Chapter 7

General discussion and summary

The main objective of this thesis was to unravel relationships between obesity, insulin resistance, hyperglycemia, and atherosclerosis. It is well-established that patients with type 2 diabetes have a 2- to 3-fold increased risk of cardiovascular disease. We investigated whether insulin resistance and hyperglycemia are associated with atherosclerosis and incident cardiovascular disease before the onset of type 2 diabetes. Obesity can be considered as a common cause of both insulin resistance and atherosclerosis. Therefore, we investigated to what extent associations between insulin resistance, hyperglycemia and atherosclerosis were explained by body fat. We further aimed to study the specific role of visceral fat in the development of insulin resistance and atherosclerosis, and directly assessed abdominal subcutaneous and visceral adipose tissue depots. This final chapter provides a summary of the findings, discusses strengths and limitations of the performed studies, and presents recommendations for future studies and clinical care.

SUMMARY OF MAIN FINDINGS

In **Chapter 2**, we performed a meta-analysis and showed that fasting glucose, fasting insulin, and the Homeostasis Model Assessment of insulin resistance (HOMA-IR) were associated with incident cardiovascular disease in persons without diabetes. These findings indicate that disturbances in glucose metabolism play a role in the development of cardiovascular disease before the onset of type 2 diabetes. These results are in line with results from previous meta-analyses [13, 16, 19, 105-107]. Moreover, in our meta-analysis comprising more than 500,000 persons we directly compared the strength of associations of fasting glucose, fasting insulin, and HOMA-IR with incident coronary heart disease. We showed that the relative risk of coronary heart disease associated with one standard deviation of HOMA-IR (1.64, 95% CI: 1.26, 1.69) was higher than the relative risks per standard deviation of fasting glucose (1.21, 95% CI: 1.13, 1.30) or of fasting insulin (1.04, 95% CI: 0.96, 1.12). Nevertheless, the relative risk associated with HOMA-IR may reflect the sum of direct effects of insulin resistance [16] and the effects of its consequences such as hyperglycemia [16, 17] on the arterial wall. For this reason, it is difficult to conclude from **Chapter 2** that insulin resistance is most important in the etiology of cardiovascular disease. Joint modeling of both hyperglycemia and insulin resistance may help to distinguish their relative contributions to the development of atherosclerosis and cardiovascular disease. Previous studies that reported associations of insulin resistance and hyperglycemia with carotid intima-media thickness (cIMT) as a measure of subclinical atherosclerosis, did not distinguish their relative contributions [20-23, 228].

Therefore, in **Chapter 3**, the relative contributions of hyperglycemia (assessed with fasting plasma glucose and HbA_{1C}) and of insulin resistance (assessed with HOMA-IR) to subclinical atherosclerosis were distinguished in a joint model using data from the Netherlands Epidemiology of Obesity (NEO) study. After mutual adjustment, the associations of hyperglycemia and

insulin resistance with cIMT were of similar strength. Importantly, waist circumference largely explained the associations of insulin resistance and hyperglycemia with cIMT. This supports that abdominal obesity is a common cause of insulin resistance, hyperglycemia, and atherosclerosis. Furthermore, waist circumference remained associated with cIMT after adjustment for HOMA-IR, fasting glucose, and HbA_{1C}, implying that pathways underlying the association of abdominal adiposity with atherosclerosis exist, beyond pathways via insulin resistance and hyperglycemia.

Dysfunctional adipose tissue secretes pro-inflammatory cytokines and non-esterified fatty acids (NEFAs) that may induce both insulin resistance [49] and atherosclerosis [138]. Adipose tissue may therefore promote the development of atherosclerosis through insulin resistance and consequential hyperglycemia [16, 17], but also via other mechanisms such as inflammation, dyslipidemia, and hypertension [54, 138]. Whereas waist circumference cannot distinguish visceral fat from abdominal subcutaneous fat, it has been postulated that the excess risk of type 2 diabetes and cardiovascular disease associated with abdominal obesity is due to the presence of large amounts of visceral adipose tissue [38]. Visceral fat is characterized by a high secretion rate of lipolysis [69] and pro-inflammatory cytokines [70], which reach the liver via the portal vein [71, 72]. Nonetheless, the majority of body fat is stored subcutaneously (80 to 90%) [39] and therefore subcutaneous fat may contribute most to the circulating pool of adipokines and NEFAs. Most previous studies investigating effects of visceral fat did not take the effects of total body fat into account [56-58, 63, 65-67]. Therefore, the specific role of visceral fat in the development of insulin resistance and atherosclerosis remained unclear. Furthermore, although clear differences in body fat distribution between men and women exist [25, 39], it was unclear whether associations between visceral fat, subcutaneous fat and glucose metabolism actually differ between men and women.

In the NEO study, we directly assessed abdominal subcutaneous and visceral fat by magnetic resonance imaging (MRI). This enabled us to investigate associations of abdominal subcutaneous and visceral fat with insulin resistance and insulin secretion (**Chapter 4**) and with subclinical atherosclerosis (**Chapter 5**). We observed that, on average, men had less total body fat than women, but twice the amount of visceral fat. Furthermore, we observed that for a given waist circumference, the amount of visceral fat varied considerably among both men and women. Next, we investigated the contributions of abdominal subcutaneous and visceral fat to insulin resistance, insulin secretion and subclinical atherosclerosis and showed that visceral fat contributed to insulin resistance and subclinical atherosclerosis beyond total body fat. Unexpectedly, although women had less visceral fat than men, particularly in women visceral fat accumulation was associated with insulin resistance and subclinical atherosclerosis.

In **Chapters 3 and 5**, we used the cIMT as a measure of subclinical atherosclerosis. The reproducibility of the cIMT is well studied in the general population [215, 216, 222-227] and in patients with type 2 diabetes [229-231]. In the NEO study, however, there is an oversampling of individuals with a high body mass index (BMI). It was therefore important to investigate the reproducibility of cIMT in this group. We hypothesized that excess fat in the neck region may

render cIMT more difficult to assess. In **Chapter 6**, we showed a good intra-observer agreement between paired cIMT measurements in overweight and obese individuals; mean difference -9 µm (SD: 54 µm), intraclass correlation coefficient (ICC): 0.74 (95% CI: 0.62, 0.83), coefficient of variation (CV): 5.8%. The inter-observer agreement was fair; mean difference: 0 µm (SD: 84 µm), ICC: 0.49 (95% CI: 0.33, 0.63), CV: 9.0%. Despite the oversampling of individuals with a high BMI, our intra-observer and inter-observer reproducibility measures were similar to estimates from other population-based studies [215, 216, 222-227]. In addition, in both our study and other population based studies that included persons with normal and high BMI [215, 216] there was no association between BMI and the reproducibility of the cIMT, suggesting there was no systematic error in the cIMT measurement. Therefore, the cIMT measurement can be validly used as an outcome variable in large epidemiological studies such as the NEO study.

STRENGTHS AND LIMITATIONS

In 2008, the NEO study had been set up to investigate pathways that lead to obesity-related diseases. The baseline measurements of the NEO study were used to answer the research questions of **Chapters 3 to 6.** A major strength of the NEO study is the extensive phenotyping of a large study population. Whereas large epidemiological studies often merely rely on questionnaires and self-reported BMI and waist circumference, the direct assessment of abdominal fat depots is a distinguishing feature of the NEO study. Furthermore, accurate methods to assess total body fat and a parameter of subclinical atherosclerosis were available, in addition to both fasting and postprandial blood sampling from which several measures of glucose metabolism can be calculated, and detailed information on potential confounding factors. This enabled us to (1) distinguish relative contributions of total body fat, visceral fat, insulin resistance, and hyperglycemia to atherosclerosis, (2) adjust for potential confounding factors, and (3) investigate sex differences. A major limitation is the observational, cross-sectional nature of the analyses using baseline measurements of the NEO study. As a result, residual confounding and reverse causation may hamper causal inference from our findings. The following paragraphs will reflect on these and other limitations of the study design and data collection.

Assessment of overall and regional body fat

In **Chapters 3 to 6**, we used BMI and total body fat percentage as measures of overall adiposity. BMI is an internationally accepted measure of adiposity and is used to define overweight and obesity [232]. A major drawback of BMI is that it cannot distinguish between fat mass and fat free mass [78]. In addition, BMI gives no information about the distribution of fat within the body [78]. As a result, persons with the same BMI may vary in total body fat and associated risk. For example, very muscular persons may be misclassified as overweight, or persons with a low BMI but with large amounts of visceral fat as lean. We also assessed total body fat with bioelec-

trical impedance analysis (BIA) while participants were standing bare footed on two electrodes. Although a hand-to-foot BIA is more accurate in estimating total body fat [233], the foot-to-foot BIA showed a good agreement with densitometry, a gold standard method to assess total body fat [234, 235]. MRI has also been shown to assess body fat accurately [236]. In order to study the specific effects of visceral fat, we directly assessed abdominal subcutaneous and visceral adipose tissue depots with MRI. To that extent, three cross-sectional images were made at the level of the fifth lumbar vertebra. Although these are highly correlated with total volumes of visceral fat (r between 0.85 and 0.90) [163, 164] and abdominal subcutaneous fat (r =0.80) [164], a limitation of our study may be that we did not assess gluteal-femoral subcutaneous fat. Gluteal-femoral subcutaneous adipose tissue has a lower secretion rate of pro-inflammatory cytokines and may have a distinct function than abdominal subcutaneous adipose tissue [237]. Therefore our results from **Chapters 4 and 5** pertain to abdominal subcutaneous adipose tissue. It must furthermore be noted that abdominal subcutaneous adipose tissue is further separable in a deep and superficial layer, separated by the fascia superficialis which may have distinct functions [238]. To this extent, the MRI images of the NEO study will be revised in the near future to distinct deep subcutaneous adipose tissue from superficial adipose tissue and investigate their effects in relation to atherosclerosis and insulin resistance. The main findings of this thesis, however, relate to visceral fat accumulation, which can be validly assessed by using cross-sectional MRI images [163, 164].

Assessment of insulin resistance

The Homeostasis Model assessment was used to estimate the degree of insulin resistance and beta-cell function in **Chapters 2, 3, and 4**. HOMA-IR is estimated from fasting plasma glucose and insulin concentrations [80] and it corresponds well to estimates of total body insulin resistance derived from the hyperinsulinemic euglycemic glucose clamp, the gold standard method to assess insulin resistance [81, 82]. A limitation of HOMA is that insulin resistance and beta-cell function are estimated solely from fasting plasma glucose and insulin concentrations [80], and can therefore not account for disturbances in non-fasting conditions. High fasting glucose and insulin concentrations reflect mainly hepatic insulin resistance (caused by a reduced suppression of gluconeogenesis), whereas high postprandial glucose and insulin concentrations may reflect insulin resistance of peripheral tissues, such as adipose tissue and muscles [83]. In **Chapter 4**, we also used postprandial glucose and insulin concentrations to calculate measures of beta-cell function and peripheral insulin resistance [83].

Assessment of atherosclerosis

In **Chapters 3 and 5**, the cIMT measurement was used as a marker of subclinical atherosclerosis and in **Chapter 6** its reproducibility was investigated in a subsample of the NEO study, resulting in a fair inter-observer agreement. An advantage of the cIMT is that it is measured at only two locations, namely in the left and right common carotid artery, but corresponds to the degree

of atherosclerosis in several other arteries [239]. In the analyses in **Chapter 3 and 5** we studied associations with cIMT, which is an intermediate outcome of cardiovascular disease, instead of clinical cardiovascular disease endpoints. The observed associations may therefore differ from those in relation to cardiovascular disease, which is a complex, heterogeneous disease involving not only atherosclerosis, but also plaque rupture and thrombosis. Nevertheless, using the cIMT as an outcome enabled us to specifically study pathways leading to atherosclerosis before the onset of cardiovascular disease. Another limitation is that the cIMT provides no information about the composition of the arterial wall. Autopsy studies have shown that not necessarily the thickest lesions are most prone for rupture, but possibly the lesions with a thin fibrous cap, a large lipid core and high macrophage content [240, 241]. The cIMT measurement is unable to identify such lesions and only expensive or invasive techniques such as MRI, computed tomography (CT), and intravascular ultrasound are able to measure the composition of the arterial wall. At present, none of these techniques is feasible to use in large scale epidemiological studies. A final limitation may be that, the carotid arteries were not screened for the presence of plaques. Plaques represent a more advanced stage of atherosclerosis and combining the cIMT measurement with a plaque score may have resulted in a more complete assessment of atherosclerosis. Consequently, our results relate to early and not advanced atherosclerosis.

Consequences of measurement error

All of the aforementioned measurements suffer to some extent from measurement error. The consequences of measurement error depend on whether the error is random or systematic and whether the measurement is used as an exposure, confounding, or outcome variable. Random measurement error in our exposure variables (e.g. visceral fat, glucose, insulin, and HOMA-IR) may have resulted in an attenuation of the regression coefficients derived from linear regression analysis (**Chapters 3 to 6**), as well as the pooled relative risks in the meta-analysis (**Chapter 2**). This phenomenon is also known as regression dilution bias [114] and results in an underestimation of the associations under study. In **Chapter 2**, the strength of the associations of fasting glucose, fasting insulin and HOMA-IR with coronary heart disease were compared, and such comparison is not valid when the variability in measurements is of different magnitude. On the basis of two repeated measurements in 100 participants in the NEO study we estimated intraclass correlation coefficients (ICCs) and these ranged between 0.81 and 0.98 for all exposure variables. Because the ICCs of the exposure variables were of similar magnitude, it is unlikely that differences in the variability of measurements have influenced our results.

In **Chapters 4 and 5**, associations of visceral fat with insulin resistance, beta-cell function, and subclinical atherosclerosis were adjusted for potential confounding by physical activity, using metabolic equivalent hours (MET-hours) per week, derived from the Short QUestionnaire to ASsess Health-enhancing physical activity [161]. Self-reported physical activity contain a sizeable level of random measurement error [242] and may also contain systematic measurement error, because persons with a higher BMI may overestimate their physical activity [243].

As a result, it is unclear whether measurement error in self-reported physical activity may have led to an over- or underestimation of the associations of visceral fat with insulin resistance and subclinical atherosclerosis. Nevertheless, it is unlikely that such over- or underestimation is different for total body fat, subcutaneous fat and visceral fat and would alter our conclusions about visceral fat in relation to insulin resistance and atherosclerosis. Future studies using an objective measurement of physical activity instead of a measurement based on self-report may more accurately estimate the effects of visceral fat. It must be noted though, that in our analyses we adjusted for multiple confounding factors (e.g. age, sex, ethnicity, smoking habits, alcohol consumption, and physical activity) that were all to some extent correlated. Using correlated confounding factors reduces the bias resulting from their measurement errors [244]. In **Chapters 3 and 5**, we used the cIMT as an outcome variable. In general, measurement error in an outcome variable has no effect on the size of the regression coefficient, but decreases its precision [114]. This loss of precision can be overcome by minimizing the measurement error with duplicate cIMT scans or by increasing the sample size. The large sample size of the NEO study therefore minimizes the effect of measurement error in the cIMT measurement on the precision of our estimates.

Confounding

Confounding may occur in observational studies when the exposure and outcome variable share a common cause that may obscure the causal association and explain the observed association [245]. Although, the extensive phenotyping of the participants of the NEO study enabled us to adjust for many important confounding factors such as age, sex, ethnicity, education, smoking habits, alcohol consumption, physical activity, and medication use, as in all observational studies residual confounding by unknown, unmeasured or inaccurately measured confounding factors may remain. In our meta-analysis in **Chapter 2**, we included only studies that adjusted for at least age and sex. Eighty-five percent of the included studies also adjusted for smoking habits, but only 46% adjusted for total body fat. Because body fat is a common cause of insulin resistance (and consequential hyperglycemia) and cardiovascular disease [24], there may have been residual confounding in these studies. Consequently, effect estimates of individual studies could be overestimated, which in turn, may have led to an overestimation of the pooled effect estimates. The linear regression analyses in **Chapter 3** were not adjusted for physical activity, because this information was at time of publication not yet available. Recent additional adjustment of these analyses for physical activity did not materially change the results. Because residual confounding may remain after adjustment for crude measures of adiposity as BMI and waist circumference, in **Chapters 4 and 5**, we adjusted for more precise measures of body fat, as assessed with BIA and MRI, thereby minimizing residual confounding by overall and abdominal body fat. After adjustment for total body fat, we observed that visceral fat remained associated with insulin resistance (**Chapter 4**) and subclinical atherosclerosis

(**Chapter 5**), suggesting visceral fat is an important factor in the etiology of insulin resistance and atherosclerosis.

Reverse causation

In the cross-sectional analyses of **Chapters 3 to 5**, using baseline measurements of the NEO study, the exposures and outcome variables were measured at the same time. The results from these chapters suggest that body fat, and visceral fat in particular are involved in the development of insulin resistance and atherosclerosis. However, the cross-sectional nature of these analyses hampers drawing conclusions about the direction of the observed associations. Consequently, the direction of associations may be reversed, i.e., insulin resistance or atherosclerosis may precede (visceral) fat accumulation. The alternative explanation that an increased cIMT causes visceral fat accumulation, insulin resistance, or hyperglycemia (**Chapters 3 and 5**), seems, however, unlikely. Three prospective follow-up studies showing that visceral fat accumulation was associated with an increased risk of cardiovascular disease [246-248], together with our results from **Chapter 2**, showing that insulin resistance, hyperglycemia, and hyperinsulinemia were associated with incident cardiovascular disease in a prospective meta-analysis, contribute to the hypothesized directions of the associations.

For the association between visceral fat and insulin resistance (**Chapter 4**) the alternative explanation that insulin resistance precedes visceral fat accumulation may be less unlikely. Mechanistic studies have proposed that a decreased insulin sensitivity of adipocytes may be an important factor leading to adipocyte dysfunction [46, 47, 249] and the inability of the subcutaneous adipose tissue depot to sufficiently expand its storage capacity in response to caloric excess, which may lead to visceral fat accumulation and ectopic fat deposition in the liver, heart, and skeletal muscle. According to this theory, visceral fat accumulation may be an indicator of dysfunctional subcutaneous adipose tissue. In randomized trials morbidly obese persons did not improve in insulin sensitivity after resection of visceral fat (omentectomy) in addition to gastric bypass surgery [194, 250-253], which may contribute to this alternative explanation. Also, current interest in the gut microbiome as a causal factor in weight gain and insulin resistance may relate to such alternative hypothesis [254, 255]. There is even one study in 518 Japanese men and women that showed that higher fasting insulin concentrations at baseline were associated with intra-abdominal fat accumulation after five [256] and ten years of follow-up [257]. On the contrary, the same study [59, 152, 155] and three larger prospective studies [151, 153, 258] reported that excess intra-abdominal fat was associated with the development of insulin resistance [59] and an increased risk of type 2 diabetes [59, 151-153, 155, 258] after five to ten years of follow-up. Future prospective studies should elucidate whether the observed associations in our studies are in the hypothesized direction.

Representativeness of the NEO study population

The NEO study population consisted of 6,671 participants (of the initially 6,673 included participants in the NEO study, two participants appeared to have participated twice) aged between 45 and 65 years with an oversampling of persons with a BMI of 27 kg/m^2 or higher. Participants were recruited via invitations sent by general practitioners in the area of Leiden, through advertisements in local newspapers, and through posters distributed in public areas of Leiden and surroundings [77]. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher were invited to contact the NEO study center by telephone or by completing a web-based form [77]. Because the BMI distribution of persons living in Leiden and surroundings was unknown, the participation rate of persons with a BMI of 27 kg/m^2 or higher could not be calculated. In the municipality Leiderdorp all participants aged between 45 and 65 years were invited to participate via the municipality administration, irrespective of their BMI [77]. As a result, the participation rate of Leiderdorp can be derived, which was 20.3% [77]. This participation rate is not unusual given the time volunteers were requested to donate without remuneration, including invasive (blood draws) or some unpleasant measurements (MRI). Due to this participation rate it has to be discussed whether the NEO study population is representative for the general population, and whether this is relevant. On the one hand, persons with a healthy life style who may have decided to participate because of high consciousness about their health ("healthy volunteers") [259]. On the other hand, persons with symptoms or conditions related to obesity, or with a family history of type 2 diabetes or cardiovascular disease may have been more likely to participate due to the relevance of the study to their lives [259]. When extreme, such self-selection may lead to a type of selection bias [260]. However, associations are usually assessed stratified on the variables that might have promoted self-selection, in which case only the relative size of the strata differ from the population, without biased associations. Importantly, the BMI distribution of the participants of Leiderdorp was similar to the BMI distribution of the general Dutch population [128]. This suggests that the sample from the Leiderdorp municipality is at least with respect to BMI distribution representative for the general Dutch population within the same age group. All analyses were weighted towards the BMI distribution of the participants from Leiderdorp and consequently our results apply to a population-based study without oversampling [127].

The assessment of abdominal subcutaneous and visceral fat by MRI was performed in a subgroup of participants without contra-indications for MRI such as metallic devices, claustrophobia or a body circumference of more than 1.70 m. Although few participants were excluded for the latter reason, our results in **Chapters 4 and 5** may not be representative for extremely obese persons with a body circumference of more than 1.70 m. Finally, the NEO study population consists of predominantly white individuals. Between ethnicities, differences in adipose tissue distribution and function [48, 202], as well as differences in insulin sensitivity and beta-cell function [261, 262] exist. Therefore, our findings may need confirmation in other ethnic groups.

CONCLUSIONS AND IMPLICATIONS

The main conclusions of this thesis are that associations between insulin resistance, hyperglycemia and atherosclerosis are largely explained by abdominal adiposity. We furthermore showed that within the abdomen, visceral fat contribute to insulin resistance and atherosclerosis, beyond total body fat. Finally, we observed that in men abdominal subcutaneous fat and visceral fat are similarly associated with insulin resistance and atherosclerosis, whereas particularly visceral fat is associated with insulin resistance and atherosclerosis in women. Findings of this thesis thereby emphasize the important role of abdominal obesity in the development of insulin resistance and atherosclerosis and support a specific role for visceral fat, particularly in women. In the following paragraphs we will interpret these findings in terms of mechanisms and translate them into potential clinical implications.

Potential mechanisms linking body fat to insulin resistance and atherosclerosis

The associations of insulin resistance and hyperglycemia with cIMT were weak, suggesting that insulin resistance and hyperglycemia themselves do not contribute to a large extent to the development of atherosclerosis (**Figure 1,** path C and path G). This is line with the limited success of interventions that improve glycemic control in reducing cardiovascular events in patients with type 2 diabetes [6] and in persons with pre-diabetes [7]. In contrast, intensive glycemic control did result in a smaller cIMT after six years of follow-up [263] and a cardiovascular risk reduction of 42% after 17 years of follow-up [136] in patients with type 1 diabetes. However, these patients were much younger and probably had less advanced atherosclerosis than participants in the NEO study. These results may support the hypothesis that glucose lowering therapy early in the course of atherosclerosis is beneficial [136]. Only recently, two Mendelian randomization studies were published that support a causal role of dysglycaemia and diabetes in the risk of coronary heart disease [264, 265].

Within the abdominal fat compartment, we distinguished contributions of abdominal subcutaneous and visceral fat to insulin resistance and atherosclerosis, while taking total body fat into account. We confirmed results from earlier studies [56-67], which suggested that visceral fat has a specific role in the etiology of insulin resistance (**Figure 1,** path E) [50, 51, 71, 72] and atherosclerosis (**Figure 1,** path B, path EC, and path EFG) [54, 55]. Potential underlying mechanisms may be the increased supply of cytokines and NEFAs to the liver, leading to inflammation and hepatic fat accumulation [71, 72]. Hepatic fat accumulation in the absence of significant alcohol consumption is defined as non-alcoholic fatty liver disease (NAFLD) [266]. The accumulation of intracellulair lipids, and specifically its toxic metabolites such as diacylglycerol and ceramide activate protein kinase C which interferes with insulin signaling, leads to an decreased insulin stimulated glucose uptake, glycogen synthesis, glucose oxidation, and eventually type 2 diabetes [50, 51, 267]. NAFLD is also associated with an increased cIMT and incidence of cardiovascular disease [268]. Mechanisms that link NAFLD with atherosclerosis and cardiovascular

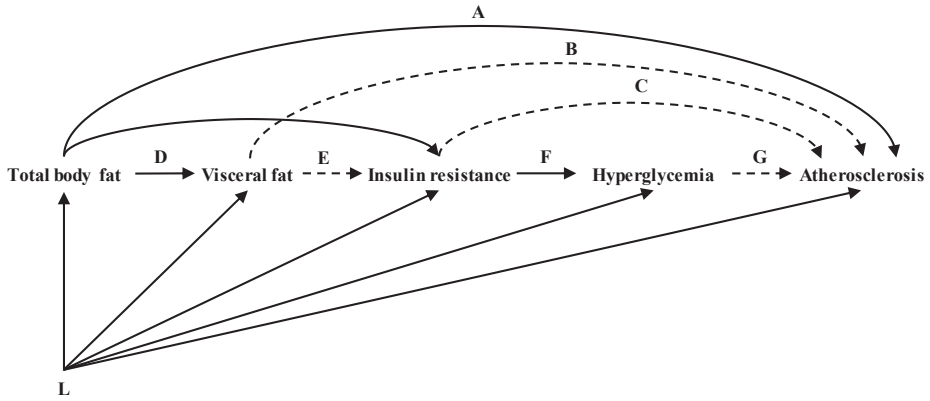

Figure 1. Hypothetical pathways between body fat, glucose metabolism, and atherosclerosis
L, known confounding factors including age, sex, ethnicity, education, tobacco smoking, alcohol consumption, and physical activity, but there may be unknown or unmeasured confounding factors.

disease are incompletely understood, but may involve dyslipidemia and an increased secretion of pro-inflammatory factors such as C-reactive protein (CRP) and plasminogen activator inhibitor type 1 (PAI-1) by the liver. This may suggest that liver fat, in addition to visceral fat, plays a role in the development of type 2 diabetes and cardiovascular disease [267-269]. The relative contributions of visceral fat, liver fat, and total body fat to insulin resistance and atherosclerosis however remain unclear, and will be studied in future analyses of the NEO study [270].

Sex differences in the role of visceral fat?

Our results suggest that visceral fat accumulation is particularly detrimental for women in relation to insulin resistance and, albeit to a lesser extent, atherosclerosis. Exact mechanisms underlying these cardiometabolic effects of VAT in women are yet unclear. Other observational studies have shown that on average, men who gain weight tend to accumulate both subcutaneous and visceral fat, whereas women predominantly accumulate subcutaneous fat [44]. Therefore, men may be more genetically [271] predisposed to store triglycerides viscerally, which in men seems similarly harmful as subcutaneously stored fat. The mechanism why some women store fat viscerally is largely unknown, but possibly hormonal and inflammatory factors play a role. Premenopausal women store fat mainly in the subcutaneous depots on the hips and thighs [272, 273]. Oestrogens are considered to be responsible for this gluteal-femoral fat distribution that seems to protect women from type 2 diabetes [173, 187]. After menopause there is a shift toward an android fat distribution, presumably due to decreasing oestrogen levels [39, 188, 189]. Recent studies have shown that high testosterone concentrations are positively associated with visceral fat amount [190] and an increased risk of type 2 diabetes [191] in postmenopausal women. In a cohort study in women, extreme low concentration of estradiol and extreme high concentrations of testosterone were associated with an increased risk of

ischemic heart disease and death [274]. In contrast, other cohort studies in women reported different results, showing an increased cardiovascular risk in women with low testosterone [275, 276], high estradiol [277], or showing no association with either estradiol nor testosterone [278]. Therefore, it remains unclear to what extent sex hormones are involved in the etiology of type 2 diabetes and cardiovascular disease and, secondly, whether sex hormones can explain the increased cardiometabolic risk associated with visceral fat in women. Finally, an alternative explanation may be considered here as well: visceral fat accumulation in women may be a more specific marker of dysfunctional subcutaneous fat than in men. Cohort studies that measure hormonal and inflammatory factors in addition to visceral fat, incident type 2 diabetes, and incident cardiovascular disease may elucidate the strength, direction, and underlying mechanisms of these relationships.

'Waist management'

The results of this thesis support the importance of maintaining a healthy body weight and in particular a healthy waist circumference for the prevention of type 2 diabetes and cardiovascular disease. The expected increase in the global prevalence of overweight up to 60%, corresponding to an absolute number of over 3 billion adults in 2030 [4] will have a huge impact on the incidence of type 2 diabetes and cardiovascular disease. As a result, projections have estimated that in the United States, when the number of obese adults will have risen with 65 million by 2030, this will add 5.4-6.8 million cases of coronary heart disease or stroke and 5.5-6.8 million cases of diabetes [9]. These striking numbers urge the need for interventions that reduce the prevalence of (abdominal) obesity and its consequences.

Unfortunately, public health campaigns aimed at general weight reduction have failed spectacularly. Therefore, an alternative may be to identify individuals at increased risk of obesity-related complications. General practitioners play an important role in the early identification of persons with abdominal obesity in primary care. Previously, only BMI was used by general practitioners to identify persons with overweight. Nowadays, in the Netherlands, waist circumference is added to a cardiometabolic risk score model which can be used in persons of 45 years or older [279]. Unfortunately, despite many earlier studies showing the importance of measuring waist circumference [26-33, 280], the use of this risk model is not yet routine in clinical practice [281]. The findings of this thesis support that the measurement of waist circumference should be routine practice in primary care to identify persons at high cardiometabolic risk and, if necessary, to evaluate the effect of treatment. We furthermore showed that for a given waist circumference, there is a large variation on visceral fat in both men and women. This highlights that, in clinical practice, simple and less expensive measures that can distinguish abdominal subcutaneous fat from visceral fat are needed to improve risk stratification. A combination of high waist circumference and serum fasting triglycerides [166-168], CRP [169], cytokines [170, 171] or sex hormones [172] may be explored for that purpose. Alternatively, identification of novel biomarkers derived from systems biology approaches may be important [282]. Finally, our

findings suggest that particularly women are susceptible to the detrimental cardiometabolic effects of visceral fat accumulation. Therefore, future research is needed to unravel underlying mechanisms in order to identify women at high cardiometabolic risk.

Different strategies such as diet, exercise, pharmaceutical or surgical interventions are able to induce weight loss, but have not been promising on the long-term with regard to maintenance of weight loss [283-285]. Nevertheless, lifestyle intervention studies have shown that cardiometabolic improvements were related to the extent of visceral fat loss [141, 142]. In general, lifestyle interventions that induce weight loss, even modest, will also result in loss of visceral fat [286]. Several intervention studies have shown that regular exercise can reduce waist circumference and visceral fat mass, leading to an improved cardiometabolic profile, even in the absence of weight loss [287]. Specific dietary factors (e.g., types of fat) have also shown to influence visceral and ectopic fat accumulation [288].

Notwithstanding these promising results, randomized intervention studies are needed to investigate whether cardiometabolic health can be improved by reducing visceral fat amount. Such trials should investigate the effects of dietary quality and physical activity patterns on visceral fat accumulation and whether a change in visceral fat affects risk of type 2 diabetes and cardiovascular disease. In the meantime, cohort studies with direct assessment of abdominal fat depots, such as the NEO study, will contribute to answer these questions.