Adult weight change and cardiometabolic disease: studies into underlying pathways

Verkouter, I.

CHAPTER 1

General introduction and outline of this thesis

ABSTRACT

Body weight gain during the life course is a well-established risk factor for type 2 diabetes mellitus and cardiovascular diseases. However, the mechanisms that underlie this relation between body weight gain and cardiometabolic diseases are still largely unknown. The main aim of this thesis was to study the cardiometabolic consequences of obesity and weight gain during the life course.

We aimed to investigate the association between body mass index (BMI) and cardiometabolic disease using Mendelian randomization in which we were particularly interested in whether the underlying causes of high BMI (e.g., different gene expression in brain or peripheral/adipose tissues) were differentially related to risk of diabetes or cardiovascular diseases. We identified 17 tissue-grouped gene sets, where BMI-associated genes were differentially expressed. However, in tissue-grouped Mendelian randomization analyses, all BMI-associated gene sets were similarly associated with increased risks of diabetes and coronary artery disease, and thus we argued that regardless of the cause of a high BMI, the risks of diseases as type 2 diabetes mellitus and coronary artery disease are similar.

We observed that abdominal adiposity in adolescence was associated with early changes in metabolomic measures indicative of an atherogenic profile already present at young adulthood, but this was only observed in young men. Also, when we investigated weight gain during adulthood, this was specifically related to an atherogenic metabolic profile, in addition to increased adipocyte size. Adult weight gain between age 20 years and middle age was associated with increased visceral fat and liver fat at middle age irrespective of total body fat. In addition, the association between adult weight gain and insulin resistance at middle age was partly mediated by the increased levels of visceral fat and liver fat at middle age. Lastly, not all individuals with obesity develop cardiometabolic disease. We observed that a favourable body fat distribution as well as metabolic profile are associated with a decreased risk of incident cardiometabolic disease in a population with obesity.

In conclusion, the results described in this thesis suggest that the cardiometabolic consequences of weight gain during both adolescence and adulthood are mediated by the amount of visceral and ectopic fat, and are reflected by a more atherogenic metabolomic profile and increased cardiometabolic risk. Once obese, preserving low glucose levels, non-smoking, and preventing abdominal obesity are important to prevent the onset of cardiometabolic disease. Overall, the results of this thesis emphasize the importance of maintaining a stable body weight during young adulthood throughout middle age. With increased attention being given to promoting a healthy lifestyle, there is potential for cardiometabolic disease prevention in promoting a healthy body weight during the life course.

GENERAL INTRODUCTION

The main objective of this thesis was to study the cardiometabolic consequences of weight gain during the life course. Body weight gain is a well-established risk factor for cardiometabolic disease, but the underlying pathways are still largely unexplored. This general introduction describes the epidemiology of overweight and obesity, the current knowledge about body weight gain during adulthood and how it may influence body fat distribution, the metabolomic profile, and risk of cardiometabolic disease. In addition, this introduction addresses the existing gaps in knowledge on this research topic and introduces the studies we conducted to address these knowledge gaps.

Overweight and obesity: a growing public health problem

Overweight and obesity are characterized by the accumulation of excess body fat, ultimately leading to chronic (cardiometabolic) disease. Body mass index (BMI), calculated as body weight in kilograms divided by the square of height in meters (kg/m^2) is commonly to classify overweight and obesity. According to the World Health Organization, a normal body weight in people from European descent is defined by a BMI between 18.5 and 24.9 kg/m^2, overweight is defined by a BMI between 25.0 and 29.9 kg/m^2, and obesity by a BMI above 30.0 kg/m^2 (1).

The worldwide prevalence of obesity doubled between 1980 and 2015 (2). In 2016, 39% of adults aged 18 years or older had overweight, and 13% of the total world's adult population were obese (3). This trend is also visible in the Netherlands: in 1990 one in three Dutch adults had overweight or obesity and this has increased to half of all Dutch adults in 2019 (4). In addition to overweight and obesity in the adult population, the global prevalence of obesity among children and adolescents has risen from 4% in 1975 to over 18% in 2016 (3). Childhood obesity is associated with insulin resistance, dyslipidaemia and the metabolic syndrome (5-7), as well as an increased risk of cardiometabolic disease and increased mortality later in life (8-10). Obesity during childhood and adolescence tends to track into adulthood (11).

Obesity in adulthood is a well-established and causal risk factor for type 2 diabetes, and weight loss interventions also show to decrease the risk of type 2 diabetes (12, 13). In addition, overweight and obesity are strong causal risk factors for cardiovascular disease (14) and its risk factors such as hypertension and dyslipidaemia (15). Obesity is also associated with increased mortality due to all cancers combined as well as cancer at specific sites, including the oesophagus, colon and rectum, liver, gallbladder, pancreas, and kidney (16). Lastly, from 1990 to 2017, the global deaths attributable to high BMI have more than doubled for both men and women (17). However, it is important to note that not everyone with overweight or obesity will develop cardiometabolic abnormalities or disease (18). Insights in additional risk factors that need to be averted to reduce the risk of future obesity-related disease are likely to yield particular targets for interventions which show the largest reduction in cardiometabolic-disease risk.

Body fat distribution and ectopic fat

BMI is a widely used, non-invasive measure of overall adiposity. However, BMI does not distinguish between body fat and lean body mass: a high BMI might be reflected by either a high total body fat percentage, high lean body mass or a combination of both. Furthermore, BMI does not yield information on whether body fat is stored centrally at the abdomen, or peripherally at the hips and thighs. Body fat distribution differs greatly between men and women as men tend to store body fat at the abdomen, whereas women are more likely to store body fat peripherally (19, 20).

Abdominal adiposity is characterized by an increased storage of excess fat in the abdominal subcutaneous (under the skin) and visceral (around the organs) adipose tissue depots. According to the 'lipid overflow hypothesis', adipose tissue becomes dysfunctional when the capacity of hypertrophic adipocytes to expand is exceeded as a result of body weight gain (21, 22). This in turn leads to 'lipid overflow': the accumulation of triglycerides in visceral adipose tissue and ectopic fat deposition in normally lean organs such as the heart, skeletal muscles, pancreas, and liver (21-23). Compared to subcutaneous adipocytes, adipocytes in the visceral depot have a high secretion rate of non-esterified fatty acids, very low-density lipoproteins and cytokines, such as IL-6 and TNF-alpha, thereby inducing a systemic low-grade inflammatory state and oxidative stress (23-26). Finally, intracellular non-esterified fatty acid accumulation in non-adipose tissues leads to impaired insulin signalling and insulin resistance (27).

Previous studies have shown that both excess visceral fat and liver fat are more strongly related to the metabolic syndrome than abdominal subcutaneous adipose tissue (28, 29). Additionally, excess visceral adipose tissue is strongly associated with an adverse metabolic profile, indicated by insulin resistance (30), increased blood pressure, triglycerides, hypertension and presence of the metabolic syndrome (31). Precise measurements, such as magnetic resonance imaging (MRI) to directly assess the amount of visceral adipose tissue, may be useful in characterization of cardiometabolic risk. Additionally, to examine the accumulation of fat deposition in organs, for example the measurement of liver fat content, proton magnetic resonance spectroscopy (^{1}H-MRS) can be used. As both MRI and ^{1}H-MRS are expensive and time-consuming methods, waist circumference can be used in addition to BMI to measure fat deposition in and around the abdomen.

Body weight gain during adulthood

Overweight and obesity at middle age are preceded by a gain in body weight during the life course. Weight gain during adulthood has been associated with a considerable increased risk of major chronic diseases in middle-aged individuals, including type 2 diabetes mellitus, cardiovascular disease, and obesity-related cancers, regardless of BMI (32-34). However, the mechanisms that underlie the relation between body weight gain and cardiometabolic disease are still largely unknown. During adult weight gain, excess adipose tissue is stored in different areas of the body, depending on various factors including genetic variation, sex, age, ethnicity and lifestyle (35-37). However, it remains unclear how weight gain during adulthood is associated with body fat distribution at middle age.

It was previously suggested that the number of adipocytes remained constant in both lean and overweight individuals after age 20 years (38). This suggests that adult weight gain after the age of 20 years might result in hypertrophy of adipocytes, and storage of excess lipids in the visceral compartment or deposition at ectopic sites after the storage capacity of subcutaneous adipocytes is reached. Therefore, we hypothesized that the accumulation of visceral fat and ectopic fat as a consequence of body weight gain may explain the link between body weight gain and increased risk of type 2 diabetes later in life.

Metabolomic measures as intermediates of weight gain and cardiometabolic disease

High throughput metabolomic measures have emerged during recent years as important intermediates between risk factors, including obesity and lifestyle exposures, and cardiometabolic disease outcomes. Metabolomic measures include small molecule substrates, intermediates or products of metabolism. Examples of metabolomic measures are lipoproteins, amino acids and fatty acids. Previous studies on the metabolomic profile associated with high BMI have helped to further elucidate underlying pathways to cardiometabolic disease. In a cross-sectional study of 12,664 adolescents, it was observed that BMI was strongly associated with the concentrations of very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) and inversely associated with high-density lipoprotein (HDL) (39). Additionally, a high BMI has been associated with higher concentrations of monounsaturated fatty acids and saturated fatty acids, as well as branched-chain amino acids (39). In turn, these adiposity-related metabolomic measures were linked to cardiometabolic disease: in an observational study, NMR-based measures of circulating cholesterol and triglycerides in VLDL and LDL particles, Apolipoprotein B and glucose were strongly associated with the risk of myocardial infarction (40). Mendelian randomization analyses showed that both LDL cholesterol, triglycerides, and Apolipoprotein B play a causal role in the development of coronary heart disease (41, 42).

Despite the fact that these metabolic alterations may link adult weight gain with the onset of cardiometabolic disease, the metabolomic profile associated with body weight gain in different stages of the life course has not been clearly defined to date. Additionally, longitudinal studies of changes in metabolomic measures are lacking, whereas these studies could aid to identify adiposity-related changes in the metabolomic profile indicative of early atherogenic progression in young adults (39).

Novel approaches to using Mendelian randomization

Results from observational studies on obesity and cardiometabolic disease may be influenced (residual) confounding and reverse causation, thereby impairing causal inference of the exposure-outcome relationship. As randomized controlled trials for overweight or obesity are usually not feasible or ethical for long study periods, alternative approaches such as Mendelian randomization can provide information on the causality of obesity-outcomes relations. In Mendelian randomization studies, genetic variants associated with the exposure of interest, assigned randomly at conception, are used as instrumental variables. These instrumental variables are then related to the outcome thereby mimicking randomized controlled trials (43). Results from Mendelian randomization studies that fulfil all necessary assumptions do not suffer from confounding or reverse causation and can therefore

be causally interpreted. To date, genetic variants associated with body weight gain during adulthood still need to be elucidated. Previous studies identified many genetic variants associated with an increased risk of developing obesity and a high body mass index (44-46) that influence different molecular mechanisms and are expressed in different tissues, and could be used as instrumental variables in Mendelian randomization studies. The most recent Genome-Wide Association Study (GWAS) identified 656 independent genetic variants associated with BMI, which collectively explained up to 6% of the total population variation in BMI (46). By using the BMI-associated genetic variants as instrumental variables, Mendelian randomization studies strengthened evidence for a causal effect of both overall obesity and abdominal obesity on coronary heart disease, stroke and type 2 diabetes (47).

In an earlier MR study on the association between BMI and anxiety, SNPs were categorized based on three mechanistic domains through which they were likely to influence BMI: appetite, adipogenesis and cardiopulmonary function, derived from an earlier GWAS on BMI (48). Based on this categorisation, we hypothesized that the characterization of genetic variants that are associated with BMI, and the genes corresponding to them, can provide insights into the heterogenous biological causes of a high BMI, which may be differentially related to disease risk. For example, BMI genes related to adipogenesis may be differentially related to cardiovascular risk than BMI genes related to cardiopulmonary function. A more detailed investigation of the differential causes of complex traits such as obesity will allow a comprehensive overview of potential specific targets for (personalized) interventions.

OUTLINE OF THIS THESIS

Previous studies have investigated the consequences of body weight gain during (young) adulthood; however, significant gaps in the scientific knowledge remain. To date, the molecular and metabolomic pathways underlying the relationship between adult weight gain and cardiometabolic disease are still largely unknown. Therefore, the objective of this thesis was to study the cardiometabolic consequences of obesity and weight gain during the life course.

Genome-wide associations studies on BMI already identified many genetic variants associated with an increased risk of developing a high BMI that influence different molecular mechanisms and are expressed in different tissues. In **Chapter 2**, we investigated the association between BMI and cardiometabolic disease using Mendelian randomization, in which we were particularly interested in whether the underlying genetic causes of high BMI (e.g., different gene expression in brain or peripheral/adipose tissues) are differentially related to risk of diabetes or cardiovascular diseases.

As childhood obesity is often carried over into adulthood, it is important to study changes in the metabolomic profile associated with obesity in young adulthood, which might provide opportunities to prevent the cardiometabolic consequences of obesity already during childhood. In **Chapter 3**, we therefore aimed to investigate the relations between trunk fat and total fat mass with changes in the concentrations of metabolomic measures during young adulthood in men and women.

Body weight gain during adulthood is associated with an increased risk of cardiometabolic disease. We hypothesized that specific metabolomic alterations as a consequence of adult weight gain indicate the onset of cardiometabolic disease. In **Chapter 4**, we investigated which metabolomic measures are specifically associated with adult weight gain, as opposed to those associated with BMI at age 20 years or BMI at middle age. We also examined the relation between adult weight gain and its identified specific metabolomic measures with adipocyte volume.

We hypothesized that the accumulation of visceral fat and ectopic fat as a consequence of body weight gain may explain the link between body weight gain and increased risk of type 2 diabetes later in life. Therefore, in **Chapter 5**, we investigated the associations of adult weight change with visceral adipose tissue and hepatic triglyceride content at middle age, taking into account total body fat at middle age. In **Chapter 6**, we further elaborated on the work described in **Chapter 5** by investigating to what extent the association of adult weight gain with insulin resistance was mediated by the amounts of visceral fat and liver fat at middle age.

Once adult weight gain has resulted in obesity, there is substantive heterogeneity in the onset of cardiometabolic disease. Not all individuals with obesity develop cardiometabolic diseases. Insights in factors may prevent the risk of cardiometabolic disease in individuals with obesity may yield particular targets for interventions. In **Chapter 7**, we investigated

which potential risk factors, including measures of body fat distribution, metabolic factors, and lifestyle factors, are needed for obesity to result in cardiometabolic disease. Finally, in **Chapter 8** we provide an overview of the main findings from the studies described in this thesis, discuss the strengths and limitations and interpretation of the results, and provide implications and future research directions.

Study designs and populations used in this thesis

For the study described in **Chapter 2**, we used summary-level data from several publicly available Genome-Wide Associations studies (body mass index [GIANT], type 2 diabetes mellitus [DIAGRAM], coronary artery disease [CARDIoGRAMplusC4D], waist circumference [GIANT] and total body fat [the Neale Lab]) (46, 49-52). The study described in **Chapter 3** has been performed in the Avon Longitudinal Study of Parents and Children (ALSPAC), a population-based birth cohort study from the United Kingdom (53) ALSPAC included 13,988 children in 1991, who have been followed since. Most studies described in this thesis (**Chapter 4, 5, 6 and 7**) were performed in the Netherlands Epidemiology of Obesity (NEO) study (54). This is a population-based cohort of 6671 individuals aged 45 to 65 years, with an oversampling of individuals with BMI≥27 kg/m^2, living in the West of the Netherlands. Participants of the NEO study were recruited between 2008 and 2012 and have been followed since. Next to data from the NEO study, for the study described in **Chapter 4** we used data from the Oxford Biobank, which is a population-based cohort study from Oxfordshire, United Kingdom (55). The Oxford Biobank recruitment began in 1999 and included 7640 participants in 2016.