Adult weight change and cardiometabolic disease: studies into underlying pathways

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CHAPTER 8

General discussion and summary of the main results

GENERAL DISCUSSION AND SUMMARY OF THE MAIN RESULTS

The main objective of this thesis was to study the cardiometabolic consequences of obesity and weight gain during the life course. Here, we will discuss and interpret the findings of the chapters described in this thesis. Furthermore, we will discuss the implications and future perspectives in the field of obesity, body weight gain and cardiometabolic disease.

Summary of main findings

In Mendelian randomization studies, genetic variants associated with an exposure of interest, assigned randomly at conception, are used as an instrumental variable to approximate the association between a life-long exposure to a certain risk factor and an outcome (43). Thereby, Mendelian randomization can be used to approximate a causal association between exposure and outcome using observational data in the absence of residual confounding and reverse causation. The field of Mendelian randomization is rapidly evolving, and novel methodological approaches are published every month in the scientific literature. For example, novel approaches to group genetic variants may provide insights in distinct processes underlying heterogenous, complex traits such as obesity (56, 57). In **Chapter 2**, we designed and applied another novel methodological approach to group BMI-associated genetic variants based on their expression in different tissues in the body. We hypothesized that a high BMI could result in a different cardiometabolic disease risk profile, depending on the underlying processes in different tissues that may have caused the high BMI. We identified 17 tissue-grouped gene sets, where BMI-associated genes were differentially expressed, mostly in several brain areas. These tissue-grouped BMI-associated genetic variants were used as exposures in two-sample Mendelian randomization analyses on cardiometabolic disease and anthropometry measures. We observed that tissue-grouped BMI-associated genetic variants were similarly associated with increased risks of type 2 diabetes and coronary artery disease. This suggests that the grouping of genetic variants based on tissue expression profiles does not yield a different risk profile for type 2 diabetes and coronary artery disease risk. These results were supported by findings from additional analyses, in which we randomly selected 100 or 200 genetic instruments from the 633 BMI-associated genetic variants. After we repeatedly performed Mendelian randomization analyses on T2DM and CAD with randomly sampled BMI-associated gene sets, the distribution of the effect estimates was similar to the results of the tissue-grouped MR analyses. We therefore concluded that our novel approach, based on tissue eQTL expression levels, does not suggest that the cause-specific increase in BMI (depending on the tissue expression level) gives an altered risk of cardiometabolic diseases. However, other novel approaches have been developed by others since we conducted this study, which may provide new and necessary insights to further grasp the heterogeneity of the obesity phenotype in future studies (56, 57). Furthermore, we cannot exclude the possibility that our novel analysis approach could be of value for addressing scientific questions other than the obesity-cardiometabolic disease relationship. A more refined Mendelian randomization analysis, in which more biological data is incorporated, might be an additional step towards a more personalized medicine approach and result in suitable and effective targets for interventions tailored to a specific cause underlying the high BMI. Evidence supporting this hypothesis has been provided recently by others examining insulin-like growth factor 1 (IGF-1) and type 2 diabetes mellitus: depending on certain data-driven clusters, the effect of IGF-1 on type 2 diabetes mellitus

was differential, and genes mapping to the instruments in the different clusters were part of different biological pathways (58).

Obesity in childhood and adolescence tends to carry over into adulthood (11), and there is a window of opportunity to reduce the cardiometabolic consequences of body size that unfold later in life already during childhood. However, it is unknown how body fat distribution during adolescence is associated with subsequent early changes in circulating metabolites. In **Chapter 3**, we investigated the consequences of overall and abdominal obesity at adolescence on changes in metabolomic measures during young adulthood in the Avon Longitudinal Study of Parents and Children (ALSPAC). We observed that abdominal adiposity in adolescence was associated with early changes in metabolomic measures indicative of a pro-atherogenic profile, including higher concentrations of very-low density lipoprotein, higher Apolipoprotein B and lower high density lipoprotein levels, in young adulthood mainly in men. This finding is in line with another prospective study in ALSPAC, in which the authors observed that the atherogenic consequences of adiposity on several metabolomic measures, including LDL cholesterol, triglycerides in VLDL and Apolipoprotein B, were stronger and apparent at a younger age in men than in women (59). These metabolomic measures were linked to adult onset cardiometabolic disease in previous studies. Circulating cholesterol and triglycerides in VLDL and LDL particles, Apolipoprotein B and glucose are strongly and consistently associated with the risk of myocardial infarction (40), whereas MR analyses showed that both LDL cholesterol and triglycerides play a causal role in the development of coronary heart disease (41, 42) as well as Apolipoprotein B (42).

Our results indicated that there is a difference in risk of cardiometabolic disease for men and women, which is already apparent at a young age. Based on these findings, we concluded that adolescence is a critical period for the prevention of adiposity-related changes in atherogenic risk factors in men. It is not completely clear why abdominal adiposity was specifically associated with atherogenic changes in young men and not in young women. One explanation might be that men are more likely to store body fat at the abdomen, whereas women are more likely to store fat at the hips and thighs (19). Abdominal adiposity, in particular visceral fat, is strongly related to metabolic disturbances (23, 30, 60), reflected by increased levels of atherogenic metabolomic measures as observed in our study.

In **Chapter 4, 5 and 6**, we studied several cardiometabolic consequences of body weight gain during adulthood. Body weight gain during adulthood is associated with an increased risk of cardiometabolic disease, possibly through adipocyte hypertrophy. Specific metabolomic alterations have the potential to indicate the onset of cardiometabolic disease as a consequence of adult weight gain. As we described in **Chapter 3**, metabolic alterations present the first indications of cardiometabolic disease in young men and also provide insights into underlying processes. However, the metabolomic profile associated with long-term adult weight gain has not been clearly defined. In **Chapter 4**, we investigated which metabolomic measures were specifically associated with weight gain during adulthood in the NEO study. We observed that adult weight gain, and not BMI at age 20 years nor BMI at middle age, was specifically associated with concentrations of 7 metabolomic measures, which we successfully replicated in Oxford Biobank. These metabolomic measures included omega-3, omega-6, total polyunsaturated fatty acids, small to medium low-density lipoproteins and

total intermediate-density lipoproteins. In addition, adult weight gain was associated with adipocyte size at middle age, and the adult weight gain-specific lipoprotein particles were associated with adipocyte size. Earlier studies have shown the metabolomic measures we found to be specifically associated with adult weight gain and cardiometabolic disease (39-41, 61, 62). Therefore, the results of our study highlight specific biochemical disturbances that could possibly indicate the onset of cardiometabolic disease related to adult weight gain.

We did observe similarities between the metabolomic measures identified to be associated with abdominal adiposity in adolescence (**Chapter 3**) and with weight gain during adulthood (**Chapter 4**). All are metabolomic measures indicative of atherogenic progression. In both studies, abdominal adiposity in adolescence and adult weight gain were strongly associated with the levels of low-density lipoproteins at either young adulthood or middle age, of which it is known to increase the risk of cardiovascular disease (40-42) and is a main target for pharmacological treatment to reduce atherogenic risk. In contrast with the results in **Chapter 3**, we did not observe differences in the association between adult weight gain and metabolomic measures between men and women at middle age in **Chapter 4**. This could be due to differences in study design or population: ALSPAC is a birth cohort study of young adults born in the 1990s and has a longitudinal design with repeated measures of metabolomics over time, whereas in the NEO study we performed a cross-sectional analysis of baseline measurements of middle-aged men and women. Other factors may have played a role as well: as the study in **Chapter 3** was performed in adolescents and young adults, changes in metabolomic measures might also be related to pubertal changes, such as (sex) hormone changes. In addition, adipocytes might respond differently to changes in body weight during young adulthood compared with adulthood or middle age (38). Future perspectives in the field of metabolomics related to body weight gain include prospective analyses of repeated measures of metabolomics in cohorts of middle-aged individuals. By this means, body weight trajectories can be established, which can then be related to outcomes of interest.

Differences in body fat distribution are an important contributor to differences in risk of cardiometabolic disease in individuals with obesity (23). During adult weight gain, excess adipose tissue is stored in different areas of the body, however only few studies investigated the depots in which body fat is preferentially stored during adult weight gain. In **Chapter 5**, we investigated the association between adult weight change and several measures of abdominal adiposity (waist circumference, abdominal subcutaneous adipose tissue, and visceral adipose tissue) and liver fat at middle age, taking into account overall body fat. In this study, we consistently observed that weight gain during adulthood was associated with a relatively higher amount of visceral fat and liver fat at middle age within all BMI categories at age 20 years. This implies that weight maintenance during adulthood plays an important role in limiting excess visceral fat and liver fat and their detrimental effects on cardiometabolic health. It is well-established that excess visceral fat was associated with increased insulin resistance and diabetes risk (28, 30, 31, 63). Therefore, we hypothesized that the association between adult weight gain and insulin resistance is mediated by the amount of visceral fat and liver fat at middle age. In **Chapter 6**, we confirmed that a small gain in body weight during adulthood was already associated with more insulin resistance compared with weight maintenance during adulthood. Stronger associations with insulin resistance were observed for more excessive weight gain during adulthood. In addition, when

we considered the mediating roles of visceral fat, we observed that the association between adult weight gain and insulin resistance at middle age was mediated by visceral fat for 32.0% (95% confidence interval 18.6-45.4) and by liver fat for 22% (95% confidence interval 15.0-30.1%). Our results highlight the importance of weight maintenance during adulthood to prevent the accumulation of excess visceral fat and liver fat and thereby insulin resistance and eventually, type 2 diabetes at middle age and older age.

Importantly, not everyone with obesity will develop cardiometabolic disease, and obesity-related consequences differ between individuals with a similar BMI (18). Apparently, there are additional risk factors needed for obesity to result in disease. In the last chapter of this thesis, **Chapter 7**, we aimed to answer the question which factors are needed for obesity to result in cardiometabolic disease. To this extent, we examined a range of risk factors, including body fat distribution, metabolic factors and lifestyle factors, in relation to incident cardiometabolic disease in ten years of follow-up in participants with a BMI of 27 kg/m^2 or higher in the NEO study. We observed that especially the amount of visceral fat, as well as metabolic factors, including fasting glucose levels and triglycerides, increase the risk of developing cardiometabolic disease in a population with obesity. Notably, lifestyle factors, such as diet quality and physical activity, were not associated with an increased risk of cardiometabolic disease in a population with obesity. When considering the joint effect of the risk factors and obesity, defined as BMI≥30 kg/m^2, we observed that fasting glucose and smoking showed additive interaction with obesity on the risk of cardiometabolic disease, as well visceral adipose tissue. This suggests that that preserving low glucose levels and non-smoking, as well as maintaining low visceral adipose tissue will result in an additive decrease in the risk of incident cardiometabolic disease in individuals with obesity, compared with individuals with both risk factors present. It must be noted that our results do not support the existence of a 'metabolically healthy obesity' phenotype, defined as obesity with absence of metabolic risk factors (18). As shown in a study with up to twenty years of follow-up, healthy obesity appears to be a transient state, with individuals with healthy obesity often naturally progressing to unhealthy obesity (64). In other words, although the risk of cardiometabolic disease was lowest in those with obesity without additional risk factors, it most likely remains increased compared with people without obesity.

Strengths and limitations

Strengths of the studies described in this thesis include the large population-based studies and the availability of precise and accurate measures of body fat distribution, including DXA-derived total fat mass and trunk fat in **Chapter 3**, and abdominal subcutaneous and visceral adipose tissue by MRI and hepatic triglyceride content by ^{1}H-MRS in Chapter **5, 6, and 7**. Additionally, all studies had information on a wide range of potential confounding factors. This enabled us to investigate the specific associations between adult weight gain and several outcomes of interest, taking into account confounding factors.

For a correct interpretation of our results, several limitations need to be considered. For most studies included in this thesis, we made use of data from the Netherlands Epidemiology of Obesity (NEO) study. In the NEO study, adult weight gain between age 20 years and middle age was based on recalled weight at age 20 years, which was reported by questionnaire. Therefore, body weight at age 20 years might be misclassified when participants do

not correctly remember their body weight at age 20 years. Nevertheless, previous studies have shown that recalled body weight is strongly related with measured weight with a similar time interval between measurement and recall as in the NEO study (65). In addition, as we only had information on body weight at two time points (age 20 years and middle age), we had no information on individual body weight trajectories over time (66, 67). For example, an individual gained a certain amount of weight between age 20 years and middle age: this could have happened gradually, or the person could have had a stable body weight for a long period of time, however rapidly gained weight in a short time period around middle age. Weight fluctuations, the so-called 'yo-yo effect', are also common and may result in different disease risks (68).

To investigate potential distinct effects of individual body weight trajectories, we are in need of detailed longitudinal studies in which body weight is frequently assessed, preferably by measuring the body weight at a study centre. By this means, body weight trajectories can be established, which can then be related to outcomes of interest. A limitation of this approach is that it is time-consuming and expensive. For example, the UK-based birth cohort ALSPAC started in the 1990s and the participants had measurements taken regularly (53, 69). However, the participants are now in their late twenties, which means that it will take approximately an additional twenty to thirty years before they will develop outcomes such as type 2 diabetes and cardiovascular disease.

In the NEO study, participants were selected on having a self-reported BMI of 27 kg/m^2 or higher and therefore, weight gain patterns might be different in this overweight population. We compared the distribution of weight gain in the NEO study population to the reference population of Leiderdorp, and observed that the distribution of weight gain was similar. In **Chapter 3** and **4**, we studied the metabolomic profile, derived from a metabolomics platform mostly containing lipoprotein (sub)particles. This platform is rather limited: to investigate other altered metabolomic pathways would require alternative platforms to have a complete overview. Lastly, all studies described in this thesis were performed in study populations predominantly including European men and women, and the results of our study need to be confirmed in other ethnic groups.

The studies about weight gain described in this thesis are all observational studies, and therefore we cannot exclude residual confounding or reverse causation and infer causality. Confounding of results may occur by a common cause of the exposure and the outcome variable, which is not in the causal pathway. In all studies we aimed to control for known and measured confounding factors as good as possible. To investigate the association between adult weight gain and visceral and liver fat at middle age, we adjusted the analyses for an array of confounding factors including ethnicity, educational level, smoking, alcohol consumption, physical activity, use of antidepressants, thyroid medication, corticosteroids or hormonal treatments, and menopausal status. However, residual confounding by unknown, unmeasured or inaccurately measured confounding factors may always remain.

Reverse causation may occur in studies of weight change when an underlying disease leads to weight loss as a result of muscle wasting and changes in muscle composition (70). This process is called sarcopenia, and can also happen in individuals with obesity (obese sar-

copenia). Sarcopenia with would result in an underestimation of the risk associated with obesity and weight gain, even in those with obesity. Nevertheless, our results were similar when we excluded participants with potential unintentional weight loss during adulthood. Therefore, we do not expect a large influence of potential reverse causation on our results. Additionally, reverse causation may occur when we studied the association between adult weight gain and insulin resistance at middle age: the development of insulin resistance may precede a gain in body weight (71, 72). However, weight gain was calculated using recalled weight at age 20 years and measured body weight at middle age, and insulin resistance was assessed at middle age, which indicates temporality.

Previously, studies have been performed in which participants were overfed in order to study the effects of body weight gain. A famous example is a study in identical twins by Bouchard et al., in which differences in response to overfeeding were investigated, as well as the involvement of genotypes in the different responses (73). Randomized controlled trials involving overfeeding also have been performed, however, such studies often assessed differences in dietary composition instead of merely weight gain versus weight maintenance (74). It is highly questionable whether such overfeeding studies are ethical, especially when participants need to be overfed for a long period of time (e.g., for a period comprising years or even decades) of time. As an alternative to randomized controlled trials on body weight gain or obesity, Mendelian randomization analyses can be performed. Mendelian randomization analyses make use of genetic variants associated with the exposure of interest (e.g., BMI) as an instrumental variable, assigned randomly at conception. These instrumental variables can then be related to the outcomes of interest (e.g., type 2 diabetes mellitus, cardiovascular disease) thereby mimicking randomized controlled trials.

Conclusions, implications and future perspectives

The results described in this thesis fit within the framework of the 'lipid overflow' hypothesis (21-23). Shortly, this hypothesis describes that when individuals gain body fat, this will be stored in subcutaneous adipose tissue, until the storage capacity is reached. Lipids will then overflow into the visceral compartment or stored ectopically, e.g., in normally lean tissues and organs, such as the liver. Here, the lipids will disrupt organ function and exert their harmful effects, eventually leading to increased risks of cardiometabolic disease. In the epidemiological studies described in this thesis, we observed an association between adult weight gain and adipocyte size at middle age. Adult weight gain was strongly associated with the amount of visceral fat and liver fat at middle age, which indicates that in individuals who gain excess body weight, the storage capacity in subcutaneous adipose tissue is more likely to be reached. We also observed a strong association between adult weight gain and insulin resistance, which was mediated by the amount of visceral fat. These results support the 'lipid overflow' hypothesis and highlight the cardiometabolic consequences of adult weight gain and lipid overflow (21-23). Additionally, adult weight gain was specifically related to atherogenic metabolic alterations specifically associated with adult weight gain and indicative of cardiometabolic disease. Lastly, overall and abdominal adiposity at adolescence was associated with an atherogenic metabolic profile. Adipocyte hypertrophy may be one of the explanations, as adipocytes respond to increases in fat mass by expansion during young adulthood, and thereby contribute to adipocyte hypertrophy, ultimately leading to metabolic disturbances (75-77).

As an alternative to randomized controlled trials on body weight gain or obesity, we suggest Mendelian randomization analyses, which make use of genetic variants associated with the exposure of interest as an instrumental variable, assigned randomly at conception. To identify genetic variants to be used as instrumental variables, Genome-Wide Association Studies (GWAS) need to be performed. A GWAS on weight gain during adulthood is currently ongoing, of which the results can be used to perform MR analyses to complement the results of our observational studies. It is also possible that genetic factors play a less significant role in adult weight gain, and that environmental factors may be more important (78). Studies on the interaction between genetic variants predisposing for adult weight gain and environmental factors, such as dietary composition, are needed to provide more information on the interplay between genetics and the environment in the development of obesity and cardiometabolic disease.

Characterization of genetic variants that are associated with adult weight gain during adulthood and the genes corresponding to them, can provide insights into the complex processes underlying adult weight gain. Identified genetic variants can then be grouped for novel MR approaches as we described in Chapter 2 of this thesis. Recently, MR techniques similar to our approach but from a different perspective, have been developed (56, 57). For example, in a recent study, principial component analysis was used to identify four groups of genetically driven variation in body shape and size: overall body size, adiposity, predisposition to abdominal fat deposition, and lean mass (56). These approaches can be used to unravel the heterogeneity in the genetics underlying complex diseases such as obesity. More detailed investigations of the differential causes of complex traits such as obesity and adult weight gain will allow a comprehensive overview of potential targets for interventions. In addition, genetic variants potentially provide personalized information on the risk of cardiometabolic disease beyond the traditional risk factors.

Not only the causes, but also the consequences of obesity are complex. Importantly, not everyone with obesity will develop cardiometabolic disease, and obesity-related consequences differ between individuals with a similar BMI. Body weight history plays an important role in the risk of cardiometabolic disease later in life: for cardiovascular disease, both BMI in adolescence and adulthood are associated with the risk of cardiovascular disease, whereas for type diabetes BMI and weight gain shortly before diagnosis seem to be more conclusive (34). We consistently observed that weight gain during adulthood was associated with an adverse body fat distribution, adverse metabolic profile and insulin resistance, eventually resulting in an increased risk of cardiometabolic disease at middle age. Precise mechanisms underlying these associations include the response of adipocytes to weight gain: although there is a constant turnover of adipocytes throughout life, adipocyte number remains fixed during adulthood, and therefore the expansion of adipose tissue in response to weight gain in adults is due to an increase in adipocyte volume (38, 79). Previous studies have shown that this adipocyte hypertrophy is associated with increased systemic insulin resistance, and thereby a worsening metabolic profile (75-77), as a results of adipose tissue becoming dysfunctional when the capacity of hypertrophic adipocytes to expand is exceeded (21, 22). This in turn, leads to “lipid overflow” and the accumulation of triglycerides in visceral adipose and ectopic fat deposition in normally lean organs such as the heart and the liver (21, 22, 24). Compared to subcutaneous adipocytes, adipocytes in the visceral

depot have a high secretion rate of non-esterified fatty acids, very low-density lipoproteins (VLDL), and cytokines, such as interleukin-6 and tumour necrosis factor-α, thereby inducing a systemic low-grade inflammatory state and oxidative stress (23-26). Finally, intracellular non-esterified fatty acid accumulation in non-adipose tissue leads to impaired insulin signalling and insulin resistance (27). Eventually, this will lead to an increased cardiometabolic risk, including both a high risk of developing type 2 diabetes and cardiovascular disease (22).

The results of this thesis emphasize the importance of maintaining a stable body weight during young adulthood throughout middle age. Body weight reduction has been proven difficult, as well as keeping weight off permanently, however, weight maintenance will already be beneficial for health as supported by or results. This is also supported by a study on type 2 diabetes (80), in which the authors estimated that about 1 in 5 cases of type 2 diabetes could be prevented if body weight in middle age was maintained at the population level. Since 2019, individuals with overweight or obesity in the Netherlands are eligible for a combined lifestyle intervention, integrating dietary habits, physical activity and behavioural changes, in order to promote and maintain a healthy weight (81). This multifactorial approach, including a combination of lifestyle changes such as maintaining a healthy diet, increasing physical activity and smoking cessation, was also promoted by the recent guidelines on the short- and long-term prevention of cardiovascular disease by the European Society of Cardiology (82). In addition, the guidelines underline the importance of increasing the knowledge on effective strategies to reduce body weight and maintain a healthy body weight. However, lifestyle changes have been proven difficult, especially in the modern obesogenic environment, in which high energy intake and sedentary behaviour are encouraged (83). This underscores the need for health-promoting population-level interventions and governmental policies promoting a healthy lifestyle (83-85). In the Netherlands, the National Prevention Agreement has been signed in 2018, with the goal to reduce smoking, obesity and alcohol consumption in the Dutch population (86). Measures against obesity in the National Prevention Agreement include providing combined lifestyle interventions, selling healthy foods at gyms, canteens and hospitals, and helping people make healthy food choices by healthy food logos on packaging (86). Altogether, the ambition of the National Prevention Agreement is that these measures should result in a decrease in overweight and obesity in Dutch adults from 50% to 38% by 2040 (86).

In conclusion, in this thesis we investigated several underlying mechanisms linking adult weight gain to cardiometabolic disease later in life. 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.

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