Insulin resistance and atherosclerosis : the role of visceral fat

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Chapter 3

Abdominal adiposity largely explains associations between insulin resistance, hyperglycemia and subclinical atherosclerosis: the NEO study

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ABSTRACT

Objective

The relative importance of insulin resistance and hyperglycemia to the development of atherosclerosis remains unclear. Furthermore, adiposity may be responsible for observed associations. Our aim was to study the relative contributions of adiposity, insulin resistance and hyperglycemia to subclinical atherosclerosis.

Methods

In this cross-sectional analysis of the Netherlands Epidemiology of Obesity (NEO) study, a cohort of persons of 45-65 years, BMI, waist circumference (WC), fasting glucose (FPG), HbA_{1c} and insulin concentrations were measured and the revised HOMA-IR was calculated. The carotid intima-media thickness (cIMT) was measured by ultrasound. We performed linear regression analyses between standardized values of FPG, HbA_{1c}, HOMA-IR, BMI, WC with cIMT, and subsequently included age, sex, ethnicity, education and smoking, HOMA-IR, HbA_{1c} and FPG, BMI and WC in the models.

Results

After exclusion of participants with glucose lowering therapy (n=356) or missing data (n=252), this analysis included 6,065 participants, 43% men, and mean (SD) cIMT of 616 (92) µm. Differences in cIMT (95% CI) per SD were: FPG: 16 (10,21); HbA_{1c}: 12 (7,16); HOMA-IR: 11 (6,16) µm. These associations attenuated after adjustments, and attenuated most strongly after adjustment for WC. Differences in cIMT (95% CI) per SD in the full model were: FPG: 4 (0,7); HbA_{1c}: 2 (-1,5); HOMA-IR: 0 (-3,3); BMI 16 (13,19); WC: 18 (14,21) µm.

Conclusion

In middle-aged individuals, we observed similar contributions of insulin resistance and hyperglycemia to subclinical atherosclerosis. These contributions were largely explained by abdominal adiposity, emphasizing the importance of weight management.

INTRODUCTION

Type 2 diabetes increases the risk of cardiovascular disease 2- to 3-fold [12, 13]. Both insulin resistance and hyperglycemia may promote atherosclerosis [16, 17]. However, the relative importance of hyperglycemia and insulin resistance to the development of atherosclerosis remains unclear.

Experimental studies have identified differential contributions of hyperglycemia and insulin resistance to the development of atherosclerosis. Insulin resistance may lead to atherosclerosis through various mechanisms, including dyslipidemia and inflammation [16]. Hyperglycemia may contribute to endothelial dysfunction by several mechanisms, including oxidative stress, increased production of advanced glycation endproducts and alterations in glycocalyx composition [17, 120]. However, randomized controlled trials in individuals with pre-diabetes and type 2 diabetes have not shown an effect of intensive glycemic control (i.e. lowering HbA_{1c} concentration) on the incidence of cardiovascular events [121, 122]. These results suggest that the contribution of hyperglycemia to the development of atherosclerosis may be limited.

Observational studies in persons without glucose lowering therapy have shown that measures of insulin resistance and glucose concentrations were associated with carotid intima-media thickness (cIMT) [20, 22, 23], a marker of subclinical atherosclerosis, and cardiovascular disease [13, 107, 123]. These studies, however, have not distinguished the contributions of hyperglycemia and insulin resistance. Moreover, adiposity may result in insulin resistance, consequential hyperglycemia and atherosclerosis and may therefore be responsible for observed associations. We hypothesize that observed associations between insulin resistance, hyperglycemia and atherosclerosis can be explained by adiposity (These hypothetical pathways are depicted in **Figure 1**).

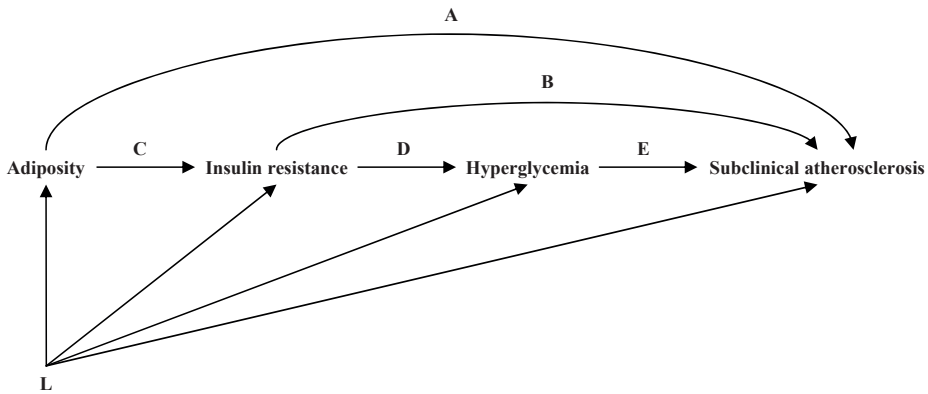

Figure 1. Hypothesis Path diagram
L: known and unknown confounding factors. Underlying mechanisms of the paths A to E are indicated in the discussion.

Therefore, the aim of this study is to investigate the relative contributions of adiposity, insulin resistance and hyperglycemia to subclinical atherosclerosis, in a cohort of men and women without glucose lowering therapy.

MATERIALS AND METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in individuals aged 45-65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. This cohort was designed to prospectively study pathways that lead to disease in persons with overweight or obesity. The present study is a cross-sectional analysis of the baseline measurements of the participants included in the NEO study from the start at September 3, 2008 until the end at September 28, 2012. Detailed information about the study design and data collection has been described elsewhere [77]. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited, irrespective of their BMI.

Prior to the NEO study visit, participants completed a questionnaire about demographic and clinical data and fasted for at least 10 hours. Participants came to the research site in the morning to undergo several baseline measurements including anthropometric measurements, blood sampling and a cIMT measurement. For the present analysis, we excluded participants who used oral hypoglycemic agents or insulin in the month prior to the study visit, in addition to participants with missing data.

The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent.

Data collection

Participants reported ethnicity by self-identification in eight categories which we grouped into white (reference) and other. Tobacco smoking was reported in the three categories current smoker, former smoker, never smoker (reference). Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high versus low education (reference). Participants reported their medical history of diabetes and cardiovascular diseases. Pre-existing cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. In addition, all use of medication in the month preceding the study visit was recorded. At the study site, height and weight were measured without shoes and with precision of 0.1 cm/kg and 1 kilogram was subtracted from the weight for clothing. BMI was calculated by dividing the weight in kilograms by the height

in meters squared. Waist circumference was measured between the border of the lower costal margin and the iliac crest with the precision of 0.1 cm. We used BMI as a measure of overall adiposity and waist circumference as a measure of abdominal adiposity.

Measures of insulin resistance and glycemic control

Fasting blood samples were drawn from the antecubal vein after 5 min rest of the participant. Fasting plasma glucose concentrations (FPG) were determined by enzymatic and colorimetric methods (*Roche Modular Analytics P800, Roch Diagnostics, Mannheim, Germany*; CV < 5%) and serum insulin concentrations were determined by an immunometric method (*Siemens Immulite 2500, Siemens Healthcare Diagnostics, Breda, The Netherlands*; CV < 5%). HbA_{1c} concentrations were measured by HPLC boronate affinity chromatography (*Primus Ultra, Siemens Healthcare Diagnostics, Breda, The Netherlands*; CV < 3%). All analyses were performed in the central clinical chemistry laboratory of the LUMC [77].

In our study population without glucose lowering therapy, we identified individuals with unknown diabetes, having a FPG of 7.0 mmol/L or higher. FPG reflects basal gluconeogenesis and HbA_{1c} represents the average glucose concentration in the preceding 3 months [124]. The updated Homeostasis Model Assessment Insulin Resistance (HOMA-IR) is a measure of insulin resistance, which corresponds well to estimates of insulin resistance derived from the hyperinsulinemic euglycemic clamp [81], and it was calculated by entering fasting glucose and fasting insulin in a Microsoft Excel spreadsheet available on the internet [125].

Carotid intima-media thickness

The cIMT was measured in the far wall of the left and right common carotid arteries (CCA's), along a 15 mm long section 10 mm, proximal of the bifurcation, and in recumbent position. A 7.5-10 MHz linear-array transducer (*Art.Lab version 2.1, Esaote, Maastricht, The Netherlands*) in B-mode setting was used to visualize the distal CCA and an online wall track system was used to detect the lumen-intima and media-adventitia boundaries. CIMT was measured in three predefined angles per side (180,135 and 90° for the right CCA and 180, 225 and 270° for the left CCA) during six heartbeats. We calculated the mean cIMT for each participant (referred to as cIMT) by averaging all 36 cIMT measurements within each individual.

Statistical analyses

In the NEO study there is an oversampling of individuals with a BMI of 27 kg/m^2 or higher. To correctly represent associations in the general population [126], adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality [127], whose BMI distribution was similar to the BMI distribution of the general Dutch population [128].

Baseline characteristics of the weighted study population were expressed as mean (SD), median (IQR) or as percentage. We divided the participants who were excluded from our analyses

into two groups: those who used glucose lowering therapy and those who did not use glucose lowering therapy, but had missing data. Differences in baseline characteristics between these groups and the participants who were included in our analyses were tested with Student t-test or Chi-squared test where appropriate. Insulin concentrations below the detection limit of the assay (2.0 mU/L) were imputed using multiple imputation methods for left censored data [129], with 10 imputation datasets. We calculated Pearson's correlation coefficients between FPG, HbA_{1c}, HOMA-IR, BMI, waist circumference, and also between the exposures and cIMT to be able to compare these correlation coefficients with correlation coefficients from previous studies. We used generalized additive models (GAM) to examine the shape of association between FPG, HbA_{1c}, HOMA-IR, BMI, waist circumference and cIMT while concurrently adjusting for age, sex, ethnicity, education, and tobacco smoking. The associations of FPG, HbA_{1c}, and HOMA-IR with cIMT were additionally adjusted for BMI and waist circumference. The associations between FPG, HbA_{1c}, HOMA-IR, BMI, waist circumference and cIMT were analyzed both in weighted tertiles of the exposures and continuously by using linear regression analyses. FPG, HbA_{1c}, HOMA-IR, and BMI were divided into tertiles. In addition, we standardized the values of FPG, HbA_{1c}, HOMA-IR, and BMI. As a result, the differences in cIMT are expressed per standard deviation of each variable. Waist circumference was standardized for men and women separately.

Regression coefficients and corresponding 95% confidence intervals (CI) were expressed as the difference in cIMT (μm) per tertile (lowest tertile as reference category) and per standard deviation of FPG, HbA_{1c}, HOMA-IR, BMI, and waist circumference. We calculated variance inflation factors (VIFs) to check for multicollinearity in our regression models and VIF values below 10 were considered appropriate. First, we adjusted the crude associations for age, sex, ethnicity, education, and tobacco smoking (model 1). Second, to study to what extent associations of FPG and HbA_{1c} with cIMT are explained by insulin resistance, we additionally adjusted these associations for HOMA-IR (model 2a). Third, in order to study direct pathways, we included intermediate variables FPG and HbA_{1c} in the model with HOMA-IR as exposure (model 2b) and FPG, HbA_{1C}, and HOMA-IR in the models with BMI or waist circumference as exposure. Fourth, to study to what extent the association between adiposity and cIMT is mediated by mechanisms of hyperglycemia or insulin resistance, we additionally included FPG and HbA_{1C} (model 2d) and HOMA-IR (model 2e) in the models with BMI or waist circumference as exposure. Finally, in order to investigate the influence of adiposity, we included waist circumference and BMI in the models with FPG, HbA_{1c} (model 3a) or HOMA-IR (model 3b) as exposure.

We performed a sensitivity analysis excluding participants with pre-existing cardiovascular disease at the baseline study visit. Analyses were performed with STATA Statistical Software (*Statacorp, College Station, Texas, USA*), version 12.0. GAM curves were constructed with R: A language and environment for statistical computing (*R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org*), version 2.15.0.

RESULTS

6,673 participants were included in the NEO study. After consecutive exclusion of participants who used oral hypoglycaemic agents (n=267), insulin (n=23) or both (n=66), participants with missing fasting blood sampling (n=117), missing cIMT data (n=66) or missing data for ethnicity (n=9), education (n=56), tobacco smoking (n=3) or waist circumference (n=1), 6,065 participants were included in the present analysis.

Characteristics of the included participants are presented in **Table 1**. When compared with the included participants, excluded participants who used glucose lowering therapy (n=356) were older (mean age 59 versus 56), more frequently male (55% versus 43%), had a higher mean BMI (31.0 kg/m^2 versus 26.2 kg/m^2), and had a higher mean cIMT (655 µm versus 616 µm). There were no differences in baseline characteristic between the included participants and excluded participants who did not use glucose lowering therapy (n=252) (data not shown).

Table 1. Baseline characteristics of the weighted NEO study population of 6,065 participants in the NEO study aged between 45 and 65 years without glucose lowering therapy

Characteristics	NEO study population (n=6,065)
Age (years)	56 (50-61)
Sex (% men)	43
Ethnicity (% whites)	95
Educational level (% low [a])	19
Tobacco smoking (%)	
Current	16
Former	46
Medical history of CVD [b] (%)	6
BMI (kg/m^2)	26.2 (4.3)
Waist circumference (cm)	
Men	98 (11)
Women	87 (13)
FPG (mmol/L)	5.4 (0.8)
≥ 7.0 mmol/L (%)	2
HbA_{1C} (% Hb)	5.3 (0.3)
HOMA-IR	0.85 (0.81)
cIMT (µm)	616 (92)

Data are shown as mean (SD), median (IQR) or number (%).

[a] Low education: none, primary school, lower vocational education.

[b] CVD, cardiovascular disease: defined as myocardial infarction, angina, congestive heart failure, stroke and peripheral vascular disease.

NEO, Netherlands Epidemiology of Obesity; CVD, cardiovascular disease; BMI, Body Mass Index; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; Hb, hemoglobin; HOMA-IR, Homeostasis Model Assessment Insulin Resistance; cIMT, carotid intima-media thickness

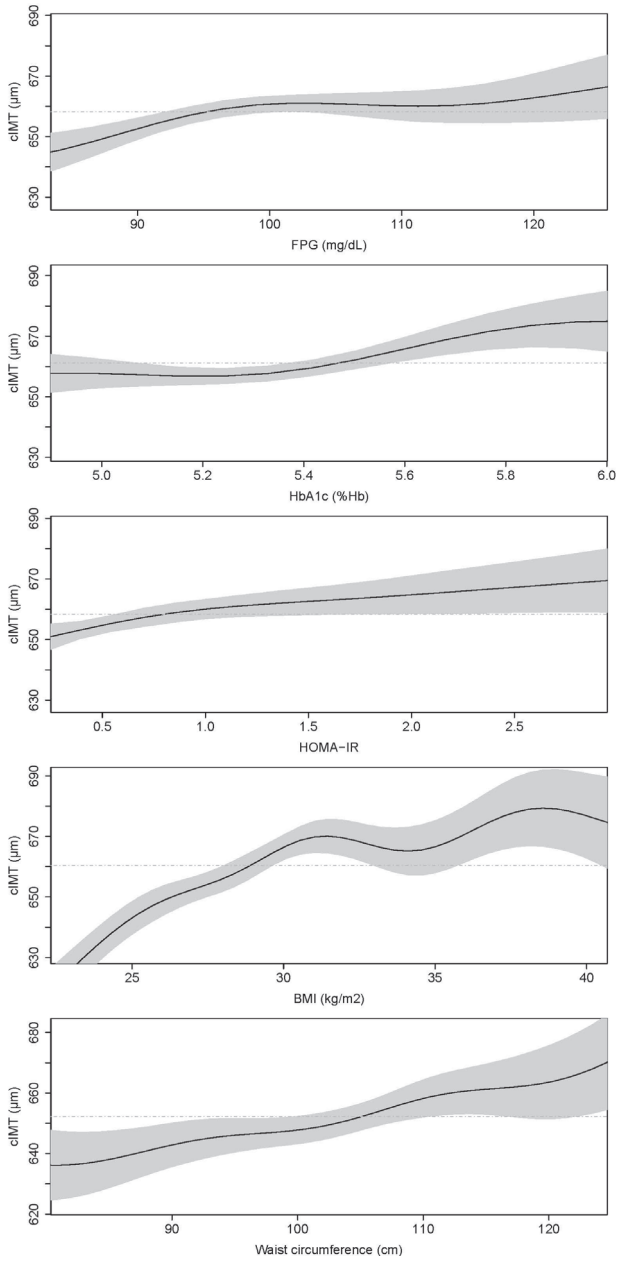

Figure 2. Generalized Additive Model curves showing the associations between FPG, HbA_{1c}, HOMA-IR, BMI, waist circumference and cIMT in 6,065 participants in the NEO study aged between 45 and 65 years without glucose lowering therapy

Curves were adjusted for age, sex, ethnicity, education, and tobacco smoking. The associations of FPG, HbA_{1c}, and HOMA-IR with cIMT were additionally adjusted for BMI and waist circumference.

cIMT, carotid intima-media thickness; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; Hb, hemoglobin; HOMA-IR, Homeostasis Model Assessment Insulin Resistance; BMI, body mass index

The Pearson correlation coefficients between FPG, HbA_{1c}, HOMA-IR, BMI, waist circumference, and cIMT are shown in the **Appendix Table**. The GAM curves showed linear associations between FPG, HbA_{1c}, HOMA-IR, BMI, waist circumference and cIMT after adjusting for confounding variables (**Figure 2**).

The results of the linear regression analyses are shown by tertiles in **Table 2** and per standard deviation of FPG, HbA_{1c}, HOMA-IR, BMI, and waist circumference in **Figure 3**. One standard

Table 2. Associations between tertiles of FPG, HbA_{1C}, HOMA-IR, BMI, waist circumference and cIMT (μm) in 6,065 participants in the NEO study aged between 45 and 65 years without glucose lowering therapy

Tertiles	Difference in cIMT (μm)[a]			
	Crude (95% CI)	Model 1 (95% CI)	Model 2a/b/c (95% CI)	Model 3a/b (95% CI)
FPG (mmol/L)			Model 2a	Model 3a
<5.1 (reference)	-	-	-	-
5.1-5.5	26 (17, 35)	18 (10, 27)	17 (8, 25)	13 (4, 21)
>5.5	43 (35, 51)	24 (16, 32)	19 (11, 28)	11 (3, 19)
HbA_{1C} (%Hb)			Model 2a	Model 3a
<5.2 (reference)	-	-	-	-
5.2-5.4	16 (7, 25)	8 (0, 17)	7 (-1, 15)	5 (-3, 13)
>5.4	31 (22, 39)	16 (8, 24)	12 (3, 20)	4 (-4, 13)
HOMA-IR			Model 2b	Model 3b
<0.78 (reference)	-	-	-	-
0.78-1.22	12 (1, 22)	8 (-2, 18)	6 (-4, 16)	1 (-9, 11)
>0.90	31 (23, 39)	23 (15, 31)	18 (9, 26)	0 (-9, 9)
BMI (kg/m^2)			Model 2c	
<24.0 (reference)	-	-	-	-
24.0-27.2	31 (21, 40)	25 (16, 34)	23 (14, 33)	-
>27.2	48 (41, 56)	42 (35, 50)	38 (30, 46)	-
Waist circumference (cm)			Model 2c	
<85 (reference)	-	-	-	-
85-97	27 (17, 36)	19 (10, 27)	16 (7, 25)	-
>97	51 (43, 59)	40 (32, 48)	34 (25, 43)	-

Model 1: Adjusted for age, sex, ethnicity, education, and tobacco smoking.
Model 2a: Adjusted for model 1 plus HOMA-IR.
Model 2b: Adjusted for model 1 plus HbA_{1C} and FPG.
Model 2c: Adjusted for model 1 plus HOMA-IR, HbA_{1c}, and FPG.
Model 3a: Adjusted for model 2a plus waist circumference and BMI.
Model 3b: Adjusted for model 2b plus waist circumference and BMI.
[a] Beta coefficients (95% CI) from linear regression.
cIMT, carotid intima-media thickness; CI, confidence interval; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; Hb, hemoglobin; HOMA-IR, Homeostasis Model Assessment Insulin Resistance; BMI, body mass index

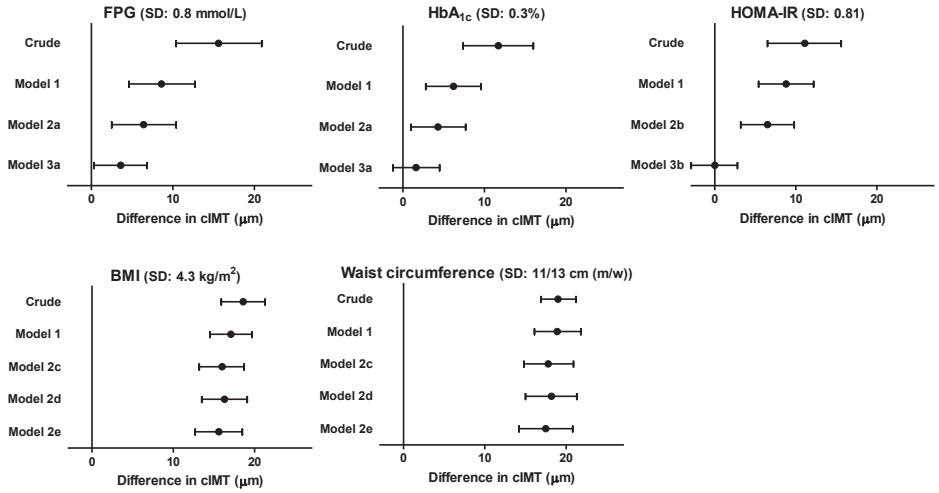

Figure 3. Associations between standardized FPG (3a), HbA_{1C} (3b), HOMA-IR (3c), BMI (3d), waist circumference (3e) and cIMT (µm) in 6,065 participants in the NEO study aged between 45 and 65 years without glucose lowering therapy

Model 1: Adjusted for age, sex, ethnicity, education, and tobacco smoking.

Model 2a: Adjusted for model 1 plus HOMA-IR.

Model 2b: Adjusted for model 1 plus HbA_{1C} and FPG.

Model 2c: Adjusted for model 1 plus HOMA-IR, HbA_{1c}, and FPG.

Model 2d: Adjusted for model 1 plus HbA_{1C} and FPG.

Model 2e: Adjusted for model 1 plus HOMA-IR.

Model 3a: Adjusted for model 2a plus waist circumference and BMI.

Model 3b: Adjusted for model 2b plus waist circumference and BMI.

FPG, fasting plasma glucose; SD, standard deviation; cIMT, carotid intima-media thickness; HbA_{1c}, glycated hemoglobin; Hb, hemoglobin; HOMA-IR, Homeostasis Model Assessment Insulin Resistance; BMI, body mass index

deviation of FPG (0.8 mmol/L) was associated with 16 (95% CI: 10, 21) µm cIMT. One standard deviation of HbA_{1c} (0.3%) was associated with 12 µm cIMT (95% CI: 7, 16). These associations attenuated after adjustment for age, sex, ethnicity, education, and tobacco smoking (FPG: 9 µm, 95% CI: 5, 13; HbA_{1c}: 6 µm, 95% CI: 3, 10) and attenuated further after adjustment for HOMA-IR (FPG: 6 µm, 95% CI: 3, 10; HbA_{1c}: 4 µm, 95% CI: 1, 8). After additional adjustment for BMI and waist circumference, the cIMT difference was 4 µm (95% CI: 0, 7) per standard deviation of FPG and 2 µm (95% CI: -1, 5) per one standard deviation of HbA_{1c} (**Figures 3a and 3b**). Of all confounding variables, association between FPG, HbA_{1c}, and cIMT attenuated most strongly after adjustment for waist circumference.

The crude association of one standard deviation of HOMA-IR (0.81 units) of 11 µm cIMT (95% CI: 6, 16) attenuated to 9 µm (95% CI: 5, 12) after adjustment for age, sex, ethnicity, education, and tobacco smoking and to 6 µm (95% CI: 3, 10) after adjustment for HbA_{1c} and FPG. After additional adjustment for both BMI and waist circumference the cIMT difference was 0 µm (95%

CI: -3, 3) (**Figure 3c**). Of all confounding variables, the association between HOMA-IR and cIMT attenuated most strongly after adjustment for waist circumference.

One standard deviation of BMI (4.3 kg/m^2) was associated with 19 µm cIMT (95% CI: 16, 21). One standard deviation of waist circumference (men: 11 cm; women: 13 cm) was associated with 19 (95% CI: 17, 21) µm cIMT. These associations attenuated after adjustment for age, sex, ethnicity, education, and tobacco smoking (BMI: 17 µm, 95% CI: 14, 20; waist circumference: 19 µm, 95% CI: 16, 22) (**Figure 3**). After additional adjustment for FPG, HbA_{1c}, and HOMA-IR, the cIMT differences was 16 µm cIMT (95% CI: 13, 19) for BMI and 18 µm cIMT (95% CI: 14, 21) for waist circumference. The differences in cIMT were similar when we adjusted separately for FPG and HbA_{1C} and HOMA-IR (**Figures 3d and 3e**).

After exclusion of participants with pre-existing cardiovascular disease results were similar (data not shown).

DISCUSSION

In this population-based study of men and women without glucose lowering medication, we observed that both insulin resistance and hyperglycemia remained associated with cIMT after mutual adjustment. Importantly, abdominal adiposity largely explained these associations.

Several studies have investigated associations between insulin resistance, hyperglycemia and cIMT, a marker of subclinical atherosclerosis. The correlation coefficients between HbA_{1C}, FPG, HOMA-IR, BMI, waist circumference and cIMT from our study were within the range of correlation coefficients observed in previous studies [20, 22, 23, 130-133]. However, relative contributions of hyperglycemia, insulin resistance and adiposity to the development of atherosclerosis were not identified in these studies. A recent study in 60 middle-aged individuals without diabetes concluded that the association between plasma glucose concentrations and cardiovascular risk was mainly explained by insulin resistance [134]. However, after adjustment for insulin sensitivity FPG remained associated with the cardiovascular risk score. In our study of 6,065 participants, in which we assessed a clinical parameter of subclinical atherosclerosis instead of a risk calculator, both insulin resistance and glucose concentrations remained associated with the cIMT after mutual adjustment. These associations were of similar strength, suggesting that both play a role in the development of atherosclerosis. However, in our study, waist circumference was most strongly associated with the cIMT, and largely explained the association of hyperglycemia with cIMT and completely explained the association of insulin resistance with cIMT. This implies that abdominal adiposity is for a large part responsible for the development of insulin resistance, hyperglycemia and subclinical atherosclerosis.

Strengths of this study are the large study population and the availability of a clinical parameter of atherosclerosis, information on measures of glycemic control, potential confounding variables, and of waist circumference as a measure of abdominal adiposity besides BMI.

This study also has some limitations that need to be considered. We assessed insulin resistance using the HOMA-IR instead of using a gold standard measurement, the hyperinsulinemic euglycemic clamp. However, the application of hyperinsulinemic euglycemic clamps in large epidemiologic studies is often not feasible. HOMA-IR is a surrogate measure of insulin resistance and may therefore not account for the total effect of insulin resistance [81]. Since waist circumference is strongly associated with insulin resistance [135], adjustment for waist circumference in addition to HOMA-IR may have resulted in a more complete adjustment for insulin resistance. However, due to the observational cross-sectional nature of our analyses, residual confounding may remain.

Data from experimental studies have shown that both insulin resistance and hyperglycemia can promote the development of atherosclerosis [16, 17]. Whereas insulin resistance seems to be involved in both early atherosclerosis and advanced plaque progression (**Figure 1**, path B), the effects of hyperglycemia seem to be limited to the development of early atherosclerosis (**Figure 1**, path E) [16]. This might explain why interventions that improve glycemic control have not resulted in a reduction of cardiovascular events in patients with type 2 diabetes [121] and in persons with pre-diabetes and a prior cardiovascular event [122], who are likely to have underlying intermediate or advanced atherosclerosis. In addition, the cardiovascular risk reduction of 42% by intensive glycemic control in a trial in patients with type 1 diabetes may support the hypothesis that glucose lowering therapy early in the course of atherosclerosis is beneficial [136]. The results of our study suggest that hyperglycemia may and insulin resistance may not contribute to the development of atherosclerosis in a population of individuals with low cIMT, possibly reflecting an early stage of atherosclerosis. Even though associations between hyperglycemia and subclinical atherosclerosis were weak, one cannot rule out the possibility that small effects can accumulate over time.

In our study, waist circumference most strongly explained the association between insulin resistance, hyperglycemia and subclinical atherosclerosis, implying underlying pathways between abdominal adiposity and atherosclerosis beyond pathways via insulin resistance and glucose. We furthermore observed that the association between waist circumference and subclinical atherosclerosis attenuated slightly after adjustment for hyperglycemia and insulin resistance, suggesting that it is partly mediated through insulin resistance and hyperglycemia, but largely through other mechanisms. Associations attenuated to the same extent when adjusting for insulin resistance and hyperglycemia together and separately, suggesting that both reflect the same mechanism.

Observational studies support that abdominal adiposity is a better marker of cardiometabolic risk and mortality than overall adiposity [137]. Adipose tissue is an endocrine organ secreting pro-inflammatory cytokines and non-esterified fatty acids (NEFAs) [49], that may promote the development of insulin resistance (**Figure 1**, path C) and atherosclerosis (**Figure 1**, path A) via mechanisms of inflammation and oxidative stress [17, 138]. Adipose tissue may therefore promote the development of atherosclerosis through insulin resistance (**Figure 1**, path CB)

and hyperglycemia (**Figure 1**, path CDE), but also directly by mechanism such as inflammation, dyslipidemia and hypertension (**Figure 1**, path A) [138]. Especially intra-abdominal adipose tissue is a major source of adipokines and NEFAs [139], and waist circumference has been shown to be an adequate marker of abdominal adiposity [140]. Several weight loss studies have shown that cardiometabolic improvements were related to the extent of intra-abdominal adipose tissue loss [141, 142]. Therefore, health benefits can be gained from weight loss interventions in individuals with abdominal adiposity.

In conclusion, in middle-aged individuals without glucose lowering medication we observed similar contributions of insulin resistance and hyperglycemia to the development of subclinical atherosclerosis. These contributions were to a large extent explained by abdominal adiposity, emphasizing the importance of weight management and prevention of weight gain in adulthood.