evolved, first in the hedgehog and 100 million years later in Old World monkeys, apes, and humans(170). One interesting proposal set out by Brown and Goldstein of lipoprotein(a) function is that it plays a role in wound healing through hemostasis(206). It was suggested that the kringle structures on lipoprotein(a) can bind to fibrin, due to its resemblance with plasminogen, and then be transported to injured sites to promote hemostasis by fibrinolysis inhibition and tissue repair by cholesterol. If this hypothesis would hold then individuals with high lipoprotein(a) levels would have low risk of bleeding.

In Paper VI we therefore tested the hypothesis that high lipoprotein(a) levels were associated with low risk of bleeding in individuals from the general population(6). We included 109,169 individuals in the Copenhagen General Population Study and the Copenhagen City Heart Study with information on lipoprotein(a) plasma levels, *LPA* KIV2 number of repeats, and the *LPA* SNP rs10455872. In this study the risk of major brain and airways bleeding when examining extremely high phenotypes/ genotypes was 0.84 (95% CI: 0.71– 0.99) for high plasma lipoprotein(a) levels, 0.83 (0.73– 0.96) for low number of KIV2 number of repeats associated with the highest plasma lipoprotein(a) levels, and 0.89 (0.81– 0.97) for rs10455872 heterozygotes and homozygotes combined (Figure 20). In a Mendelian randomization design using instrumental variable analysis for a standard deviation increase in lipoprotein(a) levels, the causal risk ratio for major brain and airways bleeding was 0.89 (95% CI: 0.80–0.98) for *LPA* KIV-2 number of repeats, 0.94 (0.87–1.02) for *LPA* rs10455872, and the observational hazard ratio for plasma lipoprotein(a) levels the corresponding value was 0.95 (0.91–1.00).

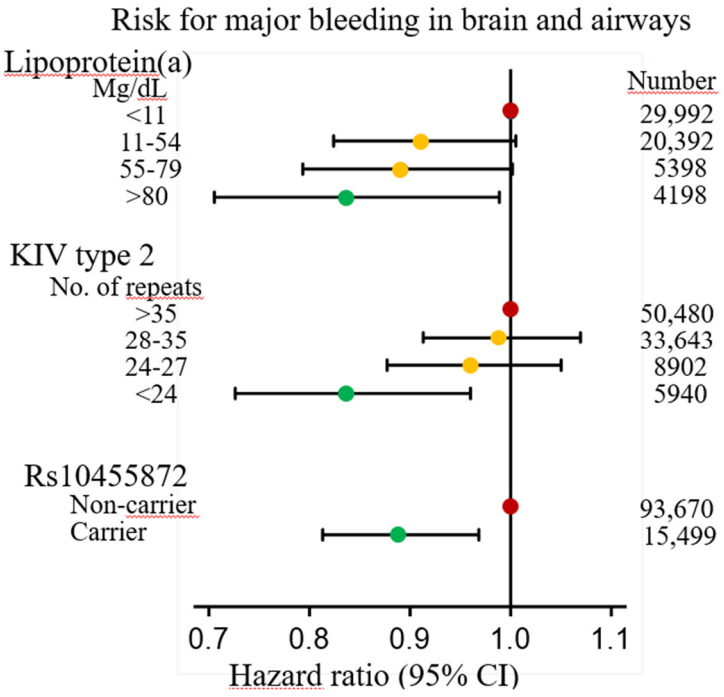

***Figure 20**. Risk of bleeding in brain and airways by lipoprotein(a) plasma levels, KIV2 number of repeats and rs10455872. CI = confidence interval. KIV-2 = kringle-IV type 2. Adapted from Langsted A et al. Clin Chem. 2017;63(11):1714-23.(6)*

In accordance with our findings a Japanese study of 10,494 individuals in a general population found high lipoprotein(a) levels associated to a very low risk of cerebral hemorrhage for both sexes separately(207). Another study supporting the wound healing hypothesis found that small apolipoprotein(a) isoforms in lipoprotein(a) which associate with high lipoprotein(a) plasma levels could inhibit fibrinolysis(208) and another study found lipoprotein(a) associated proteins during the wound healing process(209). However, other studies were not able to confirm our findings of low risk of bleeding at high levels of lipoprotein(a)(210).

If high lipoprotein(a) levels are indeed associated with better wound healing, it could be speculated that high lipoprotein(a) levels present a survival advantage as through less bleeding during childbirth, infectious diseases, and injury, perhaps leading to evolutionary advantage at young age, but more thrombosis and related diseases at old age (Figure 21). The apolipoprotein(a) part of lipoprotein(a) resembles plasminogen, and lipoprotein(a) may promote thrombosis by competing with plasminogen and inhibiting plasmin to dissolve fibrin clots(211). It could possibly be that lipoprotein(a) can bind to fibrin and thereby be transported to vulnerable plaques and to sites of turbulent blood flow due to minor injury. Because lipoprotein(a) is formed with an LDL-like particle another adjacent hypothesis is that lipoprotein(a) would act in a similar atherosclerotic manner as LDL cholesterol by transferring from the bloodstream into the arterial intima leading to cholesterol deposits eventually resulting in atherosclerotic cardiovascular disease(212).

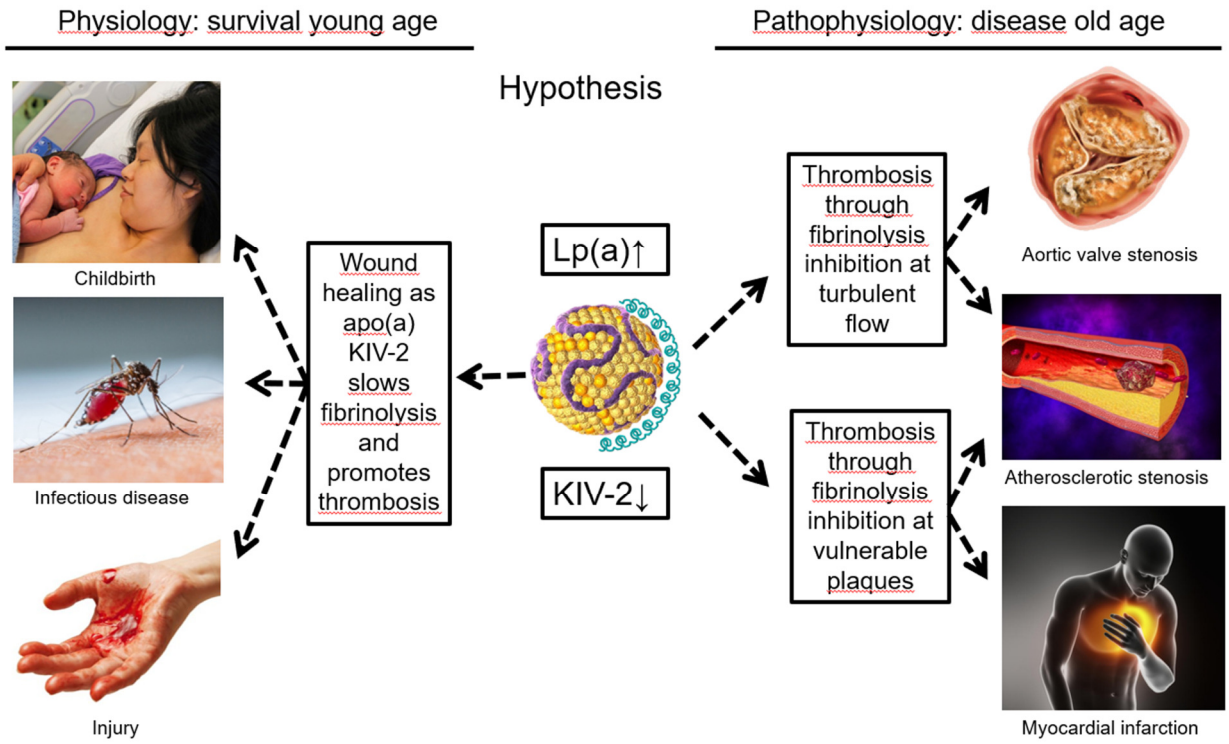

Figure 21. *Possible physiological and pathophysiology mechanisms of high lipoprotein(a) levels, with corresponding small apo(a) size due to low number of KIV-2 repeats.*

Lipoprotein(a) and mortality

Soon if randomized clinical trials show that lowering lipoprotein(a) levels will reduce cardiovascular disease, this could in theory result in reduced cardiovascular and all-cause mortality. On the other hand, as reviewed above, high levels of lipoprotein(a) might protect against bleeding and lowering of lipoprotein(a) levels could thereby in theory increase mortality risk.

In Paper VII we therefore set out to test the hypothesis that high lipoprotein(a) levels are associated with increased risk of cardiovascular and all-cause mortality(7). In this study we included 109,336 individuals in the Copenhagen General Population Study and 11,365 individuals in the Copenhagen City Heart Study and from both studies we included information on lipoprotein(a) levels in plasma, *LPA* KIV2 number of repeats, and *LPA* rs10455872. We found that observationally high plasma levels of lipoprotein(a) were associated with increased risk of cardiovascular and all-cause mortality

(Figure 22). When compared to individuals with low plasma levels of lipoprotein(a) (<10 mg/dL; <50th percentile) the hazard ratio for cardiovascular mortality was 1.5 (95% CI: 1.3-1.8) for individuals with high levels of lipoprotein(a) (>93 mg/dL; >95th percentile) and the corresponding hazard ratio for all-cause mortality was 1.2 (1.1-1.3).

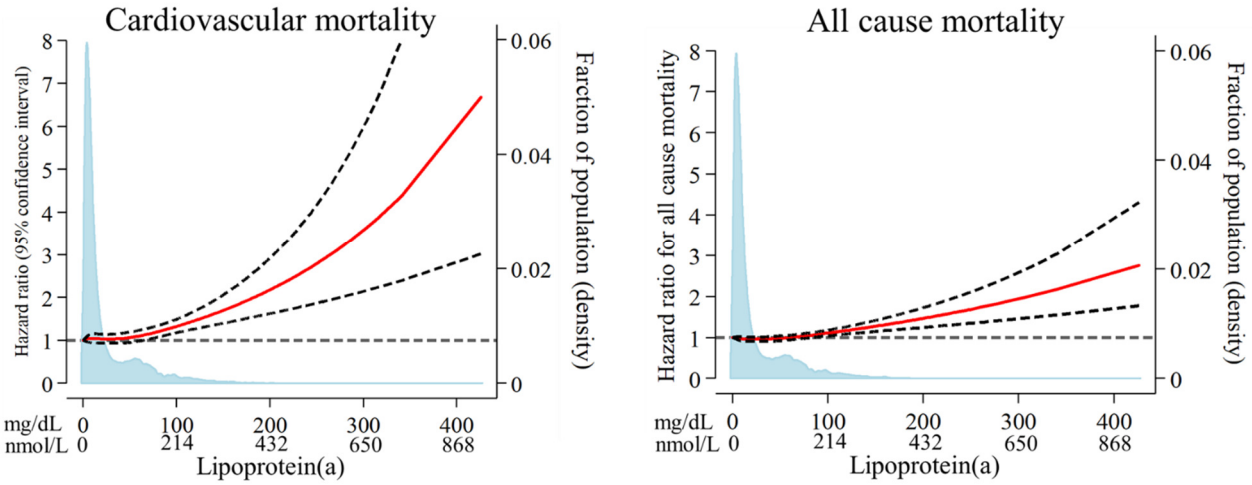

Figure 22. *Risk of cardiovascular and all-cause mortality by increasing lipoprotein(a) levels. Red lines are hazard ratios and dashed black lines are 95% confidence intervals. Adapted from Langsted A et al. Eur Heart J. 2019;40(33):2760-70.(7)*

For a 50 mg/dL higher levels of lipoprotein(a) the hazard ratio for cardiovascular mortality was 1.2 (95% CI: 1.1-1.2) in observational analysis and in genetic analysis the causal risk ratio was 1.2 (1.1-1.4) based on *LPA* KIV2 number of repeats and 1.0 (0.9-1.1) based on *LPA* rs10455872; the corresponding risks for all-cause mortality were 1.1 (1.0-1.1), 1.1 (1.0-1.2), and 1.0 (0.9-1.0), respectively (Figure 23).

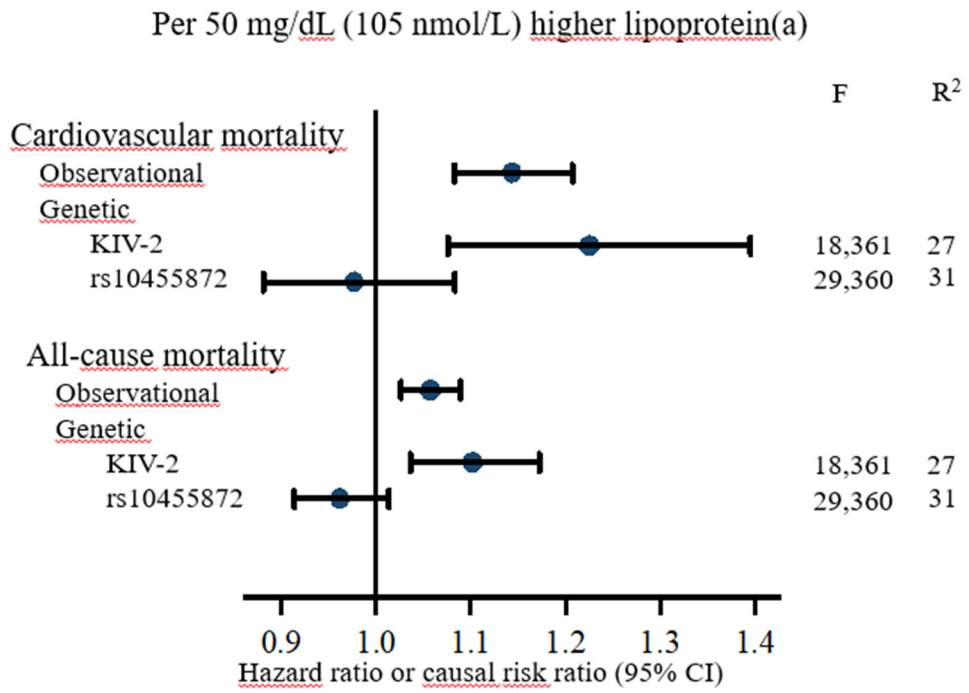

***Figure 23**. Observational hazard ratios and genetic risk ratios for cardiovascular and all-cause mortality for a 50mg/dL (105nmol/L) higher lipoprotein(a) level. Analyses were adjusted for age, sex, smoking status, body mass index, hypertension, inflammation, kidney function, liver function, thyroid function, menopausal status (women only), hormone replacement therapy (women only), diabetes mellitus, triglycerides, HDL cholesterol, and lipoprotein(a) corrected total cholesterol. CI = confidence interval; KIV-2 = kringle-IV type 2. Adapted from Langsted A et al. Eur Heart J. 2019;40(33):2760-70.(7)*

We further found that the median survival for individuals at the highest levels of lipoprotein(a) (>93 mg/dL; >95th percentile) was 83.9 years compared to 85.1 years for those with levels below. Another interesting finding was that for a 15 mg/dL increase in the cholesterol content of lipoprotein(a) we found a higher hazard ratio for cardiovascular and all-cause mortality compared to a 15 mg/dL increase in the cholesterol content of LDL (Figure 24). This indicates that the risk conferred from lipoprotein(a) cannot solely be explained by its cholesterol content and that other aspects such as its structure adds to the pathological effects.

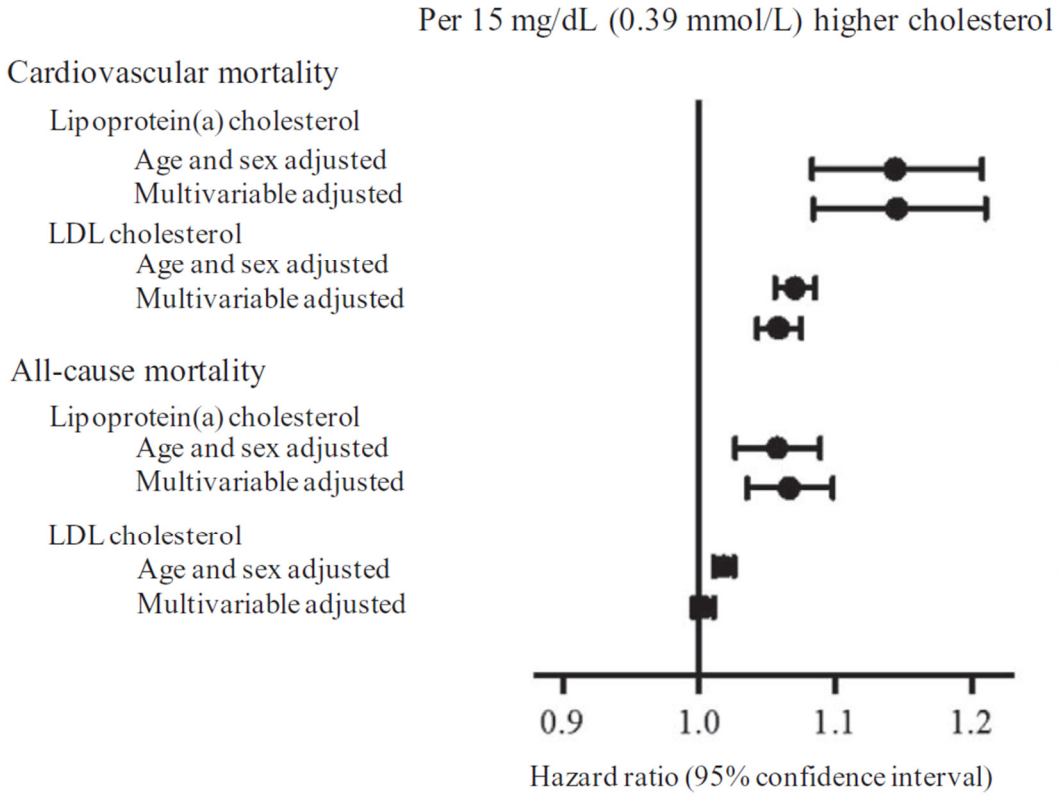

Figure 24*. Risk of cardiovascular and all-cause mortality for a 15 mg/dl (0.39 mmol/l) higher cholesterol content in lipoprotein(a) and LDL. LDL cholesterol was corrected for lipoprotein(a) cholesterol content. CI = confidence interval. Adapted from Langsted A et al. Eur Heart J. 2019;40(33):2760-70.(7)*

Other studies also examined the association of high levels of lipoprotein(a) and the risk of mortality. In the 4S trial (Scandinavian Simvastatin Survival Study) a groundbreaking randomized clinical trial showing that lowering of cholesterol by statins reduced all-cause mortality. This study also showed that individuals suffering from coronary artery disease had higher levels of lipoprotein(a) in comparison to controls in both the control and intervention group, and that high lipoprotein(a) levels associated with high risk of mortality(168). The Cardiovascular Health Study from the US examined the risk of mortality in the elderly without previous cardiovascular disease, and found that the highest versus the lowest quintile of lipoprotein(a) levels yielded a hazard ratio of 2.5 (95% CI: 1.6-4.1) for cardiovascular mortality and 1.8 (1.3-2.4) for all-cause mortality in men only, whereas

no association was found for women(213). Further, from the Emerging Risk Factor Collaboration a meta-analysis including 126,634 individuals from 24 prospective studies published from 1970 to 2009 found that for a 3.5-fold higher lipoprotein(a) level, the risk ratio was 1.1 (95% CI: 1.1-1.2) for cardiovascular mortality and 1.1 (1.1-1.2) for all-cause mortality(18). Another study showed that for individuals who had undergone coronary angiography or percutaneous coronary intervention, high versus low levels of lipoprotein(a) yielded a hazard ratio of 2.2 (1.3-4.1) for all-cause mortality(214). On the contrary, one German study of 3313 individuals following coronary angiography found no associations for highest versus lowest tertile of lipoprotein(a) and carriers versus non-carriers of *LPA* rs10455872 and rs3798220 with risk of cardiovascular or all-cause mortality(215).

The majority of studies did find that individuals with high lipoprotein(a) levels had high risk of cardiovascular and all-cause mortality; however, the genetic instrument *LPA* rs10455872 that highly influences lipoprotein(a) levels was not associated with increased mortality risk in our study (Figure 22), indicating that the mechanisms by which increased lipoprotein(a) levels lead to higher risk of mortality may be different from its involvement in cardiovascular morbidity. Our findings need independent confirmation before firm conclusions can be drawn.

Safety for lowering of lipoprotein(a) and treatment options

Currently, there is no safe, effective treatment available for lowering of lipoprotein(a) levels; however, antisense oligonucleotides or small interfering RNA technologies have in early clinical trials shown potential to lower levels up to 80–95%(25, 26) and therefore, the safety aspect of lowering lipoprotein(a) becomes increasingly important. It is estimated that >1 billion individuals worldwide have elevated lipoprotein(a) levels leading to increased cardiovascular risk(216), and it is therefore crucial to assess the general safety of lowering high lipoprotein(a) levels. Low levels of

lipoprotein(a) have been observationally and genetically associated to high risk of diabetes(217, 218), and high lipoprotein(a) levels with decreased risk of major bleeding in airways and brain, perhaps resulting in increased risk of bleeding at low levels(6).

Thus in Paper VIII we tested the hypothesis that low lipoprotein(a) levels and corresponding *LPA* genotypes associate with major disease groups including cancer and infectious disease in 109,440 individuals in the Copenhagen General Population Study(8). We included genotypes in our study to assess the causality of any potential associations, as associations of genotypes with risk of disease are in general unconfounded and not a result of reverse causality. When examining risk according to main World Health Organization (WHO) International Classification of Diseases (ICD) 10th edition chapter diseases, the lowest versus the highest quartile of lipoprotein(a) levels were as expected associated both observationally and genetically with low risk of diseases of the circulatory system (Figure 24). For all other main disease types including cancer, cancer subtypes, and infections no concordant associations were seen. When comparing the 1st with the 4th quartile of plasma lipoprotein(a) the observational hazard ratio for risk of any cancer was 1.1 (95% CI: 1.0–1.2), for genetic associations comparing the 4th with the 1st quartile of KIV-2 number of repeats 1.00 (0.97–1.07), and for rs10455872 non-carriers versus carriers 1.01 (0.96–1.07). The corresponding hazard ratios for risk of hospitalization for infections were 1.1 (95% CI: 1.0–1.1), 1.0 (1.0–1.1), and 1.0 (0.9– 1.0), respectively. Observationally, we did observe increased risk of diabetes and mental disorders at low versus high lipoprotein(a) levels; however, these findings could not be confirmed in genetic, causal analyses (Figure 25).

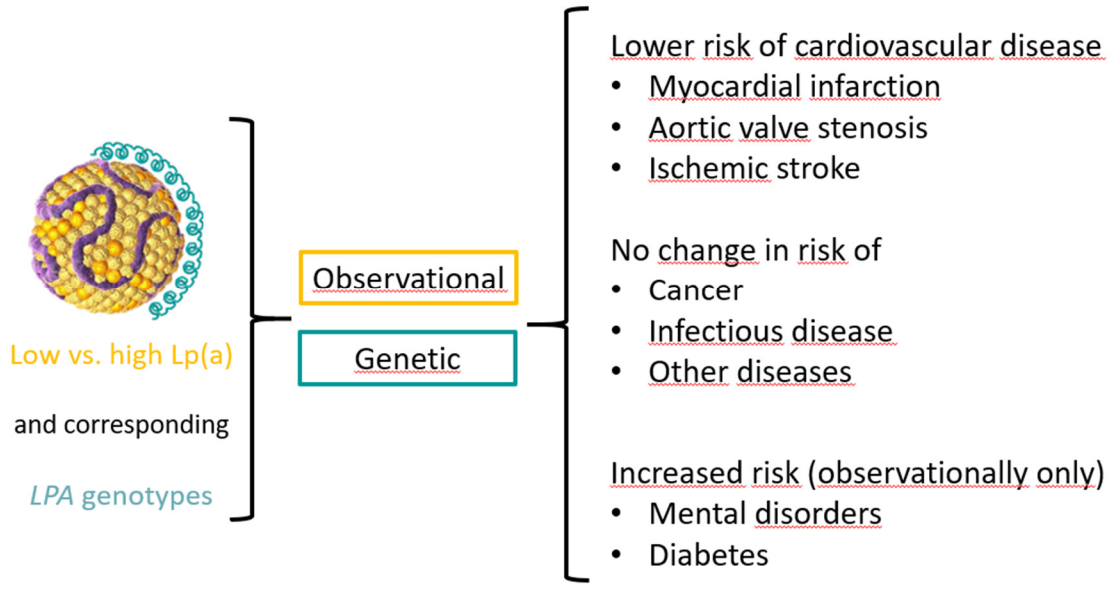

***Figure 25**. Testing low versus high levels of plasma and genetically determined lipoprotein(a) levels on all major diseases from the World Health Organization International Classification of Diseases 10th edition. Based on 109,440 individuals from the Copenhagen General Population Study. Adapted from Langsted A et al. Eur Heart J. 2021;42(12):1147-56.(8)*

In the search of an effective and safe therapy to lower levels of lipoprotein(a) several approaches have been used (Figure 26). First, statins are safe and highly effective in lowering LDL cholesterol, and since lipoprotein(a) consists of an LDL-like particle it would seem straightforward that statins would also lower lipoprotein(a) levels. On the contrary, in a large meta-analysis including data from 6 randomized placebo controlled trials, mean lipoprotein(a) levels were found to increase from 9% to 20% in the groups receiving any statin, 12% to 20% in the groups receiving pravastatin, and 19% to 24% in the groups receiving atorvastatin(219). Second, Niacin as monotherapy has been shown in randomized clinical trials to reduce cardiovascular disease; however, when given on top of statin therapy, no additional benefit was observed, and serious side-effects were found in the Niacin group(220). Third, cholesteryl ester transfer protein (CETP) inhibitors have been found to lower levels of lipoprotein(a) up to 45%, but only with modest effects on cardiovascular disease risk(221).

Fourth, mipomersen an antisense oligonucleotide that targets apolipoprotein B has been found to lower lipoprotein(a) levels by 26%; however, liver-related side effects have limited the use of this drug(222). A fifth possible lipoprotein(a) lowering agent is the PCSK9 inhibitors as reviewed previous in this thesis, but their clinical value is doubtful with respect to lowering of lipoprotein(a) sufficiently. A sixth lipoprotein(a) lowering method is lipoprotein apheresis that removes all apolipoprotein B-containing lipoproteins from plasma and also lipoprotein(a), and it has been found to reduce levels of lipoprotein(a) up to 70% immediately following apheresis; however, the average reduction is around 35% when taken into account time from one session of apheresis to the next. Apheresis has shown a significant lower number of cardiovascular events from before to after apheresis in individuals with high lipoprotein(a) levels(223); however, in this study no control group was included. Although lipoprotein apheresis is effective in lowering lipoproteins and thereby cardiovascular events, the procedure is highly expensive and very time-consuming as two monthly sessions are necessary to lower lipoprotein levels sufficiently. Finally, the seventh and very promising lipoprotein(a) lowering therapies are the antisense oligonucleotides and small interfering RNA technologies that act by binding to hepatic *LPA* mRNA and thus reducing the production of apolipoprotein(a). In a randomized trial including individuals with high levels of lipoprotein(a) and existing cardiovascular disease such antisense oligonucleotides were found to reduce lipoprotein(a) by up to 80%(25) while small interfering RNA technologies have been able to reduce lipoprotein(a) up to 95%(26). With respect to adverse effects these drugs were found to induce no major differences in platelet count, bleeding, liver, or renal parameters compared to placebo. The HORIZON is a phase 3 endpoint trial currently examining if such lowering of high lipoprotein(a) levels using antisense oligonucleotides will lead to reduced risk of cardiovascular disease.

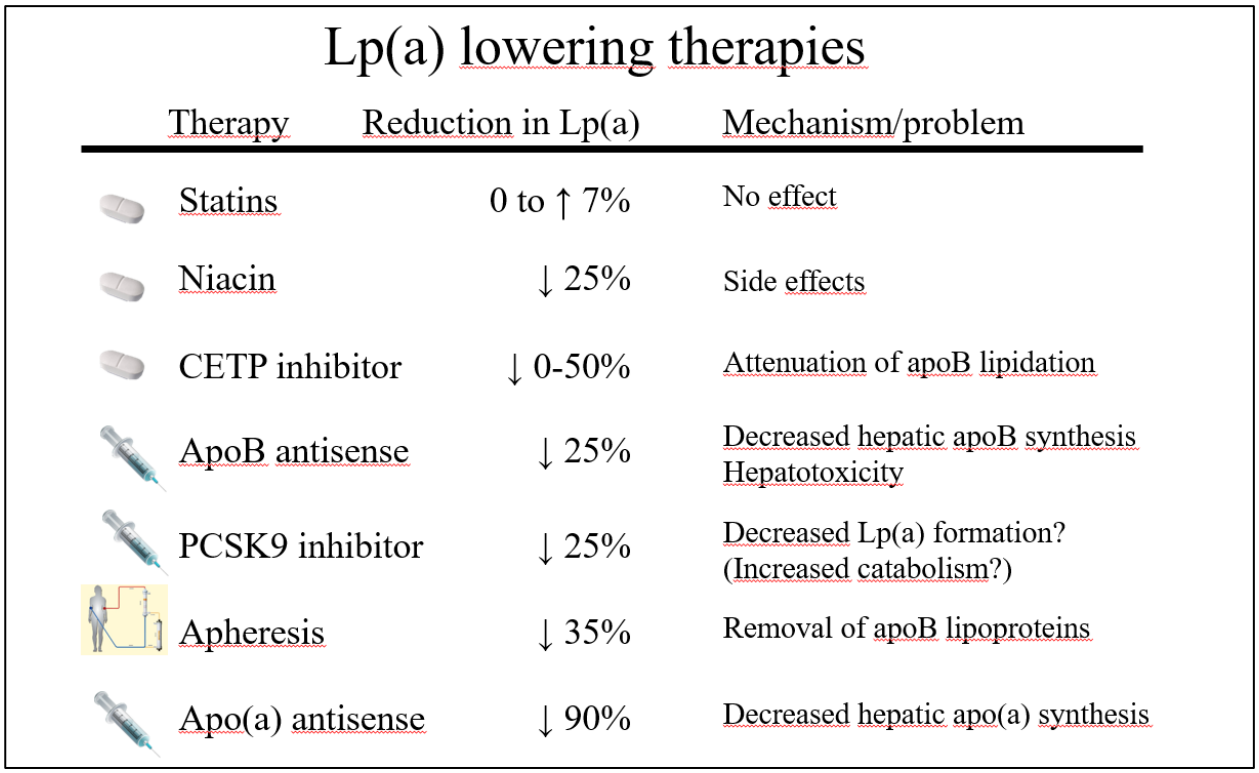

Figure 26*. Suggested treatment options for lowering of lipoprotein(a) levels with percent reductions in lipoprotein(a) levels and potential problems. Lp(a) = lipoprotein(a). CETP = cholesteryl ester transfer protein. ApoB = apolipoprotein B. PCSK9 = proprotein convertase subtilisin/kexin type 9. Apo(a) = apolipoprotein(a).*

Currently, only limited options exists for treatment of high lipoprotein(a) levels and therefore the focus for prevention of cardiovascular disease in individuals with high lipoprotein(a) levels must be on reduction of other modifiable risk factors most importantly lowering of LDL cholesterol, lowering of blood pressure, smoking cessation, and leading a healthy lifestyle including healthy eating and exercising.

Conclusion and perspectives

The papers included in the present thesis show how lipids and lipoproteins influence risk of morbidity and mortality, with special emphasis on the nonfasting state, lipoprotein(a), and

cardiovascular disease. In Papers I to III we investigated the value of nonfasting lipid profiles for cardiovascular risk prediction. We used classic observational epidemiology in these papers with large prospective studies from the general population including information from questionnaires, biochemical measurements, and follow-up from nationwide registries to demonstrate that the preanalytical handling of lipid measurements could be improved for the benefit of patients and clinicians. The journey from our first paper was published in 2008 suggesting that it is not necessary to fast before a lipid profile for risk prediction of cardiovascular disease to the implementation of this in major international guidelines in 2018-2020 is a great example of how observational studies, after due confirmation in other large studies, other populations, and other ethnicities, can impact leading experts and consequently clinical guidelines. During the transition from using fasting to nonfasting lipid profiles, it is important to consider if the risk of cardiovascular disease could be misclassified and hence result in error of initiation of lipid-lowering therapy. However, in both European and American guidelines the cardiovascular risk calculation and thereby, when to initiate lipid-lowering therapy for the individual patient is based on lipid levels but also on diabetes, smoking habits, age, and blood pressure and therefore the minor fluctuations from fasting to nonfasting observed for the lipid profile will only have minimal influence. In fact, following our publications, Denmark was the first country in the World to implement nonfasting samples for cardiovascular risk prediction as early as in 2009.

In Papers IV to VIII we investigated morbidity and mortality associated with the genetically determined lipoprotein(a). We included genetics in the analyses used thereby enabling us to investigate any causal associations and to get closer to the physiological and pathophysiological role of lipoprotein(a) in the human body. In Paper IV we suggested that elevated lipoprotein(a) should be included in the diagnosis of familial hypercholesterolemia. Due to our very large dataset, the availability of the most common FH causing mutations in Denmark and the availability of data

to categorize all participants according to the diagnostic criteria of DLCN, we were able to show that 25% of individuals getting a clinical diagnosis of familial hypercholesterolemia gets it due to high lipoprotein(a) levels. In the search for possible lowering agents of high lipoprotein(a), PCSK9 inhibitors did for a while look like a promising possibility and in Paper V we used genetic information on the *PCSK9* R45L loss-of-function mutation and found it to be associated with low lipoprotein(a) levels and low risk of both aortic valve stenosis and myocardial infarction; however, later randomized clinical trials showed that lowering of lipoprotein(a) by PCSK9 inhibitors is somewhat limited. PCSK9 genetic variation represents an example of how observational and genetic population based prospective studies can help test hypotheses that eventually can lead to a randomized controlled trial and finally to the introduction of new medication. There is currently a consensus in academic societies that lipoprotein(a) is indeed a causal risk factor for cardiovascular disease, which makes it increasingly important to uncover its role in the body. Paper VI in the present thesis, where we found high lipoprotein(a) levels to be associated with low risk of brain and airways bleeding, was an exploratory paper of the physiological role of lipoprotein(a) and helped us discover potential problems in lowering of lipoprotein(a) to very low levels. In Paper VII we found high lipoprotein(a) levels to be associated with risk of all-cause and cardiovascular mortality, which was only possible due to our very large population size, as not many phenotypes can be associated with risk of the hardest of all endpoints, namely mortality. In the last Paper VIII, due to our extensive and complete Danish nationwide registries, we were able to investigate if lowering of lipoprotein(a) is safe as we await the results of randomized trials investigating the cardiovascular effect of reducing lipoprotein(a) levels by antisense oligonucleotides. We have in all our studies tried to adjust for all possible available confounders; however, as mentioned before, residual confounding can be difficult to account for. We did try in some analyses to account for this by performing sensitivity analyses, but the metabolic changes occurring before diagnosing a disease

cannot be accounted for; however, we did use the Mendelian randomization design in many of our studies, which partly account for the mentioned shortcomings of observational studies. Nevertheless, randomized intervention trial remains the gold standard for delineating causal pathways and ultimately show potential treatment benefits and harms of substantial lipoprotein(a) lowering.

Summary in English

The current thesis includes 8 original papers published in peer-reviewed journals, a methods section, a section investigating nonfasting lipid profiles for cardiovascular risk prediction, and a section on lipoprotein(a) in relation to morbidity and mortality.

Background

For cardiovascular risk prediction the standard lipid profile most often includes plasma triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol and it has been the standard to measure the lipid profile in the fasting state; however, the scientific evidence to support this is scarce. Another lipoprotein suitable for cardiovascular risk prediction is lipoprotein(a) and since its discovery in 1963 it has been a part of cardiovascular research, with exceptionally many articles published within the last decade. High levels of lipoprotein(a) have now been causally and observationally associated with high risk of atherosclerotic stenosis, myocardial infarction, aortic valve stenosis, heart failure, ischemic stroke, and cardiovascular and all-cause mortality.

Findings of the present thesis

- After adjustment for albumin levels, the levels of total cholesterol and LDL cholesterol did not change in response to normal food intake; however, plasma triglycerides increased modestly.
- For individuals with diabetes levels of total, non-HDL, LDL, and HDL cholesterol only changed minimally in response to normal food intake, while plasma triglycerides increased modestly.
- Risk of ischemic heart disease and myocardial infarction increased with stepwise higher nonfasting total cholesterol and nonfasting triglycerides. Risk of all-cause mortality only increased with higher levels of nonfasting triglycerides, not with higher levels of nonfasting total cholesterol.

- 25% of individuals getting a clinical diagnosis of familial hypercholesterolemia gets it due to high lipoprotein(a) levels and individuals with clinical familial hypercholesterolemia combined with high lipoprotein(a) levels have the highest risk of myocardial infarction.
- The R46L loss-of-function mutation in the *PCSK9* gene was associated with low lipoprotein(a) levels and low risk of aortic valve stenosis and myocardial infarction.
- High lipoprotein(a) levels were associated observationally and genetically with low risk of major bleeding in brain and airways.
- High lipoprotein(a) levels and corresponding low *LPA* KIV-2 number of repeats were associated with high risk of all-cause and cardiovascular mortality.
- Apart from the known association with cardiovascular disease, low levels of lipoprotein(a) and associated *LPA* genotypes were not found to associate concordantly with any major diseases including cancers and infections.

Conclusions

The recommendation of introducing nonfasting samples for lipid profiles at cardiovascular risk prediction is spreading widely throughout the world. This will not only simplify blood sampling for patients, laboratories, and clinicians alike, but likely lead to better risk prediction and thereby better treatment, eventually leading to reduction of atherosclerotic cardiovascular disease if preventive treatment using lipid-lowering drugs is correctly initiated.

Lipoprotein(a) is established as a causal risk factor for cardiovascular disease, but is at general clinics not considered as part of the lipid profile for cardiovascular risk prediction, mainly because no treatment is available to lower lipoprotein(a) levels in a safe and effective manner. However, a current trial using antisense oligonucleotides assesses the impact of lowering lipoprotein(a) levels by up to 90% on risk of cardiovascular disease in high risk patients.

Dansk resume

Denne afhandling indeholder 8 originale artikler, en metode sektion, en sektion omhandlende ikkefastende lipider og en sektion om lipoprotein(a) i relation til sygdom og dødelighed.

Baggrund

Ved risikostratificering for hjertekarsygdom bruger man en standard lipid profil som oftest inkluderer plasma triglycerider, totalt kolesterol, LDL-kolesterol og HDL-kolesterol. Det har tidligere været normalt at måle lipider fastende, selvom den videnskabelige evidens herfor er sparsom. En anden potentiel risikofaktor til risikostratificering er lipoprotein(a) som siden det blev opdaget har haft en vigtig rolle i forskningen af hjertekarsygdomme. Højt niveau af lipoprotein(a) i blodet er nu fastslået som en kausal risikofaktor for stenose i pulsårer, blodprop i hjertet, hjerteklap-stenose, hjertesvigt, iskæmisk slagtilfælde og høj dødelighed.

Resultater

- Niveauerne af totalt kolesterol og LDL-kolesterol ændrer sig ikke efter normalt mad indtag, når der justeres for væskeindtag i form af albumin niveau, hvorimod plasma triglycerider stiger moderat.
- For patienter med diabetes ændrer totalt kolesterol, LDL-kolesterol og HDL-kolesterol sig kun minimalt efter normalt mad indtag, hvorimod plasma triglycerider stiger moderat.
- Der er øget risiko for blodprop i hjertet ved øgede niveauer af ikke-fastende totalt kolesterol og ikke-fastende triglycerider. For dødelighed er der kun øget risiko ved øgede niveauer af ikke-fastende triglycerider og ikke ved høje niveauer af totalt kolesterol.
- 25% af de patienter som klinisk diagnosticeres med familiær hyperkolesterolæmi får deres diagnose fordi de har høje lipoprotein(a) niveauer og dem med både familiær hyperkolesterolæmi og høje lipoprotein(a) niveauer har den højeste risiko for at få en blodprop i hjertet.

- R46L mutationen i *PCSK9* genet var associeret med lave lipoprotein(a) niveauer og lav risiko for aortastenose og blodprop i hjertet.
- Høje lipoprotein(a) niveauer var associeret med lav risiko for blødninger i hjernen og i luftvejene.
- Høje lipoprotein(a) niveauer og tilsvarende lavt antal af *LPA* KIV2 var associeret med høj risiko for at dø af enhver årsag og at dø af hjertekarsygdomme.
- Foruden den allerede kendte association med hjertekarsygdom fandt vi at lave lipoprotein(a) niveauer ikke var associeret med risiko for de store sygdomsgrupper inklusiv kræft og infektioner.

Konklusion

At indføre ikke-fastende lipid målinger til brug for risikostratificering ville gøre blodprøvetagning mere simpel for patienter, laboratorier og klinikere over hele verden og det ville også medføre bedre risikovurdering og dermed mere optimal behandling som i den sidste ende vil føre til mindre risiko for hjertekarsygdom, hvis forebyggende behandling bliver implementeret hos de rette personer. Lipoprotein(a) er en etableret kausal risikofaktor for hjertekarsygdom, men er de fleste steder ikke brugt som risikomarkør for hjertekarsygdom primært fordi der aktuelt ikke findes en effektiv og sikker behandling til at sænke lipoprotein(a) i blodet. Aktuelt er der dog et randomiseret studie, som undersøger om en ny behandling kan nedbringe risikoen for hjertekarsygdomme ved at sænke lipoprotein(a) i blodet.

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