PREDICTORS OF REGRESSION OF PREDIABETES TO NORMOGLYCEMIA

ALANINE TRANSAMINASE AND WAIST TO HIP RATIO AS PREDICTORS OF DYSGLYCEMIA AND REGRESSION TO NORMOGLYCEMIA IN ADULT PATIENTS WITH PREDIABETES

By

NATALIA YAKUBOVICH, MSc, MD, FRCPC

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Author:	Natalia Yakubovich, MSc, MD, FRCPC
Supervisor:	Dr. Hertzel C. Gerstein
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Abstract

Current evidence suggests that both prediabetes and diabetes can reverse to normoglycemia; however, predictors of remission of these conditions are poorly understood. We performed analyses on 1,209 people with impaired fasting glucose and/or impaired glucose tolerance treated with placebo rosiglitazone and placebo ramipril in the DREAM trial. Normoglycemia was defined as a fasting plasma glucose <5.6 mmol/L and 2-hour plasma glucose <7.8 mmol/L on a 75 g oral glucose tolerance test (OGTT).

The effects of baseline ALT and waist to hip ratio (WHR) on regression of prediabetes to normoglycemia 2 years later were found to be interdependent (p-value for interaction 0.01). Adjusted odds ratios ORs (95% CI) of regression to normoglycemia per 10 U/L increase in ALT were 0.79 (0.66-0.94) when WHR was at the mean minus 1 standard deviation (SD), 0.90 (0.80-1.02) when WHR was at the mean of 0.91, and 1.03 (0.90-1.18) when WHR was at the mean plus 1 SD. Adjusted ORs of regression to normoglycemia per 0.1 unit increase in WHR were 0.75 (0.60-0.95) when ALT was at the mean minus 1 SD, 0.91 (0.76-1.08) when ALT was at the mean of 25 U/L, and 1.09 (0.89-1.35) when ALT was at the mean plus 1 SD.

Similarly, the effects of baseline ALT and WHR on $AUC_{glucose0\text{-}120\ min}$ obtained from the OGTT were found to be interdependent (p-value for interaction 0.056). A 10 U/L increase in ALT was associated with an adjusted $AUC_{glucose0\text{-}120\ min}$ increase of 19.5 (95% CI 5.3 to 33.7) min*mmol/L when WHR was at the mean minus 1 SD, 11.0 (1.4 to 20.6) min*mmol/L when WHR was at the mean of 0.91, and 2.5 (-9.2 to 14.1)

min*mmol/L when WHR was at the mean plus 1 SD. A 0.1 unit increase in WHR was associated with an adjusted $AUC_{glucose0\text{-}120\ min}$ increase of 30.3 (10.2 to 50.3) min*mmol/L when ALT was at the mean minus 1 SD, 18.3 (3.8-32.9) min*mmol/L when ALT was at the mean of 25 U/L, and 6.4 (-11.5 to 24.3) min*mmol/L when ALT was at the mean plus 1 SD.

In conclusion, high baseline ALT and WHR predict a lower likelihood of regression of prediabetes to normoglycemia and an increase in $AUC_{glucose0\text{-}120\ min}$ 2 years later; however, the effects of ALT and WHR on these outcomes are interdependent.

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List of abbreviations

ALT	alanine transaminase
ΔALT	change in ALT from baseline to 1 year
AST	aspartate aminotransferase
$AUC_{glucose0-120min}$	area-under-the-glucose-curve from 0 to 120 min on a 75 g oral glucose tolerance test
BMI	body mass index
CI	confidence interval
DREAM	Diabetes REduction Assessment with ramipril and rosiglitazone Medication
EUF	end of usual follow-up
FPG	fasting plasma glucose
GGT	gamma-glutamyltransferase
HC	hip circumference
HDL	high-density lipoprotein
2-hour PG	2-hour plasma glucose
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
LDL	low-density lipoprotein
MRI	magnetic resonance imaging
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NG	normoglycemia
NGT	normal glucose tolerance
OGTT	oral glucose tolerance test
OR	odds ratio
RCT	randomized controlled trial
RR	relative risk
SD	standard deviation
T2DM	type 2 diabetes mellitus
ULN	upper limit of normal
WC	waist circumference
WHR	waist to hip ratio
ΔWHR	change in waist to hip ratio from baseline to 2 years

1. Introduction

Type 2 diabetes and prediabetes are generally treated with lifestyle therapy, with an addition of glucose-lowering medications when glycemic control deteriorates. Recent evidence suggests that prediabetes and diabetes are reversible, and prolonged normoglycemia off therapy can be induced in a large proportion of patients. However, mechanisms and predictors of remission of these conditions are poorly understood. Identifying these mechanisms may facilitate the development of new pharmacological and non-pharmacological therapies that can be used to treat type 2 diabetes. Research suggests that ectopic fat deposition may play a role in the pathophysiology of diabetes. Therefore, we hypothesized that higher measures of ectopic fat deposition may predict a lower likelihood of regression to normoglycemia in people with prediabetes. We tested this hypothesis by designing and performing secondary analyses on already collected trial data.

2. Background and Rationale

2.1 Traditional approach to treating prediabetes and type 2 diabetes

Prediabetes is characterized by an elevation of plasma glucose above normal levels but below the threshold for diabetes. Isolated impaired fasting glucose (IFG) refers to an elevated fasting plasma glucose, and people with isolated impaired glucose tolerance (IGT) have elevated postprandial plasma glucose levels. Approximately 5-10% of people with prediabetes progress to type 2 diabetes annually[1]. The prevalence of type 2 diabetes continues to increase[2, 3], which has a major health and economic impact worldwide. Prediabetes and type 2 diabetes put people at risk of cardiovascular and renal disease, retinopathy, neuropathy, cognitive decline and other serious health problems[4-6].

Currently, prediabetes and type 2 diabetes are managed by lifestyle therapy and a step-wise addition of glucose-lowering medications when glycemic control deteriorates. Despite the growing knowledge about these conditions and a wide arsenal of anti-diabetic therapies, management of patients remains suboptimal. Type 2 diabetes continues to be the leading cause of blindness, below-knee amputations and kidney failure in developed nations[7]. Mean HbA1C levels in patients with diabetes remain high, and less than 50% of patients achieve therapeutic targets[8]. This suggests that new approaches to managing diabetes may need to be considered. One approach that generated renewed interest recently is induction of remission of type 2 diabetes.

2.2 Current evidence on reversibility of glycemic derangements in prediabetes and type 2 diabetes

Several randomized controlled trials (RCTs) and before-and-after studies conducted in people with prediabetes and type 2 diabetes suggest that these conditions can be reversed. Few of these studies assessed mechanisms and predictors of regression of these conditions to normoglycemia.

2.2.1. Randomized controlled trials in people with prediabetes

The Diabetes Prevention Program was a large randomized controlled trial conducted in 3,234 people with IGT ± IFG[9]. Participants were randomized to (i) intensive lifestyle therapy, (ii) metformin, or (iii) placebo and followed for 2.8 years. Approximately 33% of people in the intensive lifestyle group, 19% of those in the metformin group, and 18% of those in the placebo group had normal glucose tolerance at the end of the trial. Perreault and colleagues studied predictors of regression of combined IFG and IGT to normal glucose tolerance in 2,528 participants in this trial[10]. Young age, low fasting and 2-hour plasma glucose, and high insulin secretion were significant independent predictors of regression to normoglycemia. Weight loss also predicted regression to normal glucose tolerance.

The DREAM (Diabetes REduction Assessment with ramipril and rosiglitazone Medication) trial[11, 12] was a multicenter 2x2 factorial design trial conducted in 5,269 people with prediabetes. After 3 years of follow-up, 50.5% of patients in the rosiglitazone group and 30.3% of patients in the placebo rosiglitazone group regressed to normoglycemia ($p<0.0001$)[11]. Ramipril also increased the likelihood of regression to normoglycemia in the study participants compared to placebo ($p=0.001$)[12]. Acarbose was

found to significantly increase regression to normal glucose tolerance (NGT) in a multicenter RCT in 1,429 patients with IGT[13]. Thirty-five percent of people randomized to acarbose and 31% of those randomized to placebo had normal glucose tolerance at the end of the trial ($p<0.0001$). In a small trial on 120 participants with prediabetes and hypertriglyceridemia, fenofibrate, metformin and dietary intervention were shown to be effective in inducing regression to normoglycemia[14].

2.2.2. Interventional studies on induction of remission in type 2 diabetes

Several studies using short-term intensive insulin therapy or oral hypoglycemic agents demonstrated successful induction of remission in early type 2 diabetes. Normoglycemia was maintained in ~67% of participants 6 months after discontinuation of glucose-lowering medications[15, 16]. Approximately 40% of participants maintained drug-free remission for 1 year[15-21]. In a randomized controlled trial on 382 participants with newly diagnosed diabetes, patients were treated intensively with a continuous subcutaneous insulin infusion, multiple daily injections of insulin, or oral hypoglycemic agents for 2-4 weeks[20]. The rates of remission 1 year after randomization were 50%, 43%, and 22%, respectively ($p=0.001$ for oral agents versus combined insulin groups). In one study, 108 participants were followed for 2 years after a short-term intensive treatment with a continuous subcutaneous insulin infusion[22]. Forty two patients (39%) maintained normoglycemia off therapy during 2 years of follow-up. Short duration of diabetes from the time of onset of symptoms to diagnosis, high body mass index (BMI), and low baseline glucose and A1C levels were predictive of successful remission of

diabetes in these studies[18, 20, 22]. Those participants who needed shorter intensive treatment to achieve normoglycemia while on therapy were also more likely to achieve and maintain drug-free remission than those who required longer intensive treatment[20]. One study suggested that intensive insulin therapy partially restored beta-cell function and insulin sensitivity, and these improvements were maintained in people who achieved long-term remission of diabetes[20, 23].

The most effective therapy to date for induction of diabetes remission is bariatric surgery. In a randomized controlled trial on 60 recently-diagnosed patients with type 2 diabetes, 73% of those treated with gastric banding maintained diabetes remission for 2 years after randomization compared to 13% of patients treated with standard diabetes therapy[24]. The improvement in glucose metabolism following bariatric surgery is immediate, and it appears to be independent of weight loss. Potential mechanisms include an enhanced production of gastrointestinal hormones including glucagon-like-peptide-1 and peptide YY, decreased ghrelin secretion, and changes in insulin sensitivity[25-27]. Bariatric surgery, however, is invasive, and is not considered to be the first-line therapy due to associated complications.

Current evidence suggests that both prediabetes and diabetes can reverse to normoglycemia [9, 20, 21]; however, mechanisms and predictors of remission of these conditions are not well understood. Understanding these mechanisms may help facilitate the development of therapies that can be used to treat these conditions.

2.3 Potential role of ectopic fat in the pathophysiology of diabetes and prediabetes

2.3.1. A link between ectopic fat and metabolic derangements of prediabetes and diabetes

Understanding the pathophysiological mechanisms underlying type 2 diabetes and prediabetes may help identify new therapeutic targets. Type 2 diabetes and prediabetes are characterized by elevated glucose levels, decreased insulin production by the pancreas, and increased resistance of tissues to insulin effects. Diabetes and prediabetes have been shown to be associated with other metabolic abnormalities, including elevated triglycerides, low high-density lipoprotein (HDL) levels, and generation of small atherogenic low-density lipoprotein (LDL) particles[28, 29]. Recently, patients with prediabetes and diabetes have also been shown to have a significant accumulation of ectopic fat. Ectopic fat refers to fat in the body viscera (internal organs) rather than subcutaneous adipose tissue. Ectopic fat, for example, can accumulate in the intraadbominal cavity, liver, pancreas, heart, skeletal muscle and around blood vessels. With weight gain, fat is initially deposited in the subcutaneous tissue; however, the expansion of subcutaneous fat stores in some people is limited and fat begins to accumulate at ectopic sites[30, 31]. The relative distribution of fat between ectopic and subcutaneous compartments depends on a number of factors including genetics, age, ethnicity, gonadal hormones, smoking and medications[32-34]. The adipocyte capacity to divide, differentiate, store fat and promote angiogenesis has also been shown to play an important role[30, 35, 36].

Non-alcoholic fatty liver disease (NAFLD) refers to fat accumulation in the liver, and it ranges from hepatic steatosis (liver fat content >5%) to steatohepatitis (fat accumulation with cell necrosis and inflammation) to liver fibrosis. Non-alcoholic

steatohepatitis is referred to as NASH[37]. The prevalence of NAFLD determined by liver ultrasound in patients with type 2 diabetes approaches 70%[38, 39]; however, the exact prevalence is unknown. Patients with prediabetes have also been shown to have visceral and liver fat accumulation. In a cross-sectional study on 330 White individuals at risk for type 2 diabetes, total body fat and visceral fat were determined by magnetic resonance imaging (MRI), and liver fat was determined by proton spectroscopy[40]. Fatty liver (liver fat content >5.6%) was present in 25% of people with NGT, 22% of those with isolated IFG, 42% of those with isolated IGT, and 69% of people with combined IFG and IGT. Mean (standard error) liver fat content was 4.73 (0.42)% in NGT and 11.1 (1.01)% in combined IFG/IGT. A total amount of visceral fat and liver fat increased as the degree of glucose intolerance increased from NGT, to IFG, IGT, and combined IFG/IGT (p values of 0.03 and <0.0001 for visceral and liver fat, respectively) whereas a total amount of body fat was not significantly different across the groups. Liver fat and visceral fat were found to be significant independent predictors of fasting plasma glucose after adjustment for age, sex and total body fat.

Intraabdominal and liver fat accumulation has been shown to be associated with increased insulin resistance, accelerated hepatic glucose production, and elevated serum glucose, insulin and C-peptide levels[41-46]. However, mechanistic pathways behind ectopic fat accumulation and the development of insulin resistance and diabetes are not completely understood. An excessive caloric intake and decreased energy expenditure result in a positive energy balance and fat storage. Accumulation of fat in the subcutaneous adipose tissue leads to macrophage infiltration and release of adipokines[47,

[48]. This process has been shown to be associated with inflammation and development of peripheral insulin resistance[41, 49-51]. Lipolysis in the insulin-resistant subcutaneous adipose tissue releases free fatty acids which are then deposited in other organs. Diminished capacity of subcutaneous adipose tissue to expand and store fat further contributes to preferential deposition of dietary fat at ectopic sites[31]. Fat accumulation in the liver leads to increased hepatic insulin resistance characterized by increased gluconeogenesis, increased lipid synthesis and decreased lipid oxidation[28, 42, 46, 52, 53]. Increasing plasma glucose and insulin levels further promote synthesis and accumulation of triglycerides in the liver leading to steatosis, NASH and eventually fibrosis (see Figure 1)[37].

Deposition of fat in the pancreas may also be toxic to beta-cells and affect insulin secretion[50]. Heni *et al.*[54] studied 51 Caucasian subjects with NGT, IFG, IGT or both and measured pancreas fat by MRI. Mean pancreatic fat content was positively associated with visceral adipose tissue, body mass index (BMI) and waist circumference (WC) after adjustment for age and sex. High mean pancreatic fat content also predicted low insulin secretion (measured during an oral glucose tolerance test) after adjusting for insulin sensitivity. Therefore, it has been hypothesized that abnormalities in glucose and lipid metabolism observed in patients with prediabetes and diabetes may be linked to fat deposition in the liver, pancreas and other organs[42, 55, 56].

2.3.2. Measures of ectopic fat

Deposition of fat in internal organs such as liver, pancreas and heart is difficult to ascertain without imaging or tissue biopsies. The gold standard test for diagnosis of the non-alcoholic fatty liver disease is a liver biopsy, and specific histological criteria exist to evaluate grades of non-alcoholic steatohepatitis and fibrosis[57, 58]. Ultrasound of the liver has been used to diagnose NAFLD in clinical practice. The sensitivity and specificity are 94% and 84% respectively for steatosis, and 57% and 88% for fibrosis when compared with histological findings[59]. Liver fat content determined by computerized tomography has also been shown to correlate with liver biopsy findings[58, 60].

NAFLD is associated with an elevation of liver enzymes, including alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT)[61]. High ALT and AST levels correlated with the presence of steatohepatitis on liver biopsy in 30 obese subjects[58]. However, the presence of histologically confirmed liver fibrosis did not correlate well with liver enzyme levels in this study. ALT levels also correlated with the percentage of hepatocyte triglyceride content determined by proton spectroscopy in 132 healthy Caucasian subjects, with a correlation coefficient of $r=0.49$ in women and $r=0.62$ in men (both p values <0.0001)[43]. Among study participants who had ALT in the upper end of the normal range, mean liver fat content was found to be 13% in women and 10% in men. Using an ALT cut-off of 36 U/L has been reported to have 63% sensitivity and 78% specificity for diagnosing fatty liver disease determined by ultrasound. GGT and AST were found to have very low sensitivities for recognizing fatty liver disease at the cut-offs used in current clinical practice (sensitivities of 20 and 17.5%, respectively)[62].

Some studies also assessed associations of anthropometric indices with liver fat content or intra-abdominal fat content. BMI (weight in kg divided by height in m^2) is considered to be a measure of overall obesity, while waist circumference, waist to hip ratio (WHR, waist circumference divided by hip circumference), and waist to height ratio are used as indices of abdominal fat deposition. Nomura and colleagues[62] reported an area under the receiver operating characteristic curve of 0.63 (95% CI 0.56-0.70) for BMI in recognizing ultrasound-diagnosed fatty liver. However, females have more subcutaneous fat than males for the same amount of liver and intra-abdominal fat[43]. This suggests that measures of overall obesity may not accurately reflect intra-abdominal fat content.

The best anthropometric measure of visceral fat is yet to be determined. In some cross-sectional studies, waist circumference was more closely correlated with radiographically-determined abdominal visceral fat than WHR[63, 64], while other studies found that WHR was a better surrogate measure of abdominal visceral adiposity[65]. Several studies reported that sagittal abdominal diameter was more closely correlated with the amount of visceral adipose tissue than WC or BMI[66, 67]. Generally, measures of abdominal obesity exhibited higher correlations with visceral adipose tissue[40, 66-68] or liver fat[40] than measures of overall obesity such as BMI. It should be noted that most of these studies did not use formal statistical methods to compare the predictive ability of different anthropometric measures for visceral adiposity. The studies were often restricted to one center or one geographic region while literature suggests that ethnic

differences may exist in the accuracy of estimating visceral fat by different surrogate anthropometric measures[69, 70].

2.3.3. Abdominal obesity and abnormal liver enzymes as predictors of type 2 diabetes

Obesity has been shown to be a strong risk factor for development of type 2 diabetes. In order to delineate the role of abdominal obesity versus overall obesity in diabetes development, Vazquez and colleagues[71] conducted a meta-analysis on 32 prospective studies and compared associations between incident diabetes and different obesity measures including BMI, WC and WHR. Some studies included in this meta-analysis used clinical criteria for diabetes diagnosis, while others accepted self-reported physician-diagnosed diabetes. The pooled relative risks of incident diabetes per standard deviation increase were 1.87 (1.67-2.10) for BMI, 1.87 (1.58-2.20) for waist circumference, and 1.88 (1.61-2.19) for WHR. MacKay *et al.*[72] compared several measures of obesity in the ability to predict the 5-year incidence of diabetes in 1,073 American participants by performing receiver operating characteristic curve analyses. BMI, WC, WHR, and waist to height ratio performed similarly in the overall cohort and within 3 ethnic groups: Hispanic, Non-Hispanic White and African American.

However, other studies suggested some differences in the predictive ability of these anthropometric measures for type 2 diabetes[73, 74]. Jia *et al.*[73] followed 61,703 Chinese adults for 2 years and compared the predictive power of BMI, WC, WHR and waist to height ratio for incident diabetes by using receiver operating characteristic curve analyses. Waist to height ratio and waist circumference had the best predictive ability in

females, and waist to height ratio was the best predictor in males. This study, however, was restricted to Asian participants. de Koning and colleagues[74] compared BMI, WC, WHR and their combinations in the ability to predict glycemic status in a cross-sectional analysis on 22,293 high risk participants from 21 countries. All participants had a baseline 75 g oral glucose tolerance test (OGTT), and 14% of participants were newly diagnosed with type 2 diabetes at the time of enrollment. The authors found that among individual measures, waist circumference had a significantly greater explanatory power for diabetes than BMI. Among combinations of measures, (i) BMI and WHR and (ii) WC and hip circumference had the best explanatory power for type 2 diabetes and the degree of dysglycemia as measured by the area-under-the-glucose-curve on the OGTT. These studies suggest that measures of abdominal obesity may be more predictive of diabetes than measures of overall obesity. A study by de Koning *et al.*[74] suggested a potential interaction of sex with WC and hip circumference in models predicting diabetes; however, there were no significant interactions of sex with BMI nor WHR. Some heterogeneity was observed in the predictive ability of BMI and hip circumference among different ethnicities. Therefore, one can potentially avoid complex interactions of anthropometric measures with sex and ethnicity by choosing WHR over other measures of abdominal obesity when studying predictors of glycemic outcomes in multiethnic cohorts.

The potential role of fatty liver in the pathogenesis of diabetes has promoted interest in evaluating associations between liver enzymes and the development of diabetes. Fraser and colleagues[75] conducted a meta-analysis on 18 prospective

population-based studies with a total of 72,841 participants evaluating baseline ALT and GGT as determinants of future incident diabetes. They found that adjusted hazard ratios (HRs) for incident diabetes were 1.83 (1.57-2.14) per one unit increase in logged ALT and 1.92 (1.66-2.21) per one unit increase in logged GGT. When comparing top versus bottom quarters of the distribution of these variables, the adjusted HRs for diabetes development were 2.02 (1.59-2.58) for ALT and 2.94 (1.98-3.88) for GGT. In three studies included in this meta-analysis, the presence of non-alcoholic fatty liver disease at baseline was evaluated by ultrasound in 9,401 Asian participants. Evidence of NAFLD on ultrasound was associated with a pooled relative risk (RR) of 2.52 (1.07-5.96) of incident diabetes, with significant heterogeneity between the three studies. It should be noted that in most studies included in this meta-analysis, a baseline and follow-up OGTTs were not performed to ascertain glycemic status; the diagnosis of diabetes was often based on self-report[75].

Several of the above summarized prospective studies tested for potential interactions of baseline ALT and GGT with other covariates in predicting type 2 diabetes. Hanley *et al.*[76] reported no evidence of interactions of baseline ALT with BMI, ethnicity or sex in predicting incident diabetes in 906 high-risk participants. Andre *et al.*[77] studied baseline ALT and GGT as predictors of incident diabetes in a prospective study on 4,201 French participants. There was no significant interaction of GGT with BMI or gender in predicting diabetes incidence. However, baseline GGT ≥ median versus < median (18.1 U/L) had adjusted odds ratios (ORs) of developing diabetes of 13.7 (1.8-99.8) when WHR was ≥0.85 and 1.7 (0.6-4.8) when WHR was <0.85 (p value for interaction of GGT

and WHR < 0.007). The authors did numerous group comparisons and tested a number of interactions between liver enzymes and other covariates in this study. Therefore, these findings need to be confirmed in future studies. Andre *et al.*[78] also assessed a change in GGT over 3 years from baseline as a predictor of type 2 diabetes in the above described study on 4,201 participants. Unchanged or increased GGT as opposed to decreased GGT was associated with OR of incident diabetes of 2.54 (1.38-4.68) in men and 2.78 (1.20-6.42) in women, after adjustment for baseline GGT, ALT, BMI and other covariates. These studies suggest that evidence of fatty liver on imaging, an increase in baseline liver enzymes, and an increase in liver enzymes over time predict future development of type 2 diabetes.

The above described studies suggest that abdominal obesity and liver fat may play a role in the pathophysiology of type 2 diabetes. A significant proportion of people with prediabetes have been shown to have high accumulation of ectopic fat. In people with prediabetes, the presence of ectopic fat may be indicative of significant metabolic derangements and predict future worsening of glycemic status.

2.4 Theoretical framework

In this study, we wanted to evaluate whether measures of ectopic fat deposition predict future glycemic outcomes in people with prediabetes. If increased ectopic fat deposition identified early in the course of the disease is predictive of future worsening of glycemic status, and decreased ectopic fat stores are predictive of an improvement in glycemic status, ectopic fat is more likely to be causal than simply associated with the

presence of type 2 diabetes. We, therefore, were interested in evaluating whether higher measures of ectopic fat deposition predict a decreased likelihood of regression of prediabetes to normoglycemia and higher degree of dysglycemia as measured by an area-under-the-glucose curve from 0 to 120 min ($AUC_{glucose0\text{-}120min}$) on an oral glucose tolerance test.

Other known important predictors of glycemic outcomes can be broadly subdivided into the following 3 categories: (i) patient demographic characteristics, including age, sex and ethnicity[79, 80]; (ii) baseline degree of dysglycemia (for example, baseline fasting plasma glucose and 2-hour plasma glucose)[1, 81, 82]; and (iii) other classic predictors of dysglycemia and type 2 diabetes such as a positive family history of type 2 diabetes[80, 83], physical activity level[84, 85] and current smoking[86]. It is important to adjust for these known predictors of dysglycemia in order to evaluate whether measures of ectopic fat deposition independently predict glycemic outcomes in people with prediabetes. One can also think of age, sex and ethnicity as confounders of the relationship between ectopic fat measures and glycemic outcomes as these three variables are known to affect both body fat distribution and glycemic parameters. Potential mediators of the relationship between ectopic fat and glycemic outcomes were beyond the scope of the current study.

2.5 Rationale for this project and overall objective

The DREAM trial[11, 12] provides a unique opportunity to test a hypothesis that higher measures of ectopic fat deposition predict future glycemic derangements and a low

likelihood of regression to normoglycemia in people with prediabetes. DREAM is an international multicenter 2X2 factorial design randomized controlled trial which was conducted in 5,269 people with prediabetes and no previous history of cardiovascular disease. This study assessed the effects of (i) rosiglitazone versus placebo and (ii) ramipril versus placebo on incident diabetes and death. The median duration of follow-up in this trial was 3 years. Participants had a scheduled 75 g oral glucose tolerance test (OGTT) at baseline, 2 years and at the end of the trial; this test is used for diagnosis of prediabetes and diabetes. Two hundred and eighty (10.6%) patients in the rosiglitazone group and 658 (25.0%) patients in the placebo rosiglitazone group were diagnosed with diabetes by the end of the trial[11]. Approximately 50.5% of patients in the rosiglitazone group and 30.3% of patients in the placebo group had regression of prediabetes to normoglycemia. The state of normoglycemia refers to normal glucose values, both fasting and postprandial. In this trial, it was defined based on the normal fasting and 2-hour plasma glucose values from the OGTT[87]. We, therefore, used the data from this completed trial to test the hypothesis that markers of ectopic fat deposition such as ALT and waist to hip ratio (WHR) predict future glycemic status in people with prediabetes.

ALT and WHR are surrogate measures of ectopic fat deposition, and we chose these measures because more accurate measures of ectopic fat were not available in the majority of the DREAM trial participants. However, ALT level correlated with the evidence of NAFLD on liver biopsies and imaging, and WHR correlated with radiographically-measured abdominal visceral fat in previous studies as described in Section 2.3.2. Since both ALT and WHR were thought to reflect one construct of ectopic

fat, we also hypothesized that the relationship of each of these variables with glycemic outcomes might be dependent on the value of the other variable, i.e. ALT and WHR may interact when predicting glycemic outcomes in people with prediabetes. We, therefore, evaluated for the presence of this interaction when performing data analyses. Glycemic outcomes included (i) regression of prediabetes to normoglycemia and (ii) degree of dysglycemia as measured by an area-under-the-glucose curve from 0 to 120 minutes ($AUC_{glucose\ 0\text{-}120\ min}$) on an OGTT. The overall objective of this thesis was to determine whether measures of ectopic fat deposition such as ALT and waist to hip ratio are independently associated with these glycemic outcomes in adult patients with prediabetes.

3. Hypothesis and Research Questions

The review of the literature suggests that ectopic fat may play a role in the pathophysiology of type 2 diabetes. Patients with prediabetes have been shown to have visceral fat deposition which increases with the degree of glucose intolerance. Ectopic fat may, therefore, play a role in the progression of glycemic derangements in people with prediabetes. We performed secondary analyses on the DREAM trial data to test the following hypothesis and research questions:

3.1 Hypothesis

We hypothesized that in people with IFG and IGT, higher measures of ectopic fat deposition such as ALT and waist to hip ratio will predict a higher degree of dysglycemia and lower likelihood of regression of prediabetes to normoglycemia 2 years later.

3.2 Primary research questions

1) In adult patients with IFG and/or IGT, is ALT an important predictor of regression to normoglycemia 2 years later?
2) In adult patients with IFG and/or IGT, is WHR an important predictor of regression to normoglycemia 2 years later?

3.3 Secondary research questions

1) Is there an interaction between ALT and WHR in predicting regression to normoglycemia 2 years later?

2) Is a change in ALT from baseline an important predictor of regression to normoglycemia at 2 years?
3) Is a change in WHR from baseline an important predictor of regression to normoglycemia at 2 years?
4) Is ALT an important predictor of dysglycemia as measured by $AUC_{glucose\ 0\text{-}120\ min}$ at 2 years?
5) Is WHR an important predictor of dysglycemia as measured by $AUC_{glucose\ 0\text{-}120\ min}$ at 2 years?
6) Is there a significant interaction between ALT and WHR in predicting $AUC_{glucose\ 0\text{-}120\ min}$ at 2 years?
7) Is a change in ALT from baseline an important predictor of $AUC_{glucose\ 0\text{-}120\ min}$ at 2 years?
8) Is a change in WHR from baseline an important predictor of $AUC_{glucose\ 0\text{-}120\ min}$ at 2 years?

4. Methods

This thesis consists of secondary analyses on already collected DREAM trial data. This chapter will outline methods that were employed to test the hypothesis that higher measures of ectopic fat deposition such as ALT and WHR predict a higher degree of dysglycemia and lower likelihood of regression of prediabetes to normoglycemia. Important methodological and statistical considerations will be discussed.

4.1 The DREAM trial

4.1.1. The DREAM trial population

The DREAM trial[11, 12] was a large, international, multi-center randomized controlled trial conducted at 191 sites in 21 countries from 2001 to 2006. A total of 5,269 adults with IFG, IGT or both participated in the trial. The diagnosis of IFG and IGT at baseline was established based on a 75 g OGTT performed during screening visit. The OGTT was performed as follows. A fasting plasma glucose (FPG) was drawn at 0 time after an overnight fast. A patient was given a 75 g oral glucose load, and a 2-hour plasma glucose (2-hour PG) was drawn at 120 min. Canadian Diabetes Association cut-offs were used to establish baseline glycemic status of the participants[88]. The impaired fasting glucose was defined as a FPG between 6.1 and 6.9 mmol/L inclusive. The impaired glucose tolerance was defined as a 2-hour plasma glucose between 7.8 and 11.0 mmol/L inclusive. Patients who had a previous history of type 2 diabetes or who were found to have a FPG ≥7.0 mmol/L or 2-hour PG ≥11.1 mmol/L (diabetes diagnosis cut-offs) on the screening visit OGTT were excluded from the trial. Patients who were

pregnant or had a history of cardiovascular disease, renal disease, active liver disease or ALT ≥ 2.5 times the upper limit of normal were also excluded.

4.1.2. The DREAM trial design

DREAM was a double-blind placebo-controlled 2x2 factorial design trial. The participants were randomized to rosiglitazone 8 mg daily or matching placebo and concurrently to ramipril 15 mg daily or matching placebo, and followed for a median of 3 years. The primary outcome was a composite outcome of incident diabetes or death from any cause. The diabetes diagnosis following randomization was made on the basis of OGTTs conducted during follow-up. All participants had an OGTT scheduled at 2 years and at the end-of-usual-follow-up (EUF) visit. Participants also had a FPG and HbA1C done at annual visits to screen for possible diabetes. If FPG was >5.3 mmol/L and HbA1C >5.6% (standardized to the upper limit of normal of 6%), then an OGTT was done at a study visit 6 months later to assess for the presence of diabetes. All follow-up OGTTs were performed while participants were on the study medications.

The diabetes diagnosis thresholds were a FPG ≥ 7 mmol/L or 2-hour PG ≥ 11 mmol/L on a follow-up OGTT. If at least one diabetes threshold was met at any time point during the study, a confirmatory OGTT was recommended within a 3-month period of the initial test or within 2 weeks of the EUF visit. The diabetes diagnosis was confirmed if at least one threshold was met on 2 consecutive tests. Participants' physicians could also make the diagnosis of diabetes in between study visits based on the results of a locally-performed OGTT, or FPG ≥ 7.0 mmol/L, or random plasma glucose ≥

11.1 mmol/L. If a participant had met at least one diabetes threshold and was started on a non-study antidiabetic medication, the diagnosis of diabetes by the outside physician was accepted. Once the participant was confirmed to have developed diabetes, no further OGTTs were performed. Participants who did not meet diabetes criteria at the EUF visit entered a washout phase of the trial and had an OGTT repeated 2-3 months after the EUF visit.

Waist circumference and hip circumference were measured in participants at baseline, 24 months and at the EUF visit. ALT was measured in all participants at baseline. People with ALT ≥2.5 times the upper limit of normal were excluded from the DREAM trial. This was done due to the concern that rosiglitazone might increase the risk of liver toxicity similar to troglitazone which was withdrawn from the market[89, 90]. ALT was therefore measured very carefully in all participants during the first year of the trial at 2,4,6,8,10 and 12 month visits in local laboratories. If ALT was found to be >3.0 times the upper limit of normal on 2 consecutive tests, rosiglitazone study drug was discontinued. Subsequent measurements of ALT during the trial were done at the discretion of the site investigator.

4.2 An overall thesis study design

The main hypothesis that was tested in this thesis is whether in people with prediabetes, higher measures of ectopic fat deposition such as ALT and WHR predict a lower likelihood of regression to normoglycemia and higher degree of dysglycemia as

measured by an area-under-the-glucose-curve $AUC_{glucose\ 0\text{-}120\ min}$. Regression modeling was employed to explore the relationship between ALT and WHR and these outcomes.

We decided to first establish whether higher baseline ALT and WHR were associated with a lower likelihood of regression of prediabetes to normoglycemia and higher $AUC_{glucose\ 0\text{-}120\ min}$ levels measured at a later date. We also wanted to test whether a change in ALT from baseline (ΔALT) and a change in WHR from baseline (ΔWHR) predicted these outcomes. The following sections will outline the rationale for selecting the study population and time points for measuring dependent and independent variables.

4.3 Statistical and methodological considerations

4.3.1. Selection of the study population sample

The DREAM trial population included people randomized to (i) placebo rosiglitazone and placebo ramipril, (ii) active rosiglitazone and placebo ramipril, (iii) active ramipril and placebo rosiglitazone and (iv) active rosiglitazone and active ramipril. Rosiglitazone is a glucose-lowering medication which has been shown to improve insulin sensitivity at the level of the liver and peripheral tissues, decrease endogenous glucose production and improve glucose clearance from the blood[91]. Ramipril has also been shown to lower 2-hour plasma glucose and increase the likelihood of regression to normoglycemia in the DREAM trial[12]. Therefore, if the proposed analyses were performed on data from all participants in the DREAM trial, it would have been important to adjust for rosiglitazone and ramipril in regression models. Both rosiglitazone and ramipril have also been shown to affect ALT levels[11, 12, 92] and change

the distribution of ectopic fat in the body[93, 94]. Therefore, when evaluating changes in ALT and WHR from baseline as predictors of glycemic outcomes it would have been important to adjust for rosiglitazone, ramipril, and possible interactions between ALT and rosiglitazone, ALT and ramipril, WHR and rosiglitazone, and WHR and ramipril. Since such models would be very complicated and difficult to interpret, it was decided to restrict the current analyses to the DREAM trial participants treated with placebo rosiglitazone and placebo ramipril.

Since both glycemic outcomes in the proposed analyses are based on plasma glucose levels obtained during a follow-up 75 g OGTT, analyses were restricted to the placebo-placebo group participants who had a follow-up OGTT during the DREAM trial. Among 1,321 placebo-placebo group participants, 1,209 participants had a follow-up OGTT and they comprised the current study population sample. The selection process for eligible participants is shown in Figure 2.

4.3.2. Data exploration and descriptive analyses

All analyses in this thesis were conducted using SAS version 9.1 (2002). Descriptive analyses were carried out on baseline characteristics of the study participants and on all variables used in the proposed regression models (see Section 4.3.4.f and Tables 1 and 2). Distribution of continuous variables was evaluated by plotting histograms and examining data skewness and kurtosis. Skewness refers to how asymmetrical the distribution is and kurtosis refers to how peaked or flat the distribution is. Normal distribution has 0 skewness (it is symmetrical) and 0 kurtosis (has a perfect

degree of flatness). Negative skew indicates skewness to the left and positive skew indicates skewness to the right. Negative kurtosis indicates a very flat distribution and positive kurtosis indicates a very peaked distribution[95]. For the purposes of regression modeling, neither dependent nor independent variables need to be normally distributed; only the errors in the dependent variable (the residuals) have to have a normal distribution to satisfy one of the assumptions of a linear regression model as discussed in Section 4.3.4.g[96]. Therefore, a non-normal variable distribution was not an indication for data transformation.

Histograms and scatter plots were examined for the presence of extreme data points. These included outliers (observations with an unusual value of the dependent variable) and data points with high leverage (observations with an unusual value of the independent variable)[97]. Such data points can have a large influence on the regression model estimates, especially when the study sample is small[96]. If these extreme values do not represent the study population, some argue for their removal from the data to derive regression models that describe a relationship between dependent and independent variables in the general population. It was thought that in the current study on 1,209 participants, any single unusual value is unlikely to have a significant influence on the study findings. Therefore, single extreme values were not removed nor corrected when performing analyses unless they were implausible and thought to be due to an error in measurement. If a set of extreme values was observed, we planned to perform sensitivity analyses with and without these observations.

4.3.3. Comparison of the characteristics between participants who regressed and who did not regress to normoglycemia

In order to identify potential predictors of regression of prediabetes to normoglycemia that can be studied in the future, we compared baseline and follow-up characteristics between participants who regressed and who did not regress to normoglycemia. We used a two-sample t-test or a non-parametric Wilcoxon two-sample t-test to compare continuous variables and a chi-square test to compare categorical variables. These comparisons were exploratory.

4.3.4. Regression modeling

a. <u>A choice of the regression model</u>

Regression modeling was employed to explore the relationship between ALT and WHR as predictor variables and (i) regression of prediabetes to normoglycemia or (ii) $AUC_{\text{glucose 0-120 min}}$ as outcome variables. These two outcomes were determined based on fasting and 2-hour plasma glucose values obtained on a follow-up OGTT. Since most participants in the DREAM trial had an OGTT at 2 years and at the end-of-usual-follow-up (EUF) visit, we considered time-to-event analyses as one approach to regression modeling of the categorical outcome: regression to normoglycemia. However, this outcome could have occurred in participants at different time-points than at 2 years and the EUF visit: the exact time of the event was not ascertained. Therefore, time-to-event analyses would not have been appropriate, and we performed logistic regression analyses to examine the relationship between ALT and WHR and the regression to

normoglycemia. Linear regression modeling was used to test whether ALT and WHR are important predictors of $AUC_{glucose\ 0\text{-}120\ min}$, a continuous outcome.

b. Definitions and time point selection for the outcome variables

Two glycemic outcomes were used in the current analyses: (i) a categorical outcome of regression to normoglycemia and (ii) a continuous outcome of the area-under-the-glucose-curve $AUC_{glucose\ 0\text{-}120\ min}$. These outcomes had to be determined from a follow-up 75 g OGTT. A scheduled OGTT was performed at 2 years after randomization and at the EUF visit in most participants; however, some participants had additional OGTTs if they were suspected to have diabetes (see Section 4.1.2.). We, therefore, had to select a time point for measuring glycemic outcomes in the current analyses. Since the timing of the EUF visit varied between participants depending on the time of recruitment into the trial, we decided to use a 2-year time point for regression to normoglycemia and $AUC_{glucose\ 0\text{-}120\ min}$ outcomes. If the results of the 2-year OGTT were not available, the OGTT performed after randomization and closest in time to the 2-year visit was chosen for outcome determination. If two such time points existed, the latest time point was chosen.

In view of the variability in OGTT performance[98], we considered using the results of several follow-up OGTTs for each participant and performing repeated measures regression analyses. This would improve the accuracy of the determination of glycemic outcomes. However, not all 1,209 participants in the placebo-placebo group had 2 follow-up OGTTs; therefore, repeated measures analyses would have a reduced power and we decided against this approach.

The glycemic outcomes were determined from the follow-up OGTT as follows. The $AUC_{glucose\ 0\text{-}120\ min}$ was calculated from the FPG and 2-hour PG values using a trapezoidal rule:

$$AUC_{glucose\ 0\text{-}120\ min} = 60*(FPG + 2\text{-hour PG}) \text{ in min*mmol/L}$$

According to the Canadian Diabetes Association guidelines, a state of normal fasting glucose and normal glucose tolerance is defined as FPG <6.1 mmol/L and a 2-hour plasma glucose < 7.8 mmol/L on a 75 g OGTT[88]. For the purposes of the current analyses, regression of prediabetes to normoglycemia was defined using more stringent American Diabetes Association criteria: a fasting plasma glucose of <5.6 mmol/L and a 2-hour plasma glucose <7.8 mmol/L on a follow-up OGTT[99]. Regression of prediabetes to normoglycemia was coded as '1' if the participant met these normoglycemic criteria on the follow-up OGTT and '0' if he/she did not. Regression to normoglycemia was modeled.

c. <u>A general strategy for selecting independent variables</u>

A number of different approaches to building a multivariable regression model can be used. One can start with a few or many independent variables to be included in the initial model, and then use either a forward selection or a backward elimination approach to determine which variables to include in the final model[96]. These decisions often influence the composition of the final models and conclusions drawn about the relationship between predictors of interest and the outcome.

Rather than allowing a statistical program to decide which variables to include in the final model, we decided to propose a specific step-wise approach to model building

starting with a minimally adjusted model and then adjusting for other important covariates to build a maximally adjusted model as discussed in Section 4.3.4.f. Selection of variables for our multivariable models was not based on the results of the univariate analyses. Control variables were chosen based on the literature. Including too many variables that are not true important predictors of the outcome in the population is considered to be less likely to introduce bias than leaving important variables out[96]. We were also not deriving a prediction model, rather testing a hypothesis on the importance of ectopic fat in predicting glycemic outcomes. We, therefore, decided to keep all proposed independent variables in the final models since this study had sufficient sample size. The only other consideration that was made for final variable selection was testing for multicollinearity between independent variables which is discussed below in Section 4.3.4.h.

d. Standardization and time point selection for the ALT and WHR predictor variables

The two predictors of interest in this study are alanine transaminase (ALT) and waist to hip ratio (WHR). Waist to hip ratio is a waist circumference in cm divided by the hip circumference in cm, and is one way to standardize a waist circumference measure. Waist circumference was measured in the DREAM trial participants over light clothing at the smallest abdominal diameter between costal margin and iliac crest. Hip circumference was measured over light clothing at the level of the greater trochanter. The tape measure was kept horizontal after attaching a 750 g spring balance. These measurements were done at baseline, 2 years and at the EUF visit. We evaluated baseline

WHR as a predictor of (i) regression to normoglycemia and (ii) $AUC_{glucose\ 0\text{-}120\ min}$ 2 years later. We also wanted to assess a change in WHR from baseline as a predictor of these glycemic outcomes. Since the outcomes were measured 2 years after randomization, a 2-year time point was selected for measuring a change in WHR from baseline. If a 2-year WHR was not available, the value of the change in WHR from baseline was set to missing and it was not included in the regression analyses.

ALT was measured in the DREAM trial by local laboratories, and the upper limit of normal varied between them. An upper limit of normal of 40 U/L in males and 35 U/L in females was proposed for ALT in the literature[100]. It was decided to standardize all measured ALT values using local lab upper limit of normal (ULN) as follows:

In males: $ALT = ALT_{measured}*40/(\text{local lab ULN})$

In females: $ALT = ALT_{measured}*35/(\text{local lab ULN})$

ALT measurements in the DREAM trial were available at baseline and at 2,4,6,8,10 and 12 months after randomization. We evaluated baseline ALT as a predictor of the two glycemic outcomes measured 2 years after randomization. A change in ALT from baseline to 1 year was also assessed as a predictor of these outcomes. If a 1-year ALT was not available, a time point closest to the 1-year visit was chosen to determine the change in ALT from baseline.

e. Selection of the control variables (covariates) and dummy coding

The following control variables were included in the maximally adjusted models: age, sex, ethnicity, FPG, 2-hour PG, family history of diabetes, current smoking and physical activity level all collected at baseline. The following control variables were

obtained on self-report. Age was recorded in years and was used as a continuous predictor. Sex was coded as 0 for males and 1 for females. Ethnicity of the participant and his/her parents was recorded at a baseline visit based on 14 different categories available. Ethnicity of both parents was taken into account to determine the final participant ethnicity. It was coded as 2 categories for the current analyses: 0 for European and 1 for Non-European. Current smoking at baseline was coded as 1 for 'yes' and 0 for 'no'. The non-smoking category (coded as 0) also included former smokers. We adjusted all multivariable models for baseline fasting plasma glucose and 2-hour plasma glucose levels measured during a baseline OGTT.

Physical activity level was defined as sedentary or non-sedentary based on the answers to 2 questions on the physical activity level obtained on self-report during a baseline visit. The first question asked how active the participant is at work with 5 different options to indicate the level and type of physical activity. The second question asked about activity during leisure time and the answers included 4 different options to indicate amount and type of physical activity. If the participant was physically active at work or reported moderate physical activity such as walking or riding a bicycle for at least 4 hours per week or regular exercise for at least 4 hours per week during leisure time, they were assigned to the non-sedentary group (coded as 0). If they were mainly sitting, working on a computer or answering the phone at work and at home, they were assigned to the sedentary group (coded as 1).

Family history of type 2 diabetes was defined as a known positive history of diabetes in a parent or a sibling and was coded as 1 for 'yes' and 0 for 'no'. Participants

were asked whether there is a family history of diabetes in their mother, father or siblings as 'Yes' or 'No'. Investigators were asked to mark 'No' if the participant was unsure. If a participant did not report any known history of diabetes in any of the first degree relatives, even if some or all answers to these questions were missing, family history of diabetes was coded as 0. For all other control variables that were not recorded, a missing value was assigned.

Active liver disease and excessive alcohol use associated with pancreatitis can potentially affect glycemic outcomes[101-103]. These conditions are also associated with elevated liver enzymes, including ALT. Therefore, active liver disease and excessive alcohol use are potential confounders of the relationship between ALT and glycemic outcomes. In the DREAM trial, patients with active liver disease and ALT $\geq$ 2.5 times the upper limit of normal were excluded from participating. We, therefore, did not adjust for alcohol intake or a history of active liver disease in our regression models.

f. Proposed linear and logistic regression models

Logistic regression models were used to evaluate the relationship between (i) baseline ALT, baseline WHR and regression of prediabetes to normoglycemia 2 years later as well as between (ii) a change in ALT from baseline, a change in WHR from baseline and regression to normoglycemia 2 years after randomization. Linear regression models were carried out to establish the relationship between (i) baseline ALT, baseline WHR and $AUC_{\text{glucose 0-120 min}}$ 2 years later and between (ii) a change in ALT from baseline, a change in WHR from baseline and $AUC_{\text{glucose 0-120 min}}$ 2 years after randomization. The following covariates were included in the maximally adjusted

models: age, sex, ethnicity, FPG, 2-hour PG, family history of diabetes, current smoking and physical activity status at baseline. Initially, univariate regression analyses were carried out followed by multivariable analyses. The proposed multivariable models are summarized in Tables 1 and 2. Proposed models E and F assessed whether there is an interaction between baseline ALT and baseline WHR in predicting glycemic outcomes. Testing for this interaction is further discussed in Section 4.3.4.j.

g. Testing assumptions of a linear regression model

The regression assumptions[96] were tested as follows. The linearity of the relationship between predictor variables and the outcome variable ($AUC_{glucose\ 0\text{-}120\ min}$) was assessed in scatter plots. The normality of the distribution of errors (residuals) of the outcome variable was evaluated in q-q plots. Q-q plot is a plot of the residuals on the y-axis and quantiles of the normal distribution on the x-axis[97]. Deviations from a straight line indicate non-normality of the residuals. Constant variance of errors in the outcome variable was evaluated by plotting residuals against predicted values of the outcome variable. Predictor variables were assumed to be measured error-free. The assumption of the independence of outcome variables was tested by performing multilevel modeling with random intercept for centre effect to account for possible clustering of data within centres (see Section 4.3.6.).

h. Testing for multicollinearity

Two variables are defined as collinear if they have a strong linear relationship[96]. Significant collinearity between independent variables (multicollinearity) is a concern in regression modeling since it affects the estimates of regression coefficients and inflates

the variance of these coefficients[96, 97]. We, therefore, performed a test of correlation between independent variables prior to carrying out regression analyses. A correlation coefficient r>0.80 was considered to indicate significant collinearity[104]. We decided as a priori that if several independent variables were found to be correlated with r > 0.80, we would only use one of these variables in the final models. If baseline FPG and 2-hour PG were found to be highly correlated with a correlation coefficient r > 0.80, it was decided as a priori to use a baseline area-under-the-glucose-curve ($AUC_{\text{glucose 0-120 min baseline}}$) as a covariate instead of the FPG and 2-hour PG.

i. Interpretation of regression model estimates

Linear regression modeling uses the least-squares method to fit the best linear regression line describing the relationship between the predictor variable x and the outcome variable y by minimizing the sum of squares of the vertical deviations of the observed data points from the fitted regression line. In a univariate linear regression model, the beta coefficient of a continuous predictor variable represents an average change in the outcome variable per 1 unit increase in the predictor variable. In a multivariable linear regression model, such beta coefficient represents an average change in the outcome variable per 1 unit increase in the predictor variable after adjusting for other independent variables in the model. A t-test in linear regression models assesses whether the observed beta coefficient (or the slope of the regression line) is significantly different from zero after adjusting for other independent variables in the model, i.e. it tests whether the predictor variable has a significant independent effect on the outcome variable with α=0.05 [96, 104].

Logistic function models the probability of a categorical outcome in participants with different levels of the independent variable. The regression coefficient in a logistic regression model represents the log of odds of the outcome in those exposed to a categorical predictor versus those unexposed or in those with a higher level of a continuous predictor versus those with a lower level of the predictor. The exponent of the coefficient of a continuous predictor variable represents the odds ratio of the modeled outcome per 1 unit increase in the predictor variable: i.e. the odds of the outcome when the predictor variable is equal to (x+1) divided by the odds of the outcome when the predictor variable is equal to x. One can also change the unit increase in the predictor variables in these regression models. A Wald chi-square test in logistic models assesses whether the log of odds of the outcome is significantly different from zero (or the odds ratio is significantly different from 1) after adjustment for other variables in the model, i.e. whether the predictor variable has a significant independent effect on the probability of the outcome with $\alpha=0.05$ [96, 104].

j. Testing for and interpreting the interaction between ALT and WHR in predicting glycemic outcomes

We wanted to assess whether there is an interaction between ALT and WHR in predicting glycemic outcomes as both variables are thought to represent ectopic fat deposition and the relationship between each one of them and the outcome might be dependent on the level of the other variable. In order to be able to interpret regression coefficients in models containing an interaction term it was important to center these variables around their respective means. To center WHR around its mean, for example,

the mean of WHR was subtracted from all observed values of WHR. If such centering of the interacting variables is done prior to running the regression models, the regression coefficients for each variable are interpreted when the other variable is held constant at its mean value[105, 106]. It was decided as a priori that an interaction term will be included in the final model if the p value for the interaction was ≤ 0.10.

The relationship between ALT and WHR and the outcomes was further explored by determining regression coefficients of each variable at different levels of the interacting variable. For example, to determine the effect of ALT on the outcome, the regression coefficients were obtained when WHR was held at the mean – 1 SD, at the mean, and at the mean + 1 SD. To determine such coefficients, centering of WHR variable was changed accordingly. We followed the same approach to determine the effect of WHR on the outcome when ALT was held at the mean – 1 SD, at the mean, and at the mean +1 SD. We confirmed results by manually calculating odds ratios, regression coefficients and 95% confidence intervals at different levels of the interacting variable using published formulas[105, 106].

4.3.5. Missing data

Current analyses were restricted to 1,209 participants in the placebo rosiglitazone, placebo ramipril group in the DREAM trial who had a follow-up OGTT. The two outcomes of interest (regression to normoglycemia and $AUC_{\text{glucose 0-120 min}}$) were obtained based on this OGTT. A total of 112 placebo-placebo participants were excluded from analyses because these people did not complete a follow-up OGTT. However, validity of

conclusions from regression models estimating the relationship between ALT, WHR and glycemic outcomes can be affected if the data are not missing at random. This would be the case, for example, if the probability of missingness of the follow-up OGTTs was related to the follow-up glycemic status of the participant, or the WHR value, or the ALT value. One can never be completely sure about the absence of a missingness bias because the missingness mechanism might be related to some important unmeasured parameter influencing the relationship between the variables of interest. However, we decided to compare the baseline characteristics between included and excluded participants to assess whether the missingness was likely to be related to the measured characteristics. Twenty-five of the excluded placebo-placebo participants were adjudicated as having developed type 2 diabetes during the study. Since these participants were unlikely to have regression of prediabetes to normoglycemia at 2 years, we assigned them to the non-regression group and performed sensitivity analyses on the logistic regression models.

Nearly all participants had a baseline age, sex, ethnicity, FPG, 2-hour PG, ALT, WHR, family history of diabetes, smoking status and physical activity status recorded during a baseline visit. Some participants were missing a follow-up ALT and/or WHR value. We decided not to impute any missing independent variables. If any independent variables were missing, these cases were dropped from the respective regression models.

4.3.6. Accounting for possible clustering of data within centres

The study population for the current analyses was drawn from a multicenter trial with 174 participating centres. We wanted to assess and account for possible clustering

of data within centres as outcome observations in participants need to be completely independent when performing linear regression analyses. If significant clustering of data exists within centres due to a similar environment, people within each centre would have less variability in glycemic outcomes than between centres, and we would underestimate the true variability between individuals if we do not account for this clustering[104]. Accounting for it generally leads to wider 95% confidence intervals, and in some cases regression coefficients might become non-significant.

We decided to account for possible variability of data due to centres by performing sensitivity analyses using multilevel modeling with random intercept for centre effect. This approach tests whether a linear relationship between the variables of interest has a different y-axis intercept for different centres. We did not perform random slope analyses as these would be difficult to conduct and interpret if regression models include an interaction term between two continuous variables. We also believe that centres are unlikely to differ in the slopes of the relationships between ALT and glycemic outcomes and between WHR and glycemic outcomes.

5. Results

We identified 1,209 participants with prediabetes in the placebo rosiglitazone placebo ramipril group who had a follow-up oral glucose tolerance test during the DREAM trial. ALT and waist to hip ratio were evaluated as predictors of regression of prediabetes to normoglycemia 2 years later in this study population. ALT and waist to hip ratio were also evaluated as predictors of dysglycemia measured by an $AUC_{glucose0\text{-}120\ min}$ during a 2-year oral glucose tolerance test.

5.1 Excluded participants

The study population of this thesis included 1,209 participants with impaired fasting glucose and /or impaired glucose tolerance treated with placebo rosiglitazone placebo ramipril who had a follow-up 75 g OGTT. Among 1,321 placebo rosiglitazone placebo ramipril participants in the DREAM trial, 112 people did not have a follow-up OGTT and were excluded from the current analyses. Baseline characteristics of the included and excluded participants were compared and are shown in Table 3. A higher percentage of the included participants had a positive family history of diabetes compared to the excluded participants (59% vs 46%, p=0.008). The two groups did not differ on the remaining nine baseline characteristics, including fasting plasma glucose, 2-hour plasma glucose, ALT and waist to hip ratio.

5.2 Characteristics of the study population

5.2.1. Baseline and follow-up characteristics of the study population

Baseline characteristics of the study population are detailed in Table 4. The included participants had a mean age of 55 years with standard deviation (SD) of 11 years. Forty-one percent of them were male, 54% were European and 59% had a positive family history of diabetes. The participants had a mean fasting plasma glucose of 5.8 mmol/L (SD 0.66) and a mean 2-hour plasma glucose of 8.7 mmol/L (SD 1.5) at baseline consistent with a prediabetes status. The mean baseline ALT was 25 U/L (SD 13) and the mean baseline WHR was 0.91 (SD 0.09). The mean change in ALT from baseline to 1 year was 0.20 U/L (SD 13) and the mean change in WHR from baseline to 2 years was 0.01 (SD 0.12).

5.2.2. A comparison between participants who regressed to normoglycemia and those who did not

Among 1,209 study participants with prediabetes, 301 (25%) regressed to normoglycemia 2 years later. Normoglycemia was defined as a fasting plasma glucose <5.6 mmol/L and a 2-hour plasma glucose <7.8 mmol/L on a 75 g OGTT performed 2 years after randomization. Participants who met normoglycemic criteria on this OGTT were assigned to the regression (to normoglycemia) group and those who did not were assigned to the non-regression group. Baseline characteristics were compared between the two groups (see Table 4). Compared to the non-regression group, people who regressed to normoglycemia were younger (mean age 54 vs 56 years), were more likely to be Non-European (51 vs 44%) and sedentary (35 vs 25%), and had a lower mean FPG (5.6 vs 5.9 mmol/L) and a lower mean 2-hour PG (8.3 vs 8.9 mmol/L) on a baseline

OGTT. They also had a lower mean baseline ALT (24 vs 25 U/L, p=0.004) compared to the non-regression group. People who regressed had a lower mean waist circumference (96 vs 99 cm, p=0.005) and a lower mean hip circumference (107 vs 109 cm, p=0.03). However, the difference in the mean baseline waist to hip ratio only approached significance between the two groups: it was 0.90 in the regression group and 0.91 in the non-regression group (p=0.05).

Follow-up characteristics were also compared between the regression and non-regression groups and are shown in Table 4. A change in ALT from baseline to 1 year was not significantly different between the two groups (p=0.28). Regression group had a mean decrease of 0.005 in the waist to hip ratio from baseline to 2 years, while the non-regression group had an increase of 0.016 on average. This was a statistically significant difference (p=0.002). A regression group had a lower area-under-the-glucose-curve on a 2-year 75 g OGTT compared to the non-regression group (656 vs 927 min*mmol/L, $p<0.0001$).

5.3 Correlation between predictor variables in regression models

Predictor variables used in the proposed regression models were assessed for multicollinearity. The correlation matrix is shown in Table 5. The highest Pearson correlation coefficient of -0.47 was observed between baseline WHR and female sex. A correlation coefficient of -0.41 was observed between a change in ALT over 1 year from baseline (ΔALT) and baseline ALT; however, we did not plan to use these two variables as predictors in the same regression model. A correlation coefficient of -0.34 was

observed between a change in waist to hip ratio over 2 years from baseline (ΔWHR) and baseline WHR. These two variables were not used as predictors in the same regression model. All remaining correlation coefficients ranged between -0.23 and +0.23. None of the variables met the correlation coefficient cut-off of >0.80 or <-0.80 to indicate significant collinearity; therefore, no predictor variables were dropped from the proposed regression models.

5.4 ALT and waist to hip ratio as predictors of regression of prediabetes to normoglycemia

5.4.1. Baseline ALT and WHR as predictors of regression to normoglycemia

Baseline ALT and baseline WHR were evaluated as predictors of regression of prediabetes to normoglycemia 2 years later in logistic regression models in 1,199-1,207 study participants (the number of participants varied between models due to missing independent variables). The relationship between baseline ALT and regression to normoglycemia in unadjusted and adjusted models is shown in Figure 3. Odds ratios of regression to normoglycemia ($OR_{regression\ to\ NG}$) are expressed per 10 U/L increase in ALT. In model 3, an interaction between baseline ALT and WHR was significant with a p-value of 0.01. Similarly, in model 4, an interaction between these variables was significant with a p-value of 0.008. In these two models, the adjusted odds ratios of regression to normoglycemia per 10 U/L increase in ALT are reported when waist to hip ratio is held at the mean of 0.91. It can be seen that baseline ALT was not found to be a significant predictor of regression to normoglycemia in these models.

The relationship between baseline WHR and regression to normoglycemia in unadjusted and adjusted models is shown in Figure 4. Odds ratios of regression to normoglycemia are expressed per 0.1 unit increase in WHR. Models 3 and 4 included an interaction term between ALT and WHR. In these models, the adjusted odds ratios of regression to normoglycemia per 0.1 unit increase in WHR are reported when ALT is held at the mean of 25 U/L. It can be seen that baseline WHR was not found to be a significant predictor of regression to normoglycemia in these models.

5.4.2. Implications of the interaction between baseline ALT and WHR in predicting regression to normoglycemia

As indicated in the previous section, there was a significant interaction between baseline ALT and WHR in predicting regression to normoglycemia 2 years later. This suggested that the effect of baseline ALT on regression to normoglycemia 2 years later was dependent on the value of baseline WHR, and the effect of baseline WHR was dependent on the value of ALT. The interaction in model 3 was further explored by obtaining adjusted odds ratios of regression to normoglycemia per 10 U/L increase in ALT at different levels of waist to hip ratio, and adjusted odds ratios of regression to normoglycemia per 0.1 unit increase in WHR at different levels of ALT. Model 3 included age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, ALT, WHR and an interaction between ALT and WHR as independent variables. Waist to hip ratio had a mean of 0.91 and standard deviation (SD) of 0.09. Adjusted odds ratios (95% confidence intervals, CIs) of regression to normoglycemia per 10 U/L increase in ALT

were 0.79 (0.66-0.94) when WHR was at the mean minus 1 SD, 0.90 (0.80-1.02) when WHR was at the mean, and 1.03 (0.90-1.18) when WHR was at the mean plus 1 SD. The results are shown in Figure 5. ALT had a mean of 25 U/L and standard deviation (SD) of 13 U/L. Adjusted odds ratios (95% CIs) of regression to normoglycemia per 0.1 unit increase in WHR were 0.75 (0.60-0.95) when ALT was at the mean minus 1 SD, 0.91 (0.76-1.08) when ALT was at the mean, and 1.09 (0.89-1.35) when ALT was at the mean plus 1 SD. The results are shown in Figure 6.

5.4.3. Changes in ALT and WHR from baseline as predictors of regression to normoglycemia

Changes in ALT and WHR from baseline were evaluated as predictors of regression of prediabetes to normoglycemia 2 years later in logistic regression models in 1,120-1,168 study participants (the number of participants varied between models due to missing independent variables). The relationship between a change in ALT from baseline to 1 year (ΔALT) and the regression to normoglycemia 2 years after randomization in unadjusted and adjusted models is shown in Figure 7. Odds ratios (95% CIs) of regression to normoglycemia are expressed per 10 U/L increase in ALT from baseline. It can be seen that the relationship between an increase in ALT from baseline and the regression to normoglycemia was not significant in these models.

A change in WHR from baseline to 2 years (ΔWHR) was found to be a significant predictor of the regression to normoglycemia 2 years after randomization in unadjusted and adjusted models as shown in Figure 8. Odds ratios (95% CIs) are expressed per 0.1

unit increase in WHR from baseline. In models 3 and 4, an interaction between ΔALT and ΔWHR was not significant (p values of 0.34 and 0.29, respectively). Therefore, this interaction term was not included in the final models. After adjusting for age, sex, ethnicity, FPG, 2-hour PG and ΔALT, a 0.1 unit increase in WHR from baseline was associated with an odds ratio of regression to normoglycemia of 0.83 (0.71-0.96).

5.4.4. Sensitivity analyses on excluded participants

A total of 112 people in the placebo-placebo group did not have a follow-up OGTT and were excluded from the main study analyses. Twenty-five of these people were adjudicated to have developed type 2 diabetes during the study. We performed sensitivity analyses by assigning these people to the non-regression group and repeating the following logistic regression analyses. Model E from Table 1 was repeated in 1,229 placebo-placebo participants (1,204 original participants plus 25 excluded participants). An interaction between baseline ALT and WHR remained significant with a p-value of 0.01. Baseline ALT was not a significant predictor of regression to normoglycemia when WHR was at the mean of 0.91, with an odds ratio of regression to normoglycemia of 0.90 (95% CI 0.80-1.01) per 10 U/L increase in ALT. Baseline WHR was not a significant predictor of regression to normoglycemia when ALT was at the mean of 25 U/L, with an OR of regression to normoglycemia of 0.91 (95% CI 0.77-1.09) per 0.1 unit increase in WHR. These results were similar to model 3 results shown in Figures 3 and 4.

Model I from Table 1 was repeated in 1,136 placebo-placebo participants (1,125 original participants plus 11 excluded participants). An interaction between ΔALT and

ΔWHR remained non-significant with a p-value of 0.39 and it was dropped from the model. A change in ALT over 1 year from baseline was not a significant predictor of regression to normoglycemia with an OR of 0.98 (95% CI 0.88-1.09) per 10 U/L increase in ALT, similarly to model 3 results in Figure 7. An increase in WHR over 2 years from baseline decreased the likelihood of regression to normoglycemia with an OR of regression to normoglycemia of 0.82 (95% CI 0.71-0.96) per 0.1 unit increase in WHR, similarly to model 3 results in Figure 8.

5.5 ALT and waist to hip ratio as predictors of dysglycemia measured by an area-under-the-glucose curve $AUC_{glucose\ 0\text{-}120\ min}$

5.5.1. Testing assumptions of a linear regression model

Baseline ALT and waist to hip ratio as well as changes in ALT and waist to hip ratio from baseline were evaluated as predictors of $AUC_{glucose0\text{-}120\ min}$ 2 years after randomization in linear regression models unadjusted and adjusted for other covariates. Data exploration did not reveal any violations of assumptions of linear regression models. Specifically, scatter plots suggested a linear relationship between continuous predictor variables and $AUC_{glucose0\text{-}120\ min}$. Q-q plots revealed that errors in $AUC_{glucose0\text{-}120\ min}$ approximated a normal distribution. Errors in $AUC_{glucose0\text{-}120\ min}$ also had constant variance in all models.

5.5.2. Baseline ALT and WHR as predictors of $AUC_{glucose0\text{-}120min}$

Baseline ALT and WHR were evaluated as predictors of $AUC_{glucose0\text{-}120\ min}$ on a follow-up OGTT 2 years later in 1,199-1,207 study participants. Baseline ALT was found to be a significant predictor of $AUC_{glucose0\text{-}120\ min}$ in unadjusted and adjusted models as shown in Figure 9. It was found that an interaction between baseline ALT and baseline WHR had a p value of 0.056 in model 3 and 0.048 in model 4; therefore, this interaction term was included in these models. The beta coefficient for the interaction term was -9.2 in model 3 and -9.5 in model 4. After accounting for this interaction and adjusting for age, sex, ethnicity, FPG, 2-hour PG and baseline WHR in model 3, a 10 U/L increase in baseline ALT was associated with an increase of 11.0 (95% CI 1.4 to 20.6) min*mmol/L in $AUC_{glucose0\text{-}120\ min}$ when baseline WHR was held at the mean of 0.91. An association between baseline ALT and $AUC_{glucose0\text{-}120\ min}$ remained significant after additionally adjusting for family history of diabetes, smoking and sedentary lifestyle (p=0.04).

Baseline WHR was also found to be a significant predictor of $AUC_{glucose0\text{-}120\ min}$ 2 years later in unadjusted and adjusted models as shown in Figure 10. After accounting for an interaction between baseline ALT and WHR and adjusting for age, sex, ethnicity, FPG, 2-hour PG and baseline ALT in model 3, a 0.1 unit increase in baseline WHR was associated with an increase of 18.3 (3.8 to 32.9) min*mmol/L in $AUC_{glucose0\text{-}120\ min}$ when baseline ALT was held at the mean of 25 U/L. An association between baseline WHR and $AUC_{glucose0\text{-}120\ min}$ remained significant after additionally adjusting for family history of diabetes, smoking and sedentary lifestyle (p=0.04).

5.5.3. Implications of the interaction between baseline ALT and WHR in predicting $AUC_{glucose0\text{-}120min}$

The interaction between baseline ALT and WHR in predicting $AUC_{glucose0\text{-}120\ min}$ 2 years later observed in models 3 and 4 suggested that the effect of baseline ALT on $AUC_{glucose0\text{-}120\ min}$ 2 years later was dependent on the value of baseline WHR, and the effect of baseline WHR was dependent on the value of ALT. The interaction in model 3 was further explored by looking at a change in $AUC_{glucose0\text{-}120\ min}$ per 10 U/L increase in baseline ALT at different levels of WHR (Figure 11). After adjusting for other covariates and the interaction term in this model as described above, a 10 U/L increase in baseline ALT was associated with an $AUC_{glucose0\text{-}120\ min}$ increase of 19.5 (95% CI 5.3 to 33.7) min*mmol/L when baseline WHR was at the mean minus 1 SD, 11.0 (1.4 to 20.6) min*mmol/L when WHR was at the mean of 0.91, and 2.5 (-9.2 to 14.1) min*mmol/L when WHR was at the mean plus 1 SD. Similarly, a 0.1 unit increase in baseline WHR was associated with an $AUC_{glucose0\text{-}120\ min}$ increase of 30.3 (10.2 to 50.3) min*mmol/L when baseline ALT was at the mean minus 1 SD, 18.3 (3.8-32.9) min*mmol/L when ALT was at the mean of 25 U/L, and 6.4 (-11.5 to 24.3) min*mmol/L when ALT was at the mean plus 1 SD.

5.5.4. Changes in ALT and WHR from baseline as predictors of $AUC_{glucose0\text{-}120min}$

Changes in ALT and WHR from baseline as predictors of $AUC_{glucose0\text{-}120\ min}$ 2 years after randomization were evaluated in linear regression models in 1,120-1,168 study participants. A change in ALT from baseline to 1 year (ΔALT) was found to be a

significant predictor of $AUC_{glucose0\text{-}120\ min}$ 2 years after randomization in unadjusted and adjusted models as shown in Figure 13. An interaction between a change in ALT from baseline to 1 year and a change in WHR from baseline to 2 years (ΔWHR) was found to be significant with a p-value of 0.046 in model 3 and 0.049 in model 4. This interaction term was therefore included in these models post-hoc. After accounting for this interaction and adjusting for age, sex, ethnicity, FPG, 2-hour PG and ΔWHR in model 3, a 10 U/L increase in ALT from baseline was associated with an increase of 11.1 (95% CI 2.0 to 20.1) min*mmol/L in $AUC_{glucose0\text{-}120\ min}$ when ΔWHR was at the mean of 0.01 (p=0.02).

A change in WHR from baseline to 2 years was found to be a significant predictor of $AUC_{glucose0\text{-}120\ min}$ 2 years after randomization in unadjusted and adjusted models as shown in Figure 14. After accounting for an interaction between ΔALT and ΔWHR and adjusting for age, sex, ethnicity, FPG, 2-hour PG and ΔALT in model 3, a 0.1 unit increase in WHR from baseline was associated with an increase of 13.0 (3.4 to 22.6) min*mmol/L in $AUC_{glucose0\text{-}120\ min}$ when ΔALT was at the mean of 0.26 U/L (p=0.008).

5.5.5. Implications of the interaction between a change in ALT and a change in WHR from baseline in predicting $AUC_{glucose0\text{-}120min}$

An interaction between a change in ALT from baseline and a change in WHR from baseline in predicting $AUC_{glucose0\text{-}120\ min}$ in model 3 was further explored by looking at a change in $AUC_{glucose0\text{-}120\ min}$ per 10 U/L increase in ALT from baseline at different levels of a change in WHR from baseline. Model 3 included age, sex, ethnicity, fasting

plasma glucose, 2-hour plasma glucose, ΔALT, ΔWHR, and an interaction between ΔALT and ΔWHR. A change in waist to hip ratio from baseline to 2 years had a mean of 0.01 and standard deviation of 0.13. The results are shown in Figure 15. A 10 U/L increase in ALT from baseline to 1 year in this model was associated with an $AUC_{glucose0\text{-}120\ min}$ increase of 0.96 (95% CI -12.9 to 14.8) min*mmol/L when ΔWHR was -0.12, 11.1 (2.0 to 20.1) min*mmol/L when ΔWHR was at the mean of 0.01, and 21.2 (8.2 to 34.2) min*mmol/L when ΔWHR was 0.13.

We also looked at the change in $AUC_{glucose0\text{-}120\ min}$ per 0.1 unit increase in WHR from baseline at different levels of a change in ALT from baseline. A change in ALT from baseline had a mean of 0.26 U/L and standard deviation of 13 U/L. A 0.1 unit increase in WHR from baseline to 2 years in model 3 was associated with an $AUC_{glucose0\text{-}120\ min}$ increase of 2.5 (-12.9 to 17.9) min*mmol/L when ΔALT was -13.1 U/L, 13.0 (3.4 to 22.6) min*mmol/L when ΔALT was at the mean of 0.26 U/L, and 23.5 (10.9 to 36.2) min*mmol/L when ΔALT was 13.6 U/L. The results are shown in Figure 16.

5.5.6. Accounting for possible clustering of data within centres

In order to account for possible clustering of data within centres, we performed sensitivity analyses using multilevel modeling with random intercept for centre effect. The results are summarized in Table 6. After accounting for clustering in model E from Table 2, an interaction between baseline ALT and WHR remained to be borderline significant with a p-value of 0.06. Baseline ALT was no longer a significant predictor of $AUC_{glucose0\text{-}120\ min}$ when WHR was at the mean of 0.91 (p-value 0.10). High baseline

WHR remained a significant predictor of $AUC_{glucose0\text{-}120\ min}$ when ALT was at the mean of 25 U/L (p-value 0.009).

After accounting for clustering in model I from Table 2, an interaction between ΔALT and ΔWHR now had borderline significance with a p-value of 0.06 (see Table 6). An increase in ALT over 1 year from baseline remained a significant predictor of $AUC_{glucose0\text{-}120\ min}$ when ΔWHR was at the mean of 0.01 (p-value 0.002). An increase in WHR over 2 years from baseline remained a significant predictor of $AUC_{glucose0\text{-}120\ min}$ when ΔALT was at the mean of 0.26 U/L (p-value 0.01).

6. Discussion

As discussed in the background chapter, ectopic fat may play a role in the pathophysiology of prediabetes and type 2 diabetes. We, therefore, wanted to test the hypothesis that people with prediabetes who have high baseline measures of ectopic fat deposition such as ALT and WHR will have a high degree of dysglycemia and a low likelihood of regression to normoglycemia measured at a later date. We tested this hypothesis by performing secondary analyses on the DREAM trial data. The implications of the study findings, study strengths and limitations will be discussed in this chapter.

6.1 Summary of the study findings

A total of 1,209 participants with impaired fasting glucose and/or impaired glucose tolerance treated with placebo rosiglitazone and placebo ramipril in the DREAM trial who had a follow-up OGTT were included in the current analyses. We first evaluated whether ALT and waist to hip ratio reduce the likelihood of regression of prediabetes to normoglycemia 2 years later. We found that the effect of baseline ALT on regression to normoglycemia was dependent on the value of baseline WHR, and the effect of baseline WHR was dependent on the value of ALT (p-value for interaction 0.01). After adjustment for age, sex, ethnicity, fasting plasma glucose and 2-hour plasma glucose, high baseline ALT predicted a lower likelihood of regression to normoglycemia 2 years later when baseline waist to hip ratio was low. Similarly, high baseline waist to hip ratio predicted a lower likelihood of regression to normoglycemia when baseline ALT

was low normal. We then assessed the effects of changes in ALT and WHR over time on the probability of regression to normoglycemia 2 years after randomization. We found that an increase in WHR over 2 years from baseline predicted a lower likelihood of regression of prediabetes to normoglycemia; however, an increase in ALT over 1 year from baseline was not a significant predictor of this outcome.

We further evaluated ALT and WHR as predictors of a continuous glycemic outcome $AUC_{glucose0\text{-}120\ min}$ 2 years after randomization. We found that the effect of baseline ALT on $AUC_{glucose0\text{-}120\ min}$ was dependent on the value of baseline WHR, and the effect of baseline WHR was dependent on the value of ALT (p-value for interaction 0.056). High baseline ALT predicted an increase in $AUC_{glucose0\text{-}120\ min}$ 2 years later when baseline WHR was low or slightly elevated, and high baseline WHR predicted an increase in $AUC_{glucose0\text{-}120\ min}$ when baseline ALT was low normal or normal. Similarly, the effects of changes in ALT and WHR on $AUC_{glucose0\text{-}120\ min}$ were interdependent. An increase in ALT from baseline predicted an increase in $AUC_{glucose0\text{-}120\ min}$ 2 years after randomization if WHR did not change or increased from baseline. An increase in WHR from baseline predicted an increase in $AUC_{glucose0\text{-}120\ min}$ if ALT did not change or increased from baseline.

6.2 Interpretation of the study findings

6.2.1. An interaction between baseline ALT and waist to hip ratio in predicting regression of prediabetes to normoglycemia

When evaluating baseline ALT and baseline WHR as predictors of regression of prediabetes to normoglycemia 2 years later, it was found that there was a significant interaction between baseline ALT and WHR in predicting this outcome. In the model with age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, ALT, WHR, and ALT*WHR interaction as independent variables and regression to normoglycemia as a dependent variable (model 3), the point estimate for the interaction term was 0.143 and the exponent of it was 1.15. This suggests that the odds ratio of regression to normoglycemia per 10 U/L increase in ALT increases by a multiplicative factor of 1.15 for every 0.1 unit increase in WHR[106]. Similarly, the odds ratio of regression to normoglycemia per 0.1 unit increase in WHR increases by a multiplicative factor of 1.15 for every 10 U/L increase in ALT. So, the odds ratios of regression to normoglycemia in response to one baseline variable increase as the level of the interacting variable increases.

The implications of this interaction were evident after obtaining odds ratios of regression to normoglycemia at different levels of the interacting variables (see Figures 5 and 6). This study showed that the effect of baseline ALT on the regression to normoglycemia was statistically significant when baseline WHR was low, and the effect of ALT became non-significant when WHR was relatively high. Similarly, the effect of baseline WHR on this outcome was statistically significant when baseline ALT was low normal, and the effect of WHR became non-significant when ALT was relatively high. A possible explanation for these findings is that among people who have an elevated baseline WHR and, therefore, high levels of ectopic fat, an additional increase in baseline

ALT does not affect the likelihood of regression to normoglycemia 2 years later because it has already been reduced as a result of an elevated baseline WHR. Similarly, people with a high or high normal baseline ALT have high levels of visceral fat, and an additional increase in baseline WHR does not affect the likelihood of regression to normoglycemia 2 years later. In other words, if one of the two baseline measures of ectopic fat is found to be high, an increase in the other baseline variable does not add much more information.

6.2.2. An interaction between baseline ALT and waist to hip ratio in predicting dysglycemia as measured by an area-under-the-glucose curve $AUC_{glucose0\text{-}120\ min}$

Both baseline ALT and baseline WHR were found to be significant predictors of $AUC_{glucose0\text{-}120\ min}$ 2 years later. There was also an interaction between baseline ALT and WHR in predicting $AUC_{glucose0\text{-}120\ min}$ which suggested that their effects were interdependent. In model 3 adjusted for age, sex, ethnicity, FPG, 2-hour PG, baseline ALT and baseline WHR, the interaction term ALT*WHR had a beta coefficient of -9.2. The regression coefficients were 11.0 for ALT and 18.3 for WHR in this model. This means that $AUC_{glucose0\text{-}120\ min}$ increases on average by 11.0 min*mmol/L for every 10 U/L increase in baseline ALT when WHR is held constant at the mean value of 0.91, after adjusting for other covariates[105]. The effect of ALT on $AUC_{glucose0\text{-}120\ min}$ decreases by 9.2 min*mmol/L for every 0.1 unit increase in WHR (e.g. a total $AUC_{glucose0\text{-}120\ min}$ increase of 1.8 min*mmol/L per 10 U/L increase in ALT when WHR is 1.01). Similarly, it was found that $AUC_{glucose0\text{-}120\ min}$ increases on average by 18.3 min*mmol/L for every

0.1 unit increase in WHR when ALT is held constant at the mean value of 25 U/L. The effect of WHR on $AUC_{glucose0\text{-}120\ min}$ decreases by 9.2 min*mmol/L for every 10 U/L increase in ALT (e.g. a total $AUC_{glucose0\text{-}120\ min}$ increase of 9.1 min*mmol/L per 0.1 unit increase in WHR when ALT is 35 U/L).

The implications of this interaction were evident when we looked at the effects of each baseline variable on $AUC_{glucose0\text{-}120\ min}$ at different levels of the interacting variable. We found that the effect of baseline ALT on $AUC_{glucose0\text{-}120\ min}$ was statistically significant when WHR was 0.82 or 0.91, and it became non-significant when WHR was 1.00. The effect of baseline WHR on this outcome was statistically significant when ALT was 12 or 25 U/L, and it became non-significant when ALT was 38 U/L (see Figures 11 and 12). This could be because people with a high baseline WHR already have high levels of ectopic fat deposition, and having a high level of baseline ALT does not have any additional impact on the degree of dysglycemia 2 years later. Similarly, those who have a high baseline ALT already have high levels of visceral fat, and having a high baseline WHR has no further impact on the degree of dysglycemia.

6.2.3. An interaction between a change in ALT from baseline and a change in WHR from baseline in predicting $AUC_{glucose0\text{-}120min}$

In view of the significant interaction observed between baseline ALT and baseline WHR in predicting $AUC_{glucose0\text{-}120\ min}$, we decided to test for an interaction between a change in ALT and a change in WHR from baseline in predicting this outcome in post-hoc analyses. The interaction was found to be significant, and we included the

ΔALT*ΔWHR interaction term in the final models. In model 3 adjusted for age, sex, ethnicity, FPG, 2-hour PG, ΔALT, and ΔWHR, the interaction term ΔALT*ΔWHR had a beta coefficient of 7.9. The regression coefficients were 11.1 for ΔALT and 13.0 for ΔWHR. This means that $AUC_{glucose0\text{-}120\ min}$ increases on average by 11.1 min*mmol/L per 10 U/L increase in ALT from baseline when ΔWHR is at the mean of 0.01 (i.e. when WHR does not change much from baseline). The effect of ΔALT on $AUC_{glucose0\text{-}120\ min}$ increases by 7.9 min*mmol/L for every 0.1 unit increase in WHR from baseline (e.g. a total $AUC_{glucose0\text{-}120\ min}$ increase of 19.0 min*mmol/L per 10 U/L increase in ALT from baseline when ΔWHR=0.11). Similarly, $AUC_{glucose0\text{-}120\ min}$ increases on average by 13.0 min*mmol/L for every 0.1 unit increase in WHR from baseline when ΔALT is at the mean of 0.26 U/L (i.e. when ALT does not change much from baseline). The effect of ΔWHR on $AUC_{glucose0\text{-}120\ min}$ increases by 7.9 min*mmol/L for every 10 U/L increase in ALT from baseline (e.g. a total $AUC_{glucose0\text{-}120\ min}$ increase of 20.8 min*mmol/L per 0.1 unit increase in WHR from baseline when ΔALT=10.3 U/L).

The implications of this interaction were explored by looking at the effects of a change in each variable from baseline on $AUC_{glucose0\text{-}120\ min}$ at different levels of the interacting variable. The effect of an increase in ALT from baseline on $AUC_{glucose0\text{-}120\ min}$ was statistically significant when WHR did not change from baseline or when WHR increased from baseline. Similarly, the effect of an increase in WHR from baseline on $AUC_{glucose0\text{-}120\ min}$ was statistically significant when ALT did not change or increased from baseline. An increase in ALT over time had no effect on $AUC_{glucose0\text{-}120\ min}$ when WHR decreased from baseline, and an increase in WHR over time had no effect on this

outcome when ALT decreased from baseline, likely reflecting a process different from an accumulation of ectopic fat.

6.2.4. Ectopic fat hypothesis

The current analyses were designed to answer two primary research questions: (i) is ALT an important predictor of regression of prediabetes to normoglycemia and (ii) is WHR an important predictor of regression of prediabetes to normoglycemia 2 years later. We hypothesized that people with high levels of ALT and WHR will have a low likelihood of regression to normoglycemia. We indeed found that high levels of baseline ALT predicted a low likelihood of regression to normoglycemia when WHR was low, and high baseline WHR predicted a low likelihood of regression to normoglycemia when ALT was low normal. We also hypothesized that people with high levels of baseline ALT and WHR will have a high degree of dysglycemia as measured by $AUC_{glucose0\text{-}120\ min}$ 2 years later. We found that this was true; however, we also found that the effects of baseline ALT and WHR on $AUC_{glucose0\text{-}120\ min}$ were interdependent.

We believe that high levels of ALT and/or WHR represent high degree of ectopic fat deposition, and our study findings support the hypothesis that ectopic fat plays a role in the pathogenesis of type 2 diabetes. However, the results of this study need to be interpreted with caution since both ALT and WHR are surrogate measures of ectopic fat deposition; they might reflect a process different from ectopic fat deposition, yet still be related to the progression and regression of dysglycemia. In this case, the observed relationships between ALT, WHR and glycemic outcomes would still be valid; however,

they would not be related to ectopic fat deposition. Alternatively, there might be another unknown parameter that plays a causal role in the pathophysiology of diabetes which also affects ectopic fat deposition, ALT and WHR. Adjusting for this currently unknown confounder would weaken or nullify the observed associations between ALT, WHR and glycemic outcomes.

6.3 Generalizability of the study findings

A total of 1,209 participants with impaired fasting glucose and/or impaired glucose tolerance treated with placebo rosiglitazone and placebo ramipril in the DREAM trial who had a follow-up OGTT were included in the current analyses. Our study population had a mean FPG of 5.8 mmol/L, 2-hour PG of 8.7 mmol/L (indicating impaired glucose tolerance), ALT of 25 U/L (within normal range), and waist to hip ratio of 0.91. The trial participants included people of many ethnicities from 21 countries; this makes the results applicable internationally. As outlined in Section 4.1.1., patients who were pregnant or had a history of cardiovascular disease, renal disease, active liver disease, or ALT $\geq$ 2.5 times the upper limit of normal during screening were excluded. Excluding people with a history of cardiovascular disease and renal disease reduces generalizability of this study. Exclusion of people with active liver disease and ALT $\geq$ 2.5 times the upper limit of normal also decreases generalizability of the study findings. These participants could have been included with an adjustment for the presence of active liver disease in regression models since active liver disease is a potential confounder of the relationship between ALT and glycemic outcomes (see Section 4.3.4.e).

6.4 Comparison to literature

Although several large prospective studies evaluated ALT[76, 77, 107-111] and anthropometric measures[71-73, 107, 112] as predictors of development of type 2 diabetes, very few studies looked at predictors of regression of prediabetes or diabetes to normoglycemia[10, 18, 20, 22]. We found one large study that assessed predictors of development of normal glucose tolerance among people with prediabetes[10]. Among 2,528 people with combined impaired fasting glucose and impaired glucose tolerance participating in the Diabetes Prevention Program, 600 regressed to normal glucose tolerance over 3 years. Participants with isolated IFG and isolated IGT at baseline were excluded from analyses. Greater weight loss from baseline was found to be a significant independent predictor of regression to normal glucose tolerance (NGT) while baseline weight was not. In a model adjusted for age, sex, ethnicity, baseline FPG, 2-hour PG, insulin secretion, insulin sensitivity, weight, change in weight, and treatment group, a hazard ratio of regression to NGT was 1.34 (95% CI 1.21-1.49) per 1 SD of weight loss; however, 1 SD was not stated. This study did not assess liver enzymes as predictors of regression of prediabetes to NGT, and did not state any specific hypothesis tested.

One large study[74] evaluated anthropometric indices as predictors of an area-under-the-glucose-curve obtained during a baseline 75 g OGTT in people who had diabetes or were at risk of development of type 2 diabetes. EpiDREAM prospective cohort included healthy people, participants with prediabetes (who qualified for the DREAM trial), and those who were found to have type 2 diabetes at the time of screening

for the DREAM trial. De Koning and colleagues[74] performed a cross-sectional analysis on 22,293 people in the EpiDREAM cohort at baseline. Fourteen percent of the study participants were found to have undiagnosed type 2 diabetes. In regression models adjusted for age, sex, smoking, and ethnicity, it was found that a 1 SD increase in baseline WHR of 0.09 was associated with a 0.77 (0.71-0.84) hr*mmol/L increase in baseline $AUC_{glucose0\text{-}120\ min}$. This is equivalent to an $AUC_{glucose0\text{-}120\ min}$ increase of 46.2 in min*mmol/L. This increase is larger than observed in our study which could be because we looked at the $AUC_{glucose0\text{-}120\ min}$ prospectively and adjusted the models for baseline FPG and 2-hour PG.

6.5 Study strengths

This thesis consisted of epidemiologic analyses on already collected trial data. The strengths of this dataset included a large sample size and high follow-up rate: 91.5% of the 1,321 participants randomized to the placebo-placebo group in the DREAM trial had a follow-up OGTT and were eligible for analyses. Independent variables such as baseline FPG, 2-hour PG, ALT, WHR, age, sex and ethnicity were available in 1,204 of the 1,209 eligible participants; therefore, 1,204 people were included in regression models which evaluated baseline ALT and WHR as predictors of the two glycemic outcomes after adjustment for age, sex, ethnicity, FPG and 2-hour PG. Important covariates such as family history of diabetes, smoking status and physical activity status were ascertained in 1,199 participants at baseline; therefore, 1,199 participants were included in the maximally adjusted models testing baseline ALT and WHR as predictors

of the two glycemic outcomes. A large sample size allowed us to adjust for all known important covariates.

The study methods included a statement of the study hypothesis and primary and secondary research questions, and we stated the predicted direction of the relationship between ALT, WHR and glycemic outcomes as a priori. Statistical analyses included a careful examination of the data for multicollinearity, regression assumptions, and the presence of outliers and data points with high leverage. We assessed ALT and WHR as predictors of a continuous glycemic outcome $AUC_{glucose0\text{-}120\ min}$, and did not restrict analyses to a categorical outcome of regression to normoglycemia. We were also able to delineate an interaction between ALT and WHR in predicting the two glycemic outcomes without having to categorize one of the variables which would have led to the loss of information.

6.6 Limitations

Overall, the study has a low risk of bias. We cannot exclude selection bias as study participants were not selected for the study randomly. Exclusion of 112 placebo-placebo group participants with no follow-up OGTT could be a source of missingness bias. In order to assess whether the probability of missingness of the follow-up OGTTs was related to our variables of interest, baseline characteristics of the included and excluded participants were compared and summarized in Table 3. The results suggested that missingness of the OGTT was not related to participants' baseline glycemic status as baseline FPG and 2-hour PG were not different between included and excluded

participants. Baseline glycemic parameters have been shown to be strong predictors of the follow-up glucose tolerance status[1]; therefore, it is unlikely that included and excluded participants differed on the follow-up glycemic status which was our outcome of interest. We also compared baseline ALT and WHR between the included and excluded groups to assess whether the probability of missingness of the OGTTs was related to these predictors of interest. Baseline ALT and WHR were not significantly different between the two groups. Study results also did not change after including 25 placebo-placebo participants who did not have a follow-up OGTT in the sensitivity analyses as described in Section 5.4.4. However, this does not rule out missingness bias completely as the groups could have differed on other non-measured characteristics which could affect the relationship between ALT, WHR and glycemic outcomes. In regression models which evaluated changes in ALT and WHR from baseline as predictors of the two glycemic outcomes, up to 7% of the 1,209 eligible participants did not have a follow-up ALT or WHR and these participants were excluded from the models. We did not explore the mechanism of missingness of these values since these secondary analyses were hypothesis-generating.

Measurement of glycemic outcomes in our study was based on the results of one oral glucose tolerance test conducted during follow-up, and this test has been shown to have a relatively poor reproducibility[98]. This could be a source of misclassification error where people who generally have near-normoglycemic glucose values are classified to be in the non-regression group, and those who are generally dysglycemic are classified to be in the regression group. However, glucose levels were measured in the study objectively,

and this measurement error could be in either direction. It is, therefore, more likely to introduce noise and obscure the real relationship between ALT, WHR and regression to normoglycemia than to introduce bias. Similarly, poor reproducibility of OGTT could introduce a measurement error in calculating $AUC_{glucose0\text{-}120\ min}$; however, this error is unlikely to introduce bias in the observed relationships between ALT, WHR and $AUC_{glucose0\text{-}120\ min}$.

ALT has been shown to have high biological and analytical variability[113, 114], and our standardization of ALT values only partially accounted for the differences in analytic techniques used by different laboratories. However, baseline ALT and WHR were ascertained in all participants similarly using objective measures; therefore, measurement of these variables should not be a source of bias. It should be noted that predictors and confounders of the relationships between ALT, WHR and glycemic outcomes are not well known, and this could introduce bias due to underfitting in regression models[96].

After accounting for clustering of data within centres by performing multilevel random intercept analyses, the results have not changed much as summarized in Section 6. This suggests that clustering of data within centres was very minor for $WHR_{baseline}$, ΔALT, ΔWHR and glycemic outcomes. However, baseline ALT was no longer a significant predictor of $AUC_{glucose0\text{-}120\ min}$ as the p-value changed from 0.03 to 0.10. One possible explanation is that baseline ALT was more similar between people within centres than between centres which could be due to a similar dietary or exercise environment. The effect of clustering might be more pronounced for baseline ALT than for ΔALT over 1 year due to a prolonged effect of the environment in the case of baseline

ALT. We, therefore, may need a larger population sample in order to detect a significant effect of baseline ALT on $AUC_{glucose0\text{-}120\ min}$ in the presence of clustering.

6.7 Future directions

While this study has limitations, the finding of the interaction between ALT and WHR in predicting glycemic outcomes is interesting and warrants further research. If its existence is confirmed in other studies, accounting for this interaction might affect the nature and strengths of the observed relationships between ALT, WHR and glycemic outcomes. It would be, therefore, important to confirm study findings in other cohorts of people with prediabetes and type 2 diabetes. Since both baseline values and changes in ALT and WHR over time appear to be important in predicting the future degree of dysglycemia and likelihood of regression to normoglycemia, perhaps, regression models should include both baseline values and changes in ALT and WHR from baseline when studied in the future. Potential interactions between WHR and sex and between ALT and sex might also need to be explored. More accurate non-invasive methods of assessing the degree of ectopic fat need to be identified and employed in prospective epidemiologic studies to further delineate the importance of ectopic fat in progression of dysglycemia. This study focused on the evaluation of ALT and WHR as predictors of regression of prediabetes to normoglycemia, and studies aimed at identifying other important predictors of regression of prediabetes and diabetes to normoglycemia are needed. This information may facilitate the development of new therapies for treating these conditions.

7. Conclusions

Currently, treatment of prediabetes and type 2 diabetes is focused on lowering plasma glucose values with lifestyle therapy and medications, and treating other cardiovascular risk factors. However, it is important to study predictors of regression of prediabetes and diabetes to normoglycemia and promote the development of therapies that can induce such regression. In this study, we tested the hypothesis that measures of ectopic fat deposition such as ALT and WHR predict future glycemic outcomes in people with prediabetes. We found that higher levels of baseline ALT and baseline WHR predicted a lower likelihood of regression of prediabetes to normoglycemia and higher $AUC_{glucose0\text{-}120\ min}$ level 2 years later. The effects of baseline ALT and WHR on these outcomes were interdependent. An increase in ALT from baseline and an increase in WHR from baseline predicted $AUC_{glucose0\text{-}120\ min}$; however, only an increase in WHR from baseline predicted a lower likelihood of regression to normoglycemia. Further research is needed to confirm these findings in people with prediabetes and diabetes, and identify other important predictors of reversal of these conditions.

Table 1. Proposed multivariable logistic regression models with the regression of prediabetes to normoglycemia as the outcome.

Model	Predictors
A	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose
B	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *baseline ALT*
C	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *baseline WHR*
D	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *baseline ALT, baseline WHR*
E	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, baseline ALT, baseline WHR, *ALT*WHR interaction*
F	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, baseline ALT, baseline WHR, ALT*WHR interaction, *family history of diabetes, smoking, physical activity level*
G	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *a change in ALT from baseline to 1 year*
H	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *a change in WHR from baseline to 2 years*
I	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *a change in ALT from baseline to 1 year, a change in WHR from baseline to 2 years*
J	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, a change in ALT from baseline to 1 year, a change in WHR from baseline to 2 years, *family history of diabetes, smoking, physical activity level*

ALT – alanine transaminase; FPG – fasting plasma glucose; WHR = waist to hip ratio = waist circumference/hip circumference (cm/cm).

Table 2. Proposed multivariable linear regression models with $AUC_{glucose\ 0\text{-}120\ min}$ as the outcome.

Model	Predictors
A	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose
B	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *baseline ALT*
C	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *baseline WHR*
D	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *baseline ALT, baseline WHR*
E	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, baseline ALT, baseline WHR, *ALT*WHR interaction*
F	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, baseline ALT, baseline WHR, ALT*WHR interaction, *family history of diabetes, smoking, physical activity level*
G	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *a change in ALT from baseline to 1 year*
H	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *a change in WHR from baseline to 2 years*
I	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, *a change in ALT from baseline to 1 year, a change in WHR from baseline to 2 years*
J	age, sex, ethnicity, baseline FPG, baseline 2-hour plasma glucose, a change in ALT from baseline to 1 year, a change in WHR from baseline to 2 years, *family history of diabetes, smoking, physical activity level*

ALT – alanine transaminase; FPG – fasting plasma glucose; WHR = waist to hip ratio = waist circumference/hip circumference (cm/cm).

Table 3. Baseline characteristics of the study population (people with impaired fasting plasma glucose and/or impaired glucose tolerance treated with placebo rosiglitazone placebo ramipril who had a follow-up oral glucose tolerance test) and excluded participants (people who did not have a follow-up oral glucose tolerance test). Means (standard deviations) or n (%) are shown. Variables between the study population and excluded participants were compared using a Wilcoxon two-sample test or a chi-square test.

Variable	Study population	Excluded participants	P-value
N (%)	1209(92)	112(8)	
Age, years	55(11)	54(12)	0.47
Males (%)	500(41)	43(38)	0.54
European (%)	654(54)	55(49)	0.31
Current smoking (%)	159(13)	13(12)	0.63
Family history of diabetes (%)	718(59)	52(46)	0.008
Sedentary lifestyle (%)	329(27)	27(24)	0.46
FPG[*], mmol/L	5.8 (0.7)	5.8(0.7)	0.82
2hrPG[*], mmol/L	8.7(1.5)	8.9(1.3)	0.33
$ALT_{baseline}$[†], U/L	25(13)	26(14)	0.55
$WHR_{baseline}$[‡]	0.91(0.09)	0.90(0.08)	0.67

[*] Fasting plasma glucose (FPG) and 2-hour plasma glucose (2hrPG) were obtained on a baseline 75 g OGTT.
[†]Alanine transaminase (ALT) values were standardized as follows. In males, $ALT_{baseline} = ALT_{measured}*40/(\text{local lab ULN})$. In females, $ALT_{baseline} = ALT_{measured}*35/(\text{local lab ULN})$.
[‡]Waist to hip ratio (WHR) = waist circumference/hip circumference = WC/HC (cm/cm).

Table 4. Baseline and follow-up characteristics of the study population (people with impaired fasting plasma glucose and/or impaired glucose tolerance treated with placebo rosiglitazone placebo ramipril who had a follow-up oral glucose tolerance test). Means (standard deviations) or n (%) are shown. Variables between the regression and non-regression groups (see footnote for definition) were compared using a Wilcoxon two-sample test or a chi-square test.

	Variable	Overall	Regression group*	Non-regression group*	P-value
Baseline	N (%)	1209(100)	301(25)	908(75)	
	Age, years	55(11)	54(11)	56(11)	0.009
	Males (%)	500(41)	120(40)	380(42)	0.54
	European (%)	654(54)	146(49)	508(56)	0.02
	Current smoking (%)	159(13)	34(11)	125(14)	0.27
	Family history of diabetes (%)	718(59)	178(59)	540(59)	0.92
	Sedentary lifestyle (%)	329(27)	105(35)	224(25)	0.0006
	FPG**, mmol/L	5.8 (0.7)	5.6 (0.7)	5.9(0.6)	<0.0001
	2hrPG**, mmol/L	8.7(1.5)	8.3(1.4)	8.9(1.5)	<0.0001
	$ALT_{baseline}$†, U/L	25(13)	24(14)	25(13)	0.004
	$WC_{baseline}$‡, cm	99(14)	96(13)	99(14)	0.005
	$HC_{baseline}$‡, cm	109(14)	107(12)	109(14)	0.03
	$WHR_{baseline}$‡	0.91(0.09)	0.90(0.08)	0.91(0.10)	0.05
Follow-up	$\Delta ALT_{(1year - baseline)}$†, U/L	0.20(13.4)	0.25(13.4)	0.18(13.4)	0.28
	$\Delta WHR_{(2years - baseline)}$‡	0.010(0.13)	-0.005(0.074)	0.016(0.14)	0.002
	$AUC_{glucose0\text{-}120min}$*, min*mmol/L	860(222)	656(73)	927(213)	<0.0001

* Regression (to normoglycemia) group and non-regression group were defined on the basis of a 75g oral glucose tolerance test (OGTT) performed 2 years after randomization. Participants with prediabetes at baseline who met normoglycemic criteria (a fasting plasma glucose < 5.6 mmol/L and a 2-hour plasma glucose < 7.8 mmol/L) on this OGTT were assigned to the regression group and those who did not were assigned to the non-regression group. $AUC_{glucose0\text{-}120min}$ is the area-under-the-glucose-curve obtained on the 2-year 75 g OGTT.

** Fasting plasma glucose (FPG) and 2-hour plasma glucose (2hrPG) were obtained on a baseline 75 g OGTT.

† Alanine transaminase (ALT) values were standardized as follows. In males, ALT = $ALT_{measured}$*40/(local lab ULN). In females, ALT = $ALT_{measured}$*35/(local lab ULN).

‡ Waist to hip ratio (WHR) = waist circumference/hip circumference = WC/HC (cm/cm).

Table 5. Correlation between predictor variables in regression models. These variables represent baseline characteristics of the study population unless indicated otherwise.

	age	sex	ethnicity	FPG*	2hrPG*	ALT	WHR**	ΔALT†	ΔWHR‡	family history of T2DM#	smoking	sedentary lifestyle
age	1.0	-0.008	-0.20	0.14	0.05	-0.14	0.07	0.02	-0.03	-0.11	-0.09	-0.05
sex		1.0	0.02	-0.14	0.04	-0.23	-0.47	0.009	0.06	0.07	-0.05	0.06
ethnicity			1.0	-0.04	-0.04	-0.05	-0.06	0.06	0.03	0.04	-0.04	0.17
FPG*				1.0	-0.12	0.07	0.11	0.006	0.07	-0.04	0.07	-0.05
2hrPG*					1.0	0.02	0.009	-0.02	-0.04	0.05	-0.08	-0.02
ALT						1.0	0.23	-0.41	-0.04	-0.03	0.07	-0.06
WHR**							1.0	-0.03	-0.34	-0.05	0.06	-0.01
ΔALT†								1.0	0.02	0.05	-0.02	0.01
ΔWHR‡									1.0	0.003	-0.007	0.002
family history of T2DM#										1.0	0.02	-0.06
smoking											1.0	0.05
sedentary lifestyle												1.0

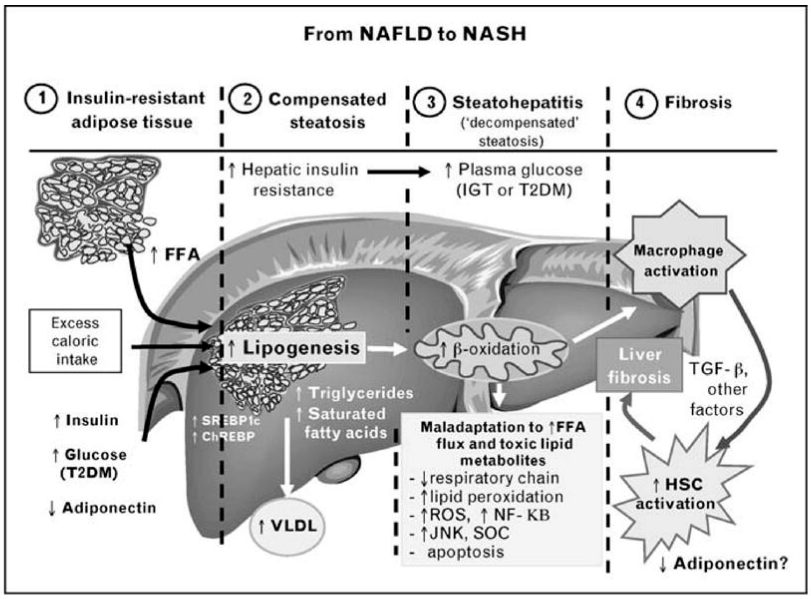
*Fasting plasma glucose (FPG) and 2-hour plasma glucose (2hrPG) were obtained on a baseline 75 g OGTT.
**Waist to hip ratio (WHR) = waist circumference/hip circumference = WC/HC (cm/cm).
†ΔALT – change in alanine transaminase (1 year – baseline)
‡ΔWHR – change in WHR (2 years – baseline)
#T2DM – type 2 diabetes mellitus

Table 6. Comparison of regression coefficients (95% confidence intervals) and p-values before and after adjustment of linear regression models for centre effect

Dependent variable	Covariates	Independent variables	No adjustment for centre effect		After adjustment for centre effect	
			β-coefficient[#] (95% CI)	P-value	β-coefficient[#] (95% CI)	P-value
$AUC_{glucose0\text{-}120\ min}$[*]	age, sex, ethnicity, FPG[**], 2hrPG[**]	$ALT_{baseline}$	11.0 (1.4 to 20.6)	0.03	8.2 (-1.5 to 17.9)	0.10
		$WHR_{baseline}$[§]	18.3 (3.8 to 32.9)	0.01	19.1 (4.7 to 33.4)	0.009
		$ALT_{baseline}$*$WHR_{baseline}$	-9.2 (-18.6 to 0.24)	0.056	-9.0 (-18.3 to 0.37)	0.06
$AUC_{glucose0\text{-}120\ min}$[*]	age, sex, ethnicity, FPG[**], 2hrPG[**]	ΔALT[†]	11.1 (2.0 to 20.1)	0.02	14.1 (5.1 to 23.1)	0.002
		ΔWHR[‡]	13.0 (3.4 to 22.6)	0.008	12.1 (2.7 to 21.6)	0.01
		ΔALT*ΔWHR	7.9 (0.15 to 15.6)	0.046	7.2 (-0.38 to 14.9)	0.06

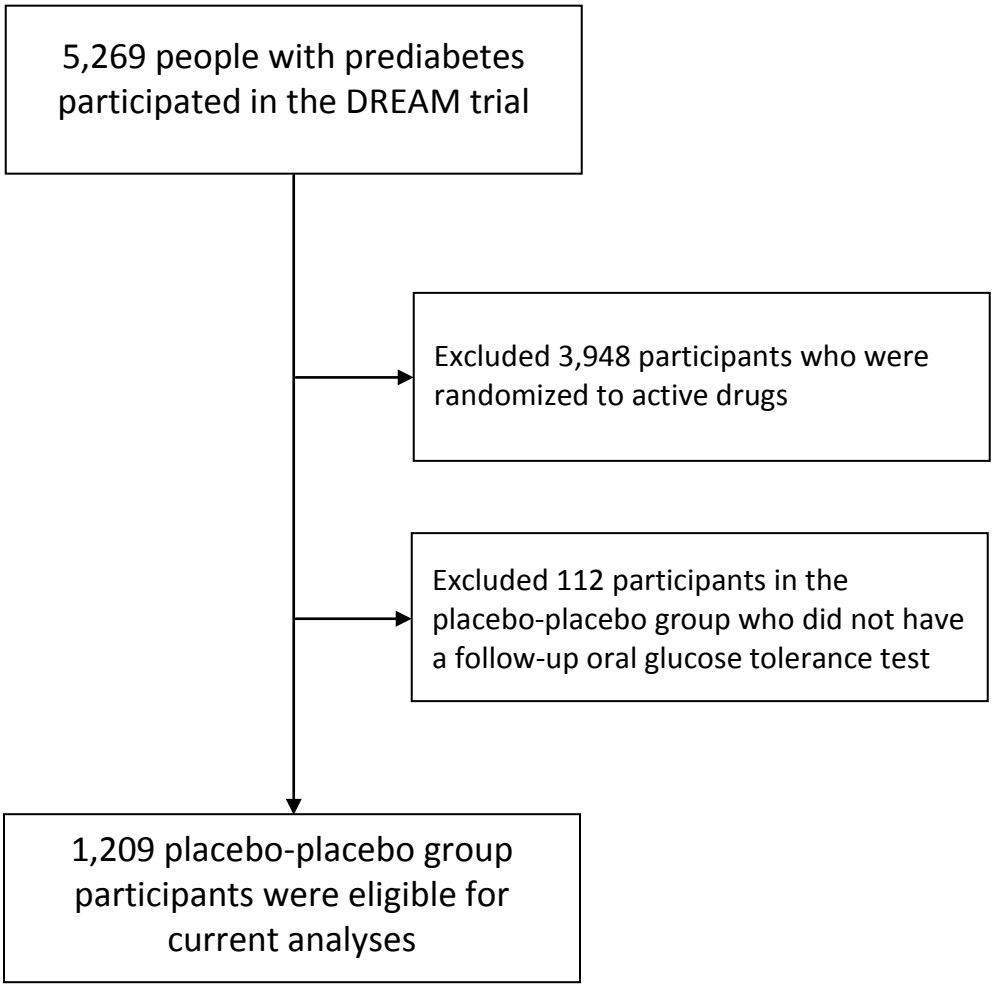
[*]$AUC_{glucose0\text{-}120min}$ is the area-under-the-glucose-curve obtained on the 2-year 75 g OGTT.
[**]Fasting plasma glucose (FPG) and 2-hour plasma glucose (2hrPG) were obtained on a baseline 75 g OGTT.
[§]Waist to hip ratio (WHR) = waist circumference/hip circumference = WC/HC (cm/cm).
[†]ΔALT – change in alanine transaminase (1 year – baseline)
[‡]ΔWHR – change in WHR (2 years – baseline)
[#]β-coefficients represent a change in $AUC_{glucose0\text{-}120min}$ (i) per 10 U/L increase in $ALT_{baseline}$ when $WHR_{baseline}$ was at the mean of 0.91, (ii) per 0.1 unit increase in $WHR_{baseline}$ when $ALT_{baseline}$ was at the mean of 25 U/L, (iii) per 10 U/L increase in ALT over 1 year from baseline when ΔWHR was 0.01, and (iv) per 0.1 unit increase in WHR over 2 years from baseline when ΔALT was 0.26 U/L.

Figure 1. Potential mechanisms involved in the progression of insulin resistance to non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. This figure was adapted from Cusi K. (2009). *Current Opinion in Endocrinology, Diabetes and Obesity*; 16:141-49.



(1) Insulin-resistant adipose tissue: in the setting of metabolic syndrome and/or T2DM, elevated rates of lipolysis/plasma FFA from insulin-resistant adipose tissue combined with hyperinsulinemia and hyperglycemia stimulate excessive hepatic triglyceride synthesis and the formation of toxic saturated fatty acids; (2) Compensated steatosis: steatosis in turn may: exacerbate hepatic insulin resistance, stimulate VLDL secretion, and increase mitochondrial beta-oxidation. If a new steady state is achieved, only benign steatosis and/or dyslipidemia (high triglyceride, low HDL-C) takes place; (3) Steatohepatitis: if mitochondrial function cannot adapt to the increased FFA flux and respiratory oxidation collapses, lipid-derived toxic metabolites activate inflammatory pathways and hepatocyte lipotoxicity with stimulation of chronic necrosis and inflammation; (4) Fibrosis: the magnitude of the cross-talk between hepatocytes, macrophages, and HSCs determines the degree of the fibrogenic response and potential progression to cirrhosis. In this setting, low plasma adiponectin levels are believed to promote steatosis and fibrosis by allowing unchecked triglyceride synthesis and activation of HSCs, respectively. FFA, free fatty acid; HDL-C, high-density lipoprotein-C; HSC, hepatic stellate cell; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

Figure 2. Selection of the study population among participants of the DREAM (Diabetes REduction Assessment with ramipril and rosiglitazone Medication) trial.



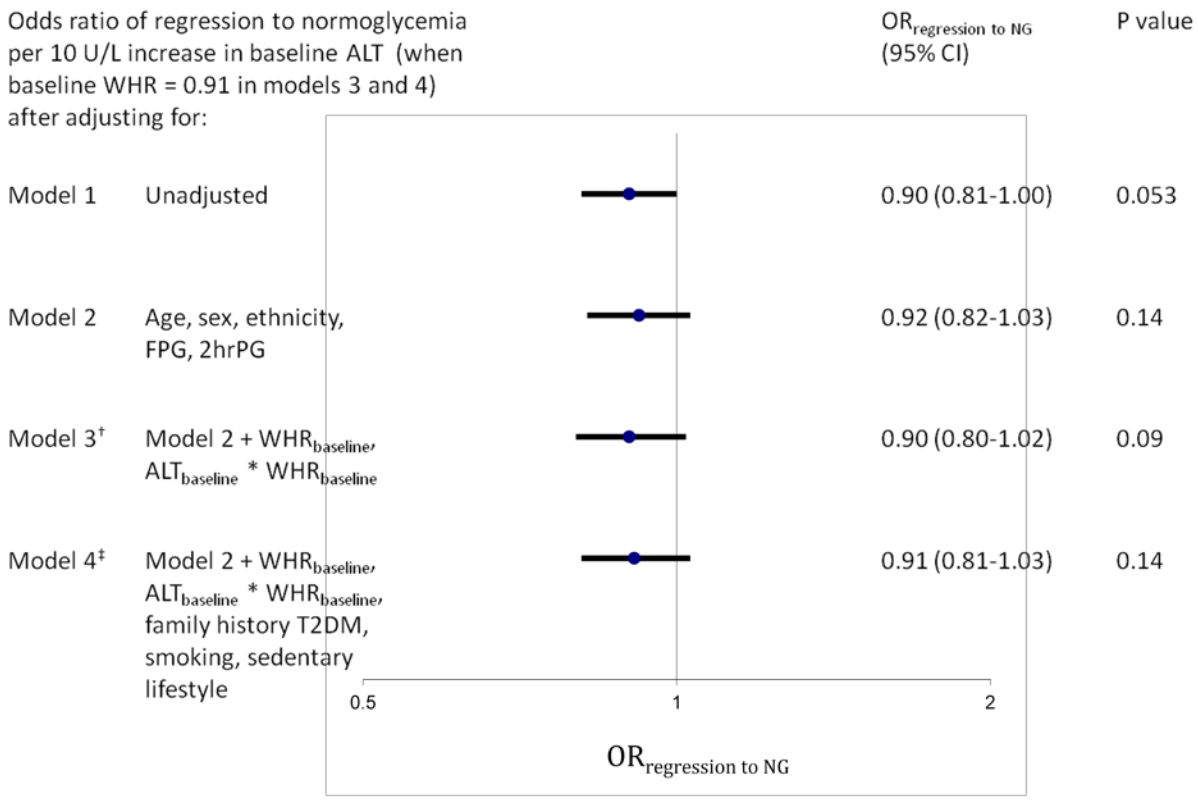
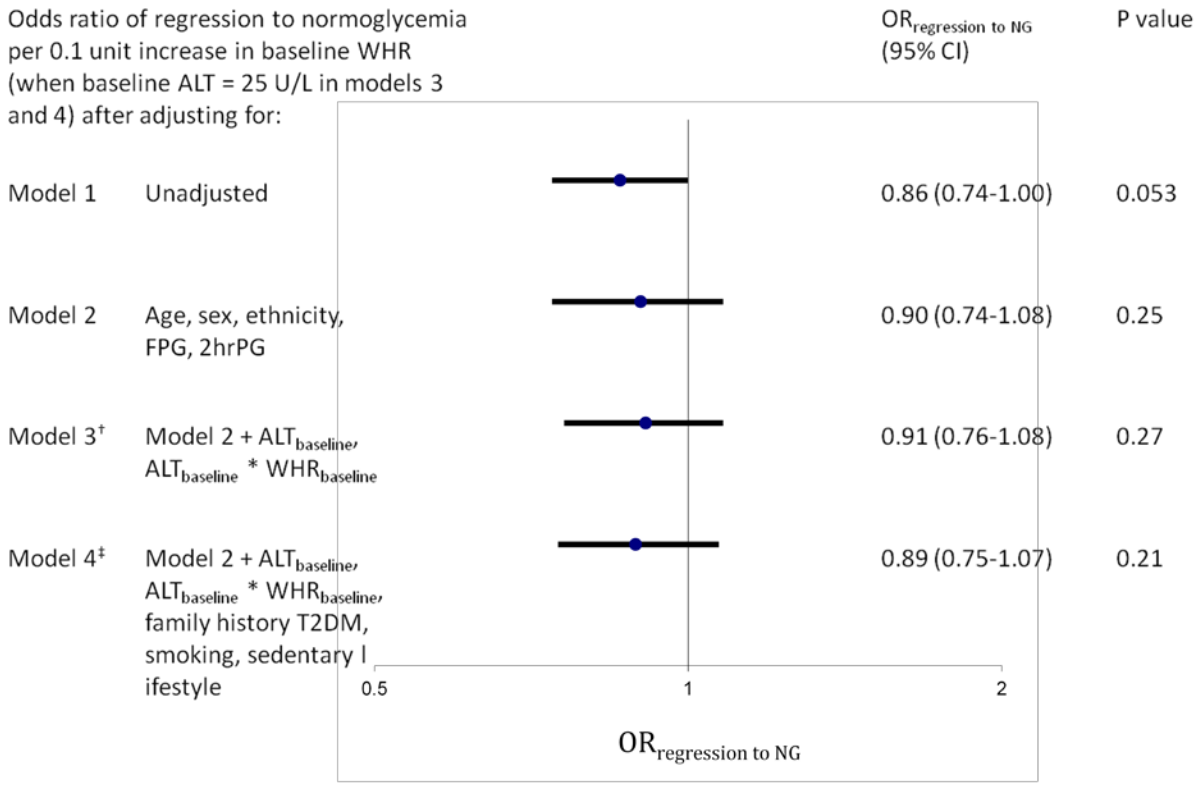
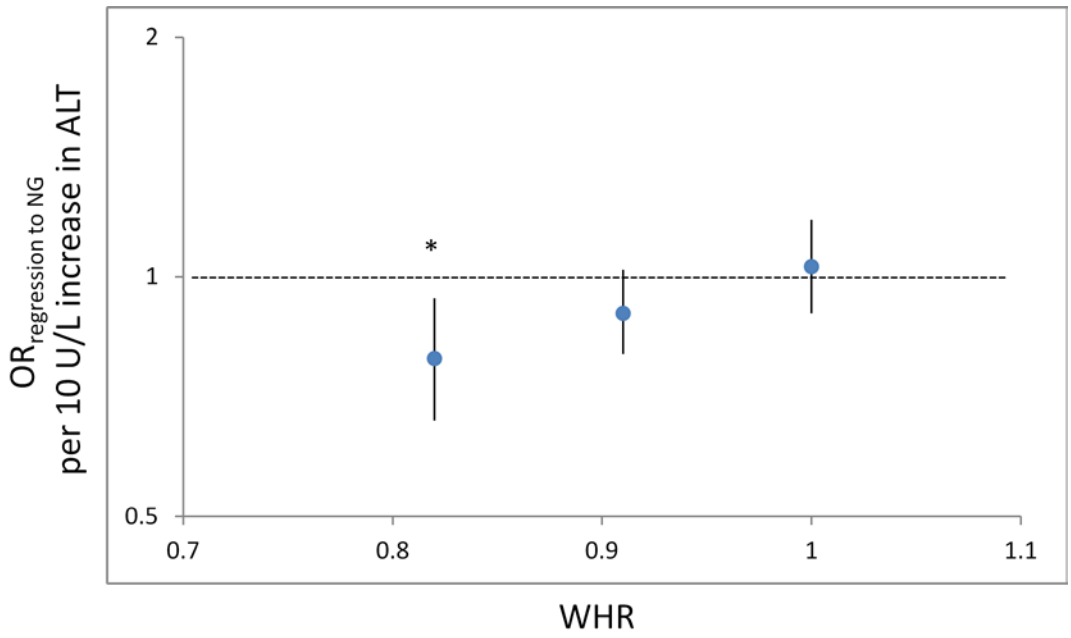


Figure 3. A relationship between baseline ALT and regression of prediabetes to normoglycemia 2 years later. Odds ratios (95% confidence intervals) of regression to normoglycemia per 10 U/L increase in ALT are shown, unadjusted and adjusted for other covariates. In models 3 and 4, an interaction between baseline ALT and waist to hip ratio was significant, and the odds ratios are reported per 10 U/L increase in ALT when waist to hip ratio is at the mean of 0.91. ALT – alanine transaminase, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, NG – normoglycemia, OR – odds ratio, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



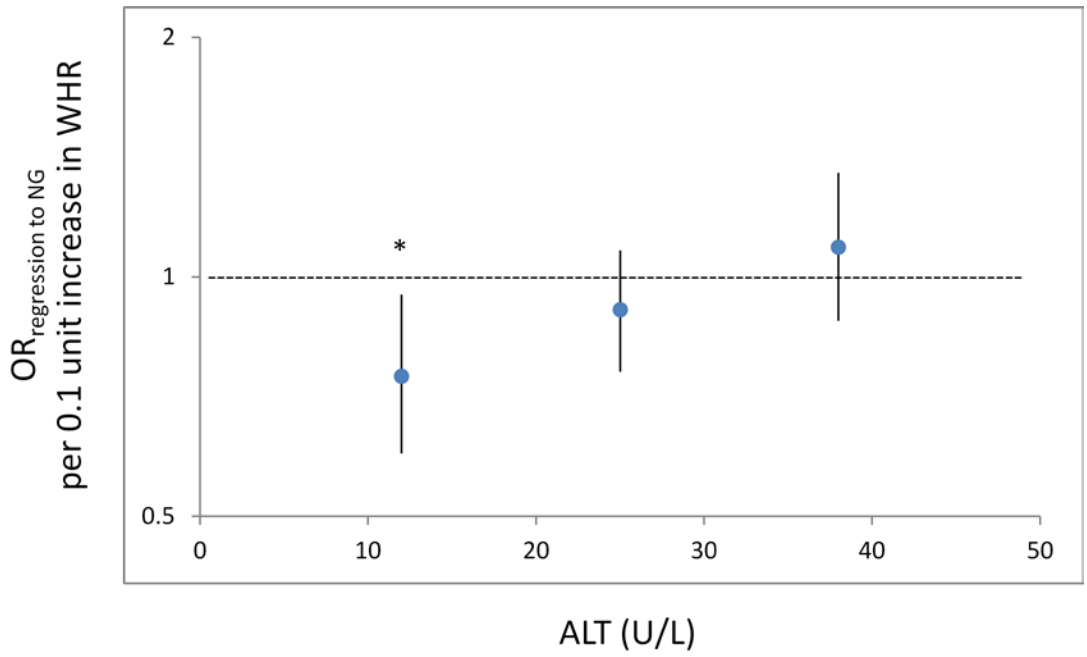
†P-value for $ALT_{baseline}$ * $WHR_{baseline}$ interaction 0.01.
‡P-value for $ALT_{baseline}$ * $WHR_{baseline}$ interaction 0.008.

Figure 4. A relationship between baseline waist to hip ratio and regression of prediabetes to normoglycemia 2 years later. Odds ratios (95% confidence intervals) of regression to normoglycemia per 0.1 unit increase in waist to hip ratio are shown, unadjusted and adjusted for other covariates. In models 3 and 4, an interaction between baseline ALT and waist to hip ratio was significant, and the odds ratios are reported per 0.1 unit increase in waist to hip ratio when ALT is at the mean of 25 U/L. ALT – alanine transaminase, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, NG – normoglycemia, OR – odds ratio, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



* P-value for $OR_{regression\ to\ NG}$ 0.008.

Figure 5. A relationship between baseline ALT and regression of prediabetes to normoglycemia 2 years later at different levels of baseline waist to hip ratio (mean=0.91, mean - 1 standard deviation, mean + 1 standard deviation). Odds ratios (95% confidence intervals) per 10 U/L increase in ALT were obtained after adjusting for age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, waist to hip ratio, and an interaction between ALT and waist to hip ratio (p-value for interaction 0.01). ALT – alanine transaminase, NG – normoglycemia, OR – odds ratio, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



* P-value for $OR_{regression\ to\ NG}$ 0.02.

Figure 6. A relationship between baseline waist to hip ratio and regression of prediabetes to normoglycemia 2 years later at different levels of baseline ALT (mean=25 U/L, mean-1SD, mean+1SD). Odds ratios (95% confidence intervals) per 0.1 unit increase in waist to hip ratio were obtained after adjusting for age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, ALT, and an interaction between ALT and waist to hip ratio (p-value for interaction 0.01). ALT – alanine transaminase, NG – normoglycemia, OR – odds ratio, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).

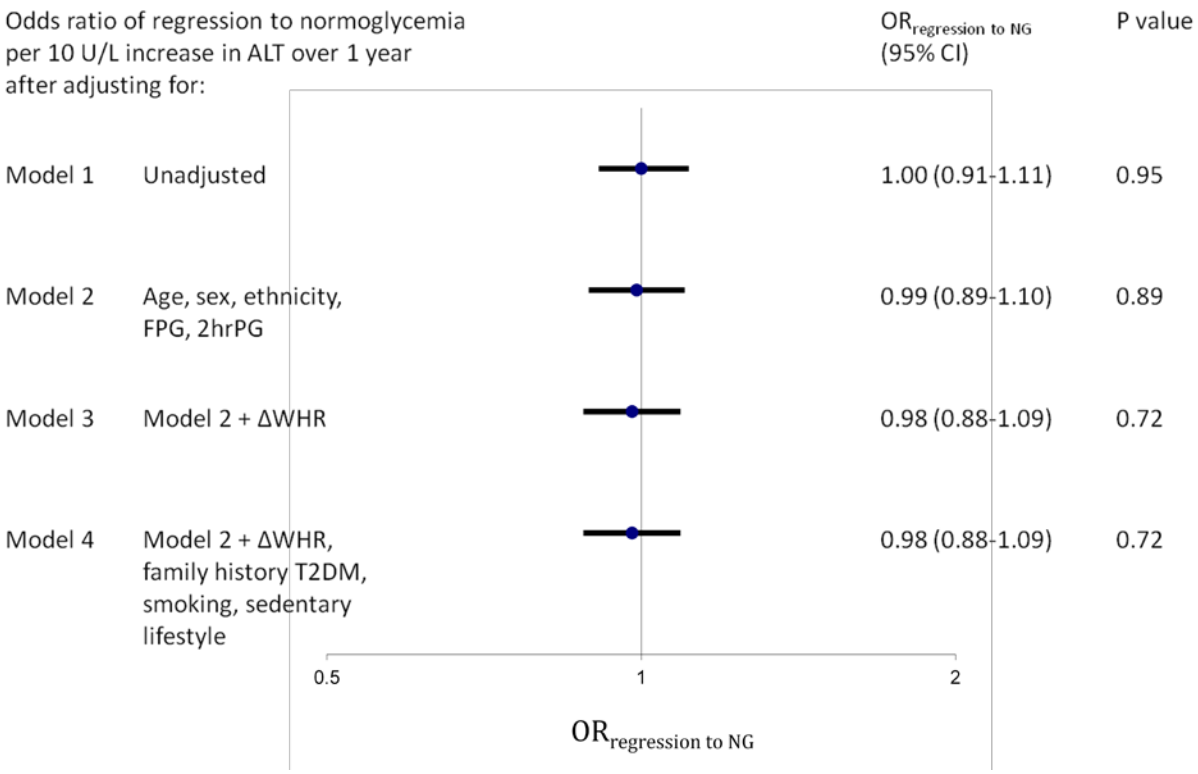


Figure 7. A relationship between a change in ALT from baseline to 1 year and regression of prediabetes to normoglycemia 2 years after randomization. Odds ratios (95% confidence intervals) of regression to normoglycemia per 10 U/L increase in ALT from baseline are shown, unadjusted and adjusted for other covariates. ALT – alanine transaminase, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, NG – normoglycemia, OR – odds ratio, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm), ΔWHR – a change in waist to hip ratio from baseline to 2 years.

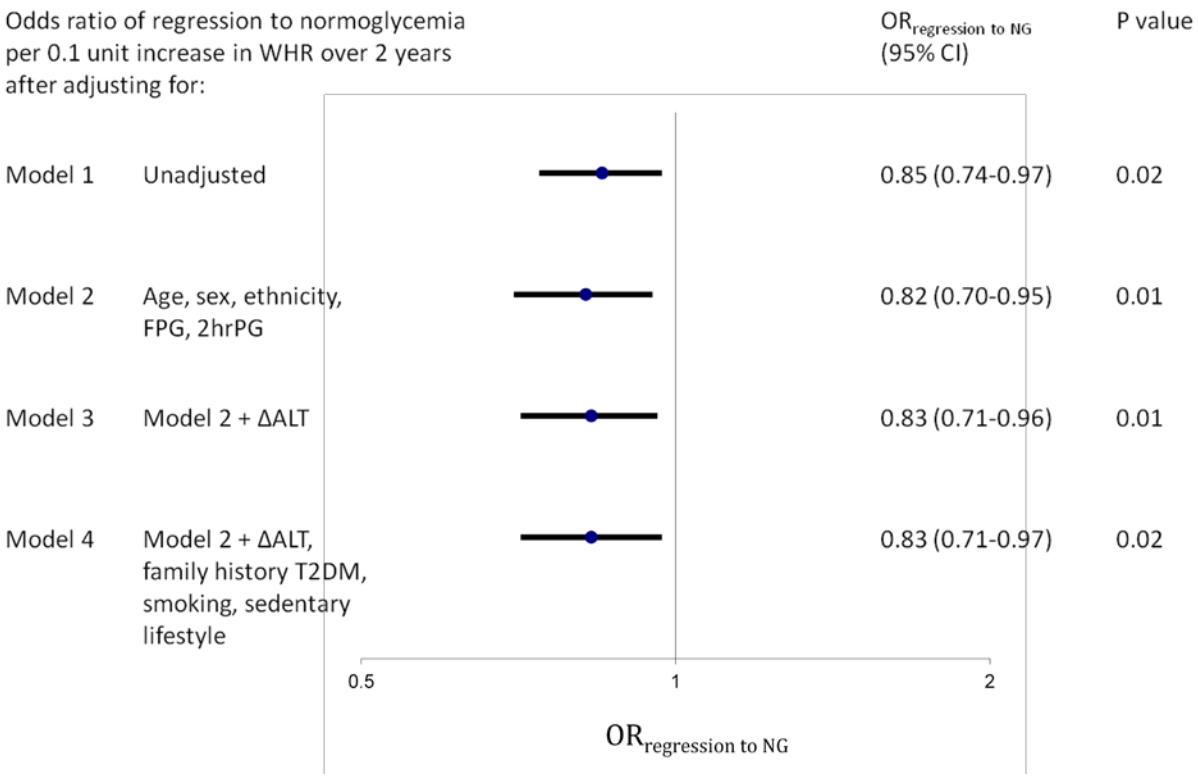
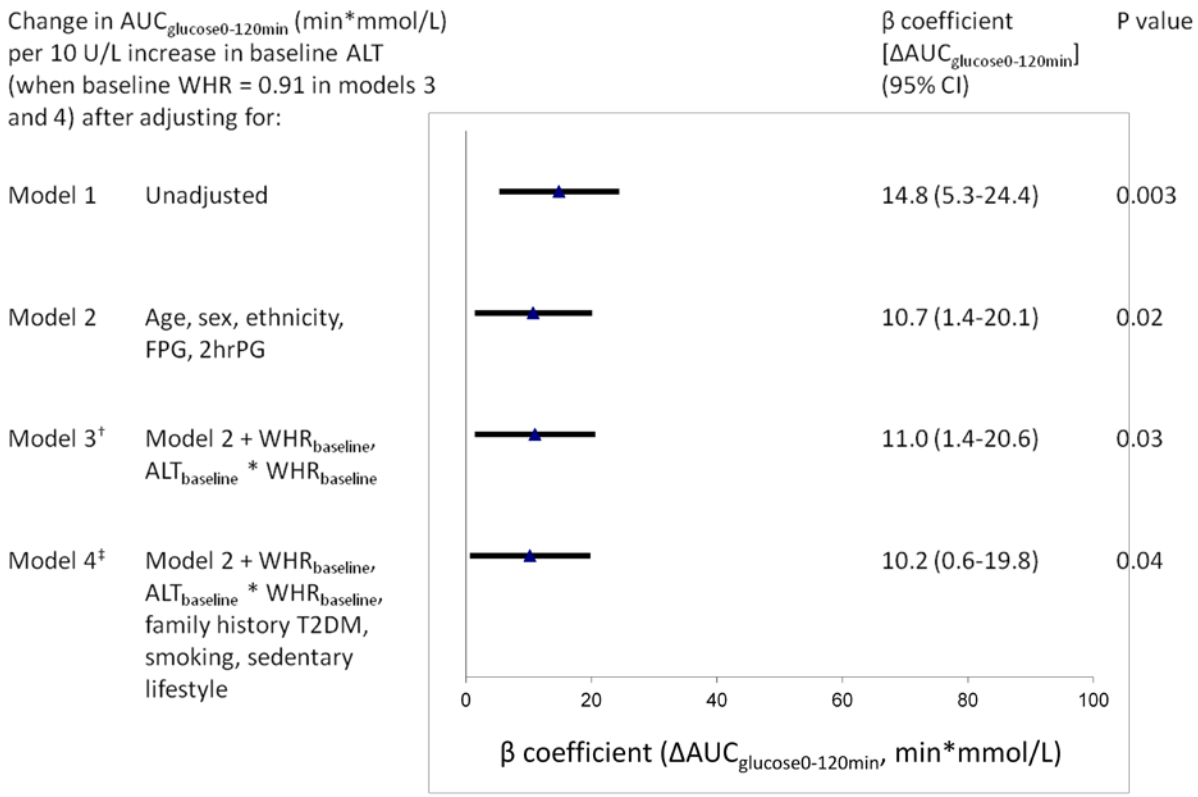
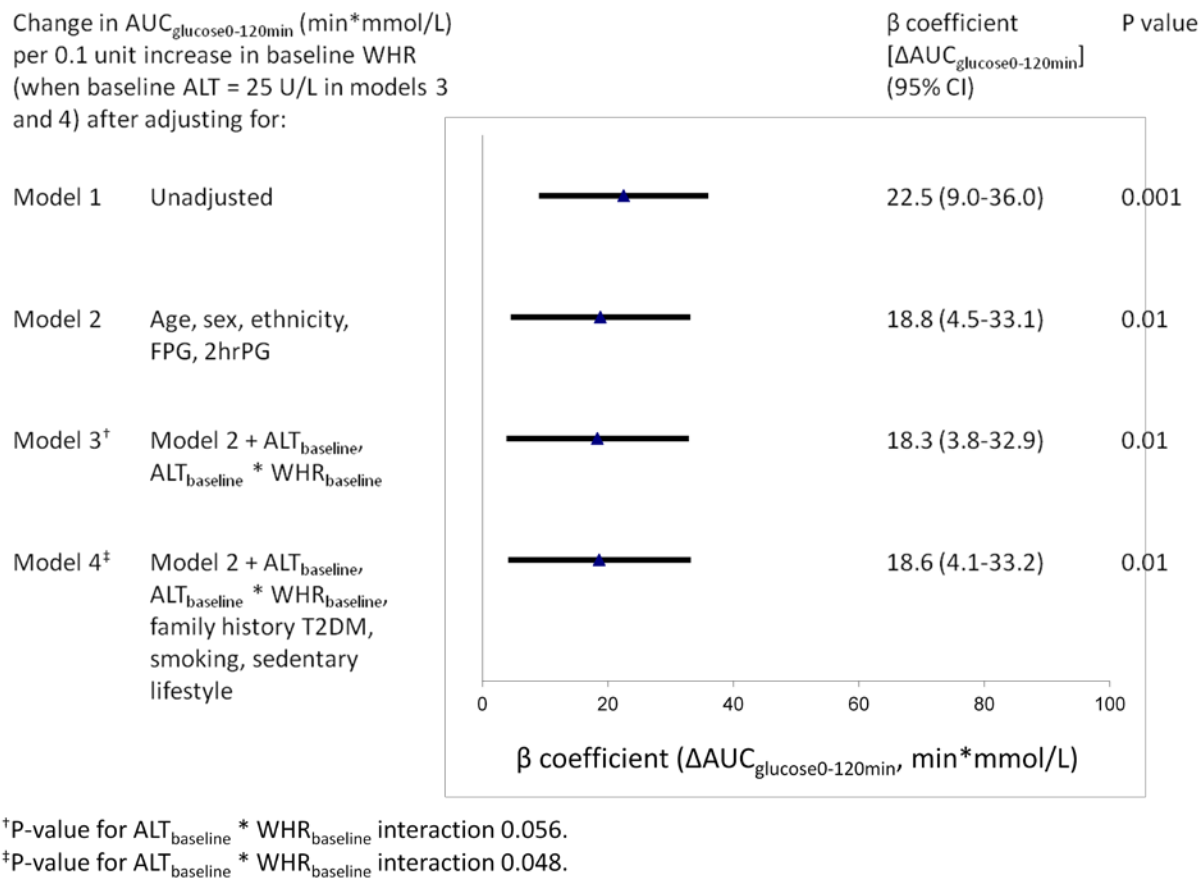


Figure 8. A relationship between a change in waist to hip ratio from baseline to 2 years and regression of prediabetes to normoglycemia 2 years after randomization. Odds ratios (95% confidence intervals) of regression to normoglycemia per 0.1 unit increase in waist to hip ratio from baseline are shown, unadjusted and adjusted for other covariates. ALT – alanine transaminase, ΔALT – a change in alanine transaminase from baseline to 1 year, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, NG – normoglycemia, OR – odds ratio, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



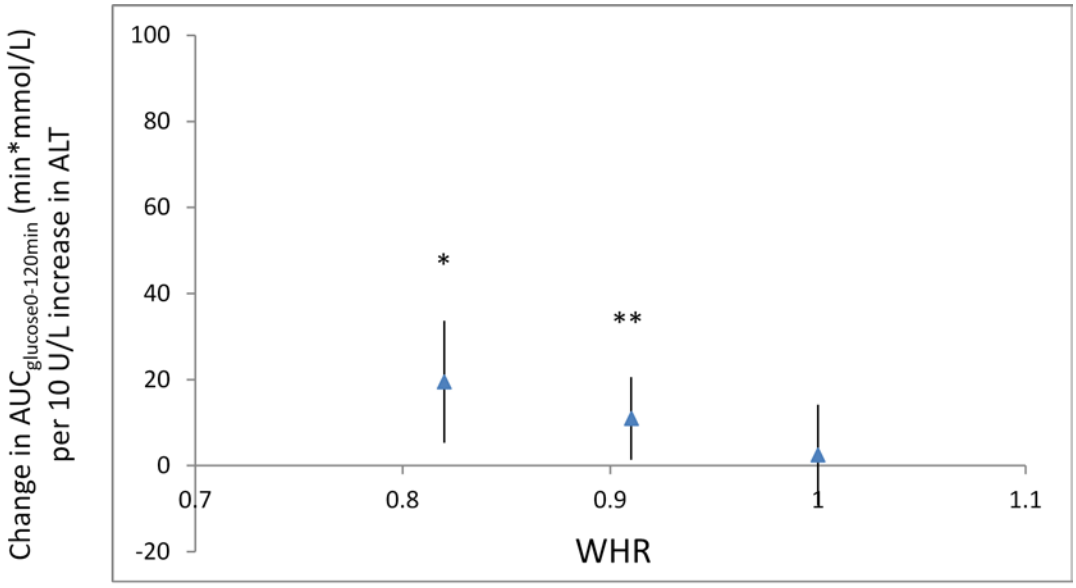
†P-value for $ALT_{baseline}$ * $WHR_{baseline}$ interaction 0.056.
‡P-value for $ALT_{baseline}$ * $WHR_{baseline}$ interaction 0.048.

Figure 9. A relationship between baseline ALT and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years later. β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 10 U/L increase in ALT, unadjusted and adjusted for other covariates. In models 3 and 4, an interaction between baseline ALT and waist to hip ratio was significant, and β-coefficients are reported per 10 U/L increase in ALT when waist to hip ratio is at the mean of 0.91. ALT – alanine transaminase, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



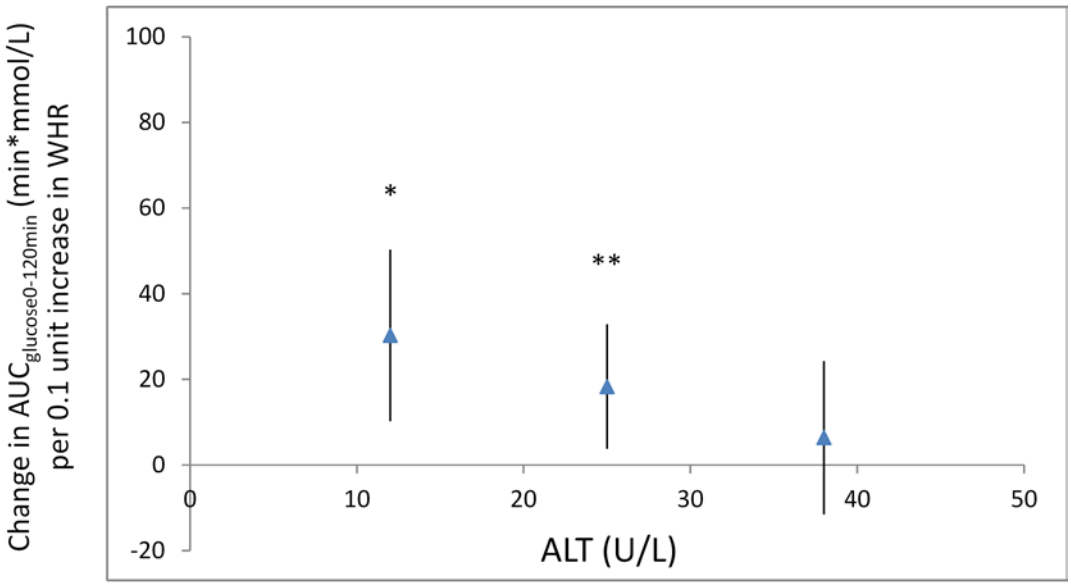
†P-value for $ALT_{baseline}$ * $WHR_{baseline}$ interaction 0.056.
‡P-value for $ALT_{baseline}$ * $WHR_{baseline}$ interaction 0.048.

Figure 10. A relationship between baseline waist to hip ratio and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years later. β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 0.1 unit increase in waist to hip ratio, unadjusted and adjusted for other covariates. In models 3 and 4, an interaction between baseline ALT and waist to hip ratio was significant, and β-coefficients are reported per 0.1 unit increase in waist to hip ratio when ALT is at the mean of 25 U/L. ALT – alanine transaminase, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



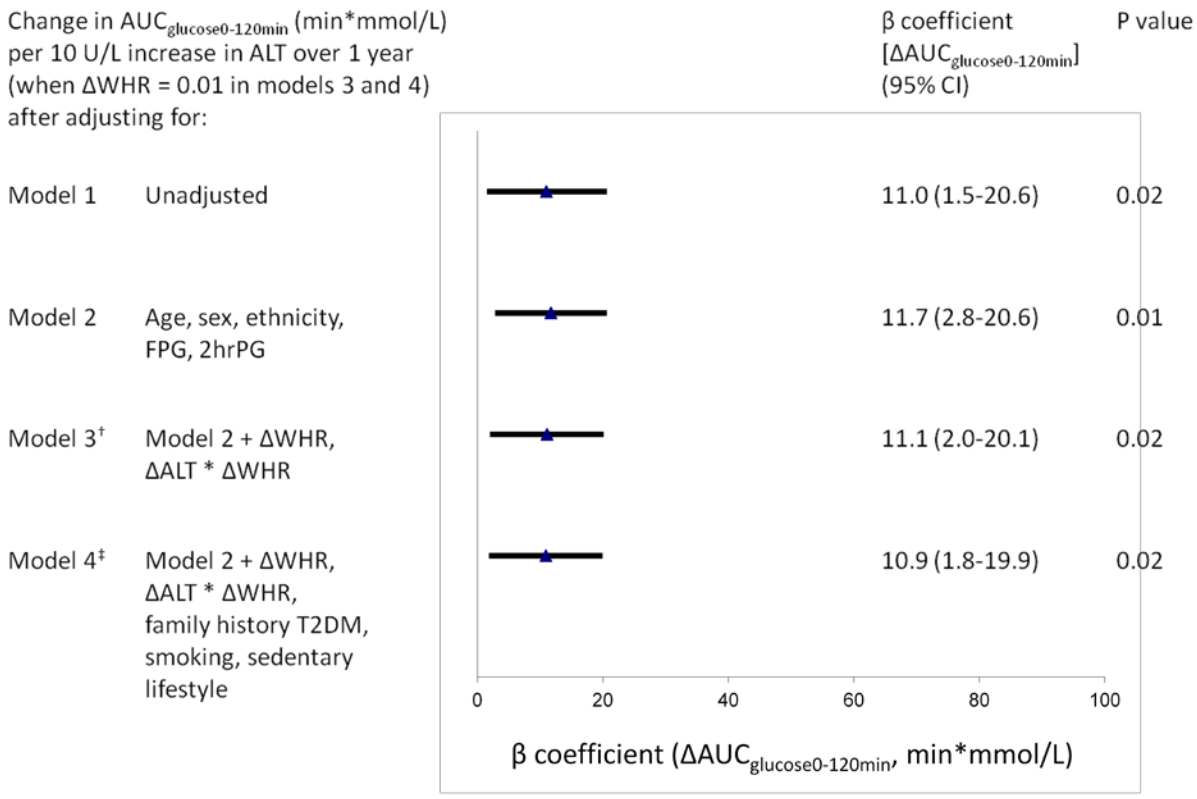
* P-value for change in $AUC_{glucose0\text{-}120min}$ 0.007.
** P-value for change in $AUC_{glucose0\text{-}120min}$ 0.03.

Figure 11. A relationship between baseline ALT and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years later at different levels of baseline waist to hip ratio (mean=0.91, mean - 1 standard deviation, mean + 1 standard deviation). β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 10 U/L increase in ALT after adjusting for age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, waist to hip ratio, and an interaction between ALT and waist to hip ratio (p-value for interaction 0.056). ALT – alanine transaminase, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



* P-value for change in $AUC_{glucose0\text{-}120min}$ 0.003.
** P-value for change in $AUC_{glucose0\text{-}120min}$ 0.01.

Figure 12. A relationship between baseline waist to hip ratio and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years later at different levels of baseline ALT (mean=25 U/L, mean-1SD, mean+1SD). β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 0.1 unit increase in waist to hip ratio after adjusting for age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, ALT, and an interaction between ALT and waist to hip ratio (p-value for interaction 0.056). ALT – alanine transaminase, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).



†P-value for ΔALT * ΔWHR interaction 0.046.
‡P-value for ΔALT * ΔWHR interaction 0.049.

Figure 13. A relationship between a change in ALT from baseline to 1 year and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years after randomization. β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 10 U/L increase in ALT from baseline, unadjusted and adjusted for other covariates. In models 3 and 4, an interaction between ΔALT and ΔWHR was significant, and β-coefficients are reported per 10 U/L increase in ALT when ΔWHR is at the mean of 0.01. ALT – alanine transaminase, ΔALT – a change in alanine transaminase from baseline to 1 year, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm), ΔWHR – a change in waist to hip ratio from baseline to 2 years.

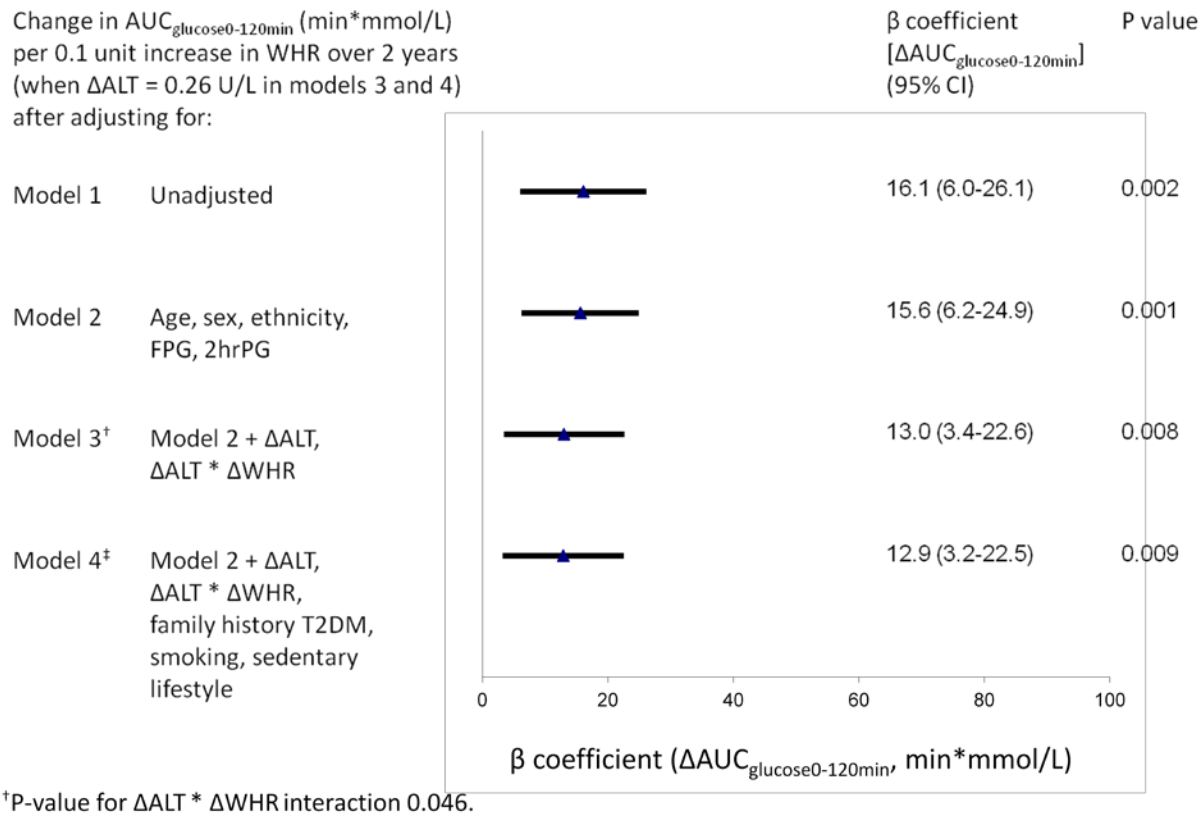
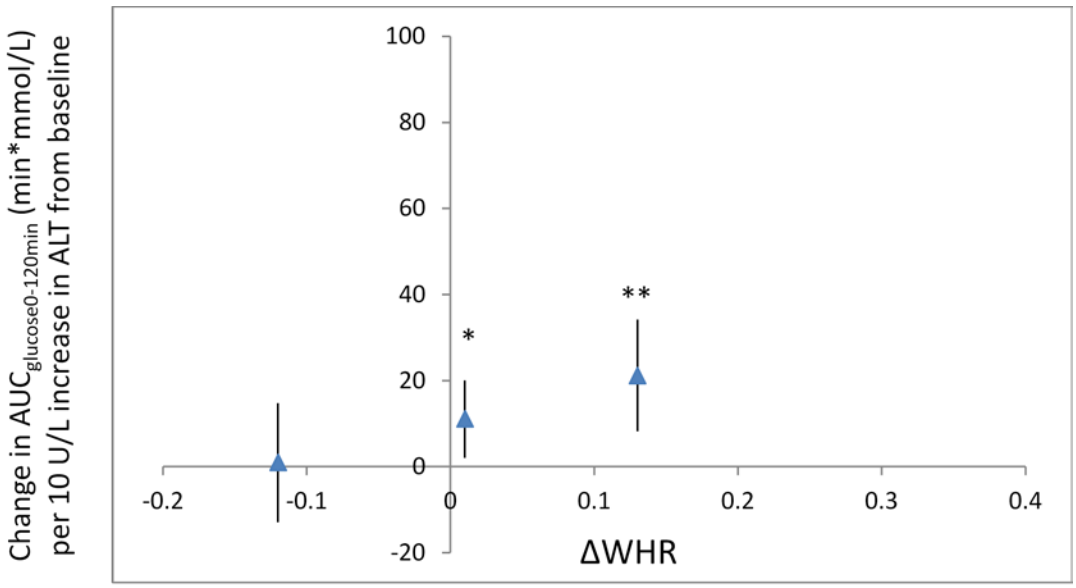
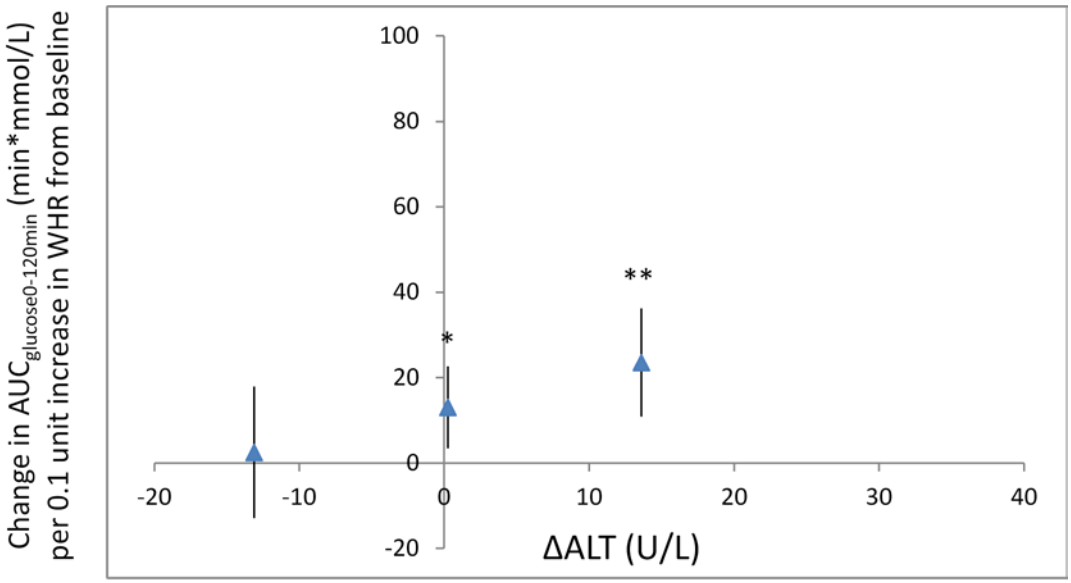


Figure 14. A relationship between a change in waist to hip ratio from baseline to 2 years and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years after randomization. β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 0.1 unit increase in waist to hip ratio from baseline, unadjusted and adjusted for other covariates. In models 3 and 4, an interaction between ΔALT and ΔWHR was significant, and β-coefficients are reported per 0.1 unit increase in WHR when ΔALT is at the mean of 0.26 U/L. ALT – alanine transaminase, ΔALT – a change in alanine transaminase from baseline to 1 year, FPG – fasting plasma glucose, 2hrPG – 2-hour plasma glucose from a baseline 75 g oral glucose tolerance test, T2DM – type 2 diabetes, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm), ΔWHR – a change in waist to hip ratio from baseline to 2 years.



* P-value for change in $AUC_{glucose0\text{-}120min}$ 0.02.
** P-value for change in $AUC_{glucose0\text{-}120min}$ 0.002.

Figure 15. A relationship between a change in ALT from baseline to 1 year and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years after randomization at different levels of a change in waist to hip ratio from baseline to 2 years (mean=0.01, mean - 1 standard deviation, mean + 1 standard deviation). β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 10 U/L increase in ALT from baseline after adjusting for age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, a change in waist to hip ratio, and an interaction between a change in ALT and a change in waist to hip ratio (p-value for interaction 0.046). ALT – alanine transaminase, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm), ΔWHR – a change in waist to hip ratio from baseline to 2 years.



* P-value for change in $AUC_{glucose0\text{-}120min}$ 0.008.
** P-value for change in $AUC_{glucose0\text{-}120min}$ 0.0003.

Figure 16. A relationship between a change in waist to hip ratio from baseline to 2 years and area-under-the-glucose-curve ($AUC_{glucose0\text{-}120min}$) from a 75 g oral glucose tolerance test 2 years after randomization at different levels of a change in ALT from baseline to 1 year (mean=0.26 U/L, mean-1SD, mean+1SD). β-coefficients (95% confidence intervals) represent a change in $AUC_{glucose0\text{-}120min}$ per 0.1 unit increase in waist to hip ratio from baseline after adjusting for age, sex, ethnicity, fasting plasma glucose, 2-hour plasma glucose, a change in ALT from baseline, and an interaction between a change in ALT and a change in waist to hip ratio (p-value for interaction 0.046). ΔALT – a change in alanine transaminase from baseline to 1 year, WHR – waist to hip ratio (waist circumference/hip circumference in cm/cm).

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