Novel insights into blood markers and cardiovascular disease: Results of the Netherlands Epidemiology of Obesity study

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CHAPTER 1

GENERAL INTRODUCTION

The research in this thesis is aimed at describing and understanding the role of factors related to body fat and body fat distribution in atherosclerosis and cardiovascular disease.

Atherosclerosis and cardiovascular disease

Cardiovascular disease (CVD) is a major cause of mortality, in 2015 17.7 million people died from CVD worldwide. [1] In the Netherlands alone, 38,647 people died from CVD in 2016. [2]

One of the most prominent markers of subclinical cardiovascular disease is atherosclerosis. Atherosclerosis is a progressive disease of the vessel wall, characterized by the accumulation of mainly low-density lipoprotein (LDL)- cholesterol and pro-inflammatory cells in the intima of the vessel. [3] Further development of atherosclerotic lesions is characterized by the proliferation of vascular smooth muscle cells in the media of the vascular wall [4], increased vascularization of the adventitia [5, 6], decreased responsiveness of the vascular smooth muscle cells to vasodilators [7], and endothelial wear. [8] Recent studies suggested that the pathology of atherosclerosis differs between women and men, in the sense that lesions typical for men are less dense and have a thin fibrous cap that is prone to rupture while lesions in women often have a more stable fibrous cap, but suffer from endothelial wear. [9]

Atherosclerosis may cause cardiovascular disease when it decreases the diameter lumen of a coronary artery and the restricted blood flow induces chest pain or dyspnoea with physical activity or cold-induced stress, i.e., angina pectoris, or when it causes total blockade of blood flow by its rupture and subsequent thrombus formation, i.e., myocardial infarction. In the brain, this may lead to ischaemic stroke. [10, 11]

Body fat distribution and atherosclerosis

One of the main risk factors for atherosclerosis is excess body fat, specifically body fat localised around the organs in the abdominal cavity, which is referred to as visceral fat. [12] Well-known underlying mechanisms in this association are low-density lipoprotein cholesterol (LDL-c), hypertension and diabetes, but there are several novel mechanisms that are under investigation for their potential role in this association, such as other blood lipids, inflammation and adipocytokines.

Traditional risk factors for atherosclerosis

Multiple factors contribute to the development of atherosclerosis. Genetic, lifestyle and environmental factors interact and therefore establish a complex causal structure that may eventually lead to cardiovascular disease. Since the conception of the famous Framingham Heart Study in 1948 [13], a plethora of studies have set out to investigate risk factors of atherosclerosis. Prominent and well-studied risk factors that resulted from these endeavours include smoking, high LDL-cholesterol concentrations, high blood pressure, and diabetes, which may in turn be caused by a lack of physical activity, stress, dietary habits, or obesity. [14, 15]

Overweight and obesity are increasingly prevalent risk factors for atherosclerosis. In 2017, almost half of the population in the Netherlands had overweight or obesity, defined as a body mass index of 25 or 30 kg/m^2 or higher. [16] Despite the ease of measurement of body mass index as a measure of overweight, in terms of cardiovascular risk visceral fat located in the abdominal cavity is regarded as a stronger cardiometabolic risk factor than the overall amount of body fat due to its higher metabolic activity and production of inflammatory markers and adipocytokines. [17] Visceral fat accumulation can be measured in research settings using computed tomography (CT) or magnetic resonance imaging (MRI), or approximated in clinical practice by measuring waist circumference.

While obesity approximately doubles the risk of cardiovascular disease, the mechanisms that explain this relation are not yet fully understood. As illustrated in Figure 1, the main cardiometabolic risk factors dyslipidaemia, hypertension, and diabetes are responsible for only half of the excess cardiovascular risk associated with obesity, acting as mediators of the relation between obesity and cardiovascular disease. [18] Consequently, half of the excess cardiovascular risk associated with obesity is yet unexplained. Quantifying the role of novel risk factors is essential to be able to improve identification of persons at risk for cardiovascular disease. Several other factors have been suggested as candidate intermediate mechanisms, such as HDL-cholesterol, postprandial triglyceride response [19], cholesteryl ester transfer protein (CETP) [20], adipokines [21], and inflammation [22]. The role of these factors in the development of subclinical atherosclerosis remains unclear.

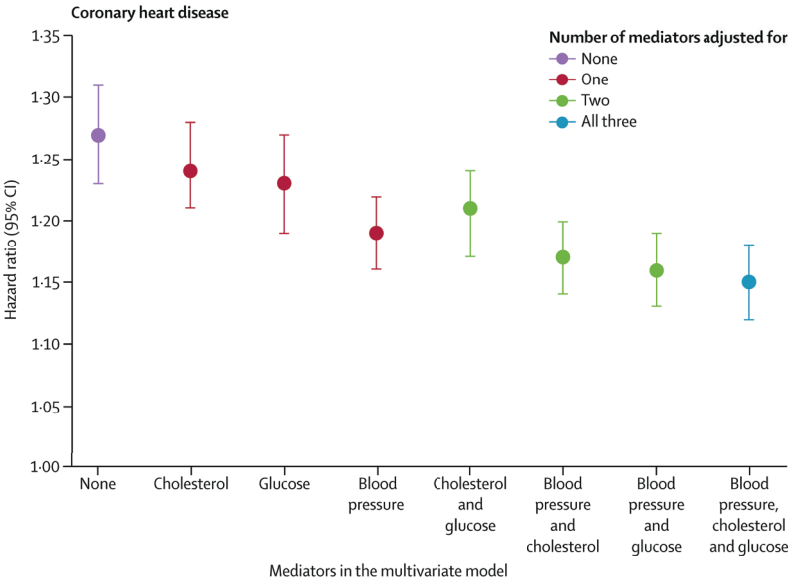

Figure 1 – Estimation of the relative contributions of metabolic mediators in the excess risk of coronary heart disease, separately and in combination. Adapted from The Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration. The Lancet 2014; 383:9921; 970-983.

Adipokines and inflammation

In the 1990s it became clear that adipose tissue does not merely store energy in the form of fat, but also secretes regulatory substances, such as inflammatory cytokines and adipose tissue specific peptide hormones. Adiponectin and leptin are hormones that are specifically secreted by adipose tissue, and therefore also referred to as adipokines or adipokine hormones. [23, 24]

Adiponectin (from Latin *adipo*, "fat"; *nectin*, "join, tie") is mainly produced by ectopic (visceral) fat, located in the abdominal cavity, and notably secretion of adiponectin is higher with lower amounts of visceral fat. [25, 26] The main physiological functions of adiponectin are yet unclear, but adiponectin has predominantly been described as a protective factor in the development of insulin resistance and type 2 diabetes. [27, 28]

In contrast, leptin (from Greek *leptos*, "small, slight, slender, delicate") is mainly produced by subcutaneous adipose tissue, and its primary function is to inhibit energy intake by signalling to the hypothalamus and activating the proopiomelanocortin (POMC) pathway. [29] Also, several studies suggested that leptin has off-target effects, which may be detrimental for cardiovascular health. [30-34]

However, these studies often have important limitations in design or available measurements. The main difficulty is the strong relation between body fat and serum leptin concentration, which may obstruct our view on potential underlying effects of leptin, because it is unknown to what extent leptin is a confounding factor, mediator, or merely a marker of body fat. Methods to circumvent this problem are statistical adjustment for body fat, or using genetic variants that predispose for higher serum leptin.

In the last three decades, the view of atherosclerosis as an inflammatory disease has emerged. [3] This is demonstrated by the higher risk of cardiovascular disease in the presence of several autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematous, which is even aggravated by the use of systemic corticosteroid treatment. [35, 36] The foremost factor suggesting a direct role for inflammation in the progression of atherosclerosis is the activation of macrophages in atherosclerotic lesions upon systemic inflammation. [37]

These macrophages incorporate LDL-cholesterol, which is subsequently oxidized causing the macrophages to form foam cells. [38] Abundance of pro-inflammatory immune cells and foam cell formation marks lesions at high risk for rupture, which motivated the development of anti-inflammatory therapies aimed at prevention of cardiovascular disease. The recent Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) indeed indicated a cardioprotective role of the anti-inflammatory drug canakinumab that targets interleukin-1β, but with a null effect on total mortality due to an increase in infection risk. [39] The potential role for inflammation is especially interesting in conditions of excess body fat, because body fat and specifically visceral fat depots have been associated with low-grade pro-inflammatory state. [40] The relative contribution of inflammation to atherosclerosis, as compared to other risk factors has not been studied in detail on a population level.

Lipids and lipoproteins

Overweight and obesity have a well-described and profound detrimental impact on the circulating lipoprotein profile, in particular an increase in atherogenic LDL-cholesterol. [41] LDL-cholesterol, together with its main structural component apolipoprotein B 100 (ApoB) is one of the most important constituents of atherosclerotic lesions. It contributes to lesion formation by its internalization in the vascular wall, and to lesion destabilization by being oxidized and being taken up by macrophages that subsequently form foam cells. Two main classes of LDL-cholesterol reducing and CVD-preventing drugs are currently being used: inhibition of the HMG-CoA reductase pathway using statin therapy, and advanced adjuvantia like PCSK9-inhibitors. [42]. However, as a side effect, both

LDL-cholesterol lowering treatments may lead to a small increased risk of type 2 diabetes. [43, 44]

Besides the well-studied effects of LDL-cholesterol on atherosclerosis, the interest has shifted towards other lipoprotein subclasses.

Specifically, the role of high-density lipoprotein (HDL)-cholesterol is highly debated. While low HDL-cholesterol concentrations are strongly associated with atherosclerosis and cardiovascular disease in observational studies, Mendelian randomisation studies have shown that on a population basis low levels of HDL-cholesterol have no causal effect on cardiovascular disease. [45] Consistently, clinical trials that aimed to increase HDL-cholesterol concentrations by inhibiting a key player in cholesterol metabolism, cholesteryl ester transfer protein (CETP) have all shown no effect of drug-induced HDL-cholesterol increase. [46-48] However, the failure of these trials is in contrast to previous reports of a cardioprotective role of genetic variants with a CETP-lowering effect. [20, 49, 50] Interestingly, the genetic studies that found a deleterious effect of CETP on cardiovascular disease were mainly performed in men and diabetic women. [20, 51] This suggests that the effect of CETP on the development of atherosclerosis and cardiovascular disease may be different in subgroups of the population.

Furthermore, fasting triglyceride concentrations have been suggested to be a risk factor for atherosclerosis. [52] However, many people are not in a fasting state for the majority of the day. Therefore non-fasting or postprandial triglyceride concentrations may be a better reflection of the total exposure of the vascular wall to triglycerides during the day. [53]

In all aforementioned mechanisms, there is a potential bias by confounding factors, i.e. common causes of the exposure and the outcome that may explain or distort the association of interest. [54] A factor that is often regarded as confounding factor is sex. In this thesis, sex is relevant as it is a determinant of adipose tissue accumulation and distribution [55], while it is also a determinant of increased cardiometabolic risk. [56] This causal structure is complicated further by the possibility that the relation between sex and atherosclerosis is partly mediated by visceral fat. There are several methods to mitigate the effects of confounding factors, of which adjusting using multivariable regression analysis is the method generally used in epidemiologic studies. Although adjustment in regression analysis is an efficient method to mitigate confounding effects while retaining statistical power, this method prohibits the interpretation of within-group effects in the case of effect modification. To facilitate interpretation of within-group effects, we aimed to stratify most analyses for sex. Other methods to reduce confounding effects include restriction, matching, and (Mendelian)

randomisation. [57] In this thesis, several manuscripts include Mendelian randomisation techniques, which are based on the random heritability of genetic variants that predispose for the exposure of interest. This implies that confounding factors would not be able to affect the genetically-determined part of the exposure phenotype. Therefore it is possible to use observational data to answer causal questions. [58]

Outline of the thesis

The aim of this thesis is to describe and improve understanding of the role of factors related to overweight and obesity in the development of cardiovascular disease.

This thesis can be divided into two main sections. The first section focuses on the role of inflammation and the adipokines leptin and adiponectin in the association between body fat and atherosclerosis. The second section includes studies that investigate determinants and effects of serum lipids and lipoproteins. Where possible, sex differences were investigated as well in the studies in this thesis. The main objectives of this thesis are visualised in the directed acyclic graph (DAG) in Figure 2.

In **chapter 2**, we studied to what extent the association between measures of total body fat and visceral fat, and subclinical atherosclerosis was mediated by measures of inflammation.

In **chapter 3** we investigated to what extent the sex difference in leptin and adiponectin concentrations were due to sex differences in body fat content and distribution. In **chapters 4 and 5**, potential cardiovascular effects of leptin and adiponectin are disentangled from cardiovascular effects of body fat and body fat distribution.

Chapter 6 builds on the long-standing controversy regarding the effects of CETP on cardiovascular disease. Considering previous genetic studies that showed marked effects of CETP in subgroups of the population we hypothesized that the effect of modulation HDL-cholesterol on atherosclerosis may be different in subgroups of the population (effect modification). Therefore we aimed to extensively investigate the relations of observational and genetically-determined CETP concentration with subclinical atherosclerosis in subgroups of the general population.

The role of the residual postprandial triglyceride response in the relation between HDL-cholesterol and coronary artery disease is investigated in **chapter**

7, and we studied the contribution of postprandial triglyceride excursions to subclinical atherosclerosis, in addition to the effects of fasting triglyceride concentrations in **chapter 8**.

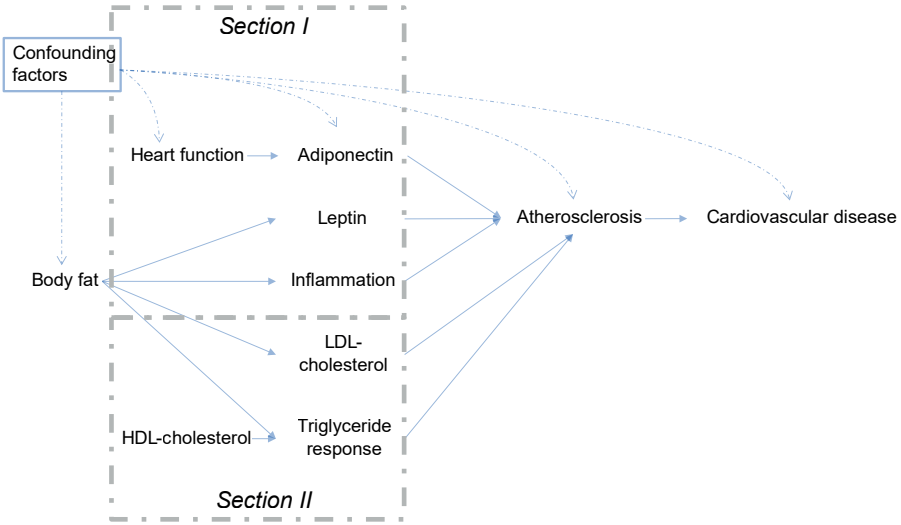

Figure 2 – Hypothesis diagram of the research presented in this thesis. In section I, mechanisms of inflammation and adipocytokines are described, while section II focuses on lipids.

Study populations

The observational analyses that are presented in this thesis were predominantly performed in the Netherlands Epidemiology of Obesity study (NEO study). The NEO study is a prospective cohort study including participants aged 45-65 years from the general area of Leiden, the Netherlands. Men and women with a self-reported body mass index (BMI) of 27 kg/m^2 or higher were eligible to participate. In addition, inhabitants of Leiderdorp (adjacent to Leiden) were invited to participate irrespective of their BMI. Unique aspects of the NEO study are the large population-based sample, detailed body fat measurements by MRI, extensive phenotyping including blood sample analyses and questionnaire data, metabolomic profiling, as well as a whole genome scan. A detailed description of the design and data collection of the NEO study can be found elsewhere. [59]

Participants with a BMI of 27 kg/m^2 were overrepresented in this study, which requires adjustment in order to be able to generalise results to a general population. All analyses presented in this thesis were performed weighted towards the BMI distribution of the participants of Leiderdorp, who had a BMI distribution that was similar to the BMI distribution of the Dutch population. [16, 60] Consequently, the results that are presented in this thesis apply to the general popula-

tion.

In addition, we performed several two-sample Mendelian randomization analyses in which we used external sources of genetic instruments and publicly-available summary statistics of genome-wide association studies. These Mendelian randomization analyses were performed on serum leptin concentrations, peak wave velocity and coronary heart disease (**chapter 4**), serum adiponectin and N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations, and measures of heart function (**chapter 5**), and serum lipid concentrations in **chapter 7**. Therefore data from several GWAS were used.

The CARDIoGRAMplusC4D consortium performed a GWAS on the risk of coronary heart disease in a case-control setting with 60,801 cases and 123,504 controls of European descent from 48 studies. [61] Coronary heart disease diagnoses included myocardial infarction, acute coronary syndrome, chronic stable angina or coronary stenosis of >50%.

The GWAS on pulse wave velocity as a measure of vascular function was performed within the UK Biobank project. [62] Pulse wave velocity was measured in 117,867 genotyped participants by using an infrared sensor at the fingertip.

The GWAS on serum leptin concentrations was performed in 32,161 participants of European descent from 23 studies. [63]

We used publicly-available summary statistics of the GWAS on adiponectin concentration that was performed by the ADIPOGen consortium in 39,883 individuals from European descent. [64]

The GWAS on circulating NT-proBNP concentrations was performed in 9,232 individuals with acute coronary syndrome, of whom 99% were of European descent, who participated in the PLATO trial. [65]

Genetic variants that predispose for heart structure and function were used from a GWAS by the EchoGen consortium, that was performed in 32,212 individuals of mostly European ancestry. [66]

The genetic variants that are associated with serum triglyceride, LDL-, and HDL-cholesterol concentrations were discovered in a GWAS that was performed by the Global Lipids Genetics Consortium, which was performed in 188,577 individuals of European, South Asian, and African ancestry [67]