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Subphenotyping of women after gestational diabetes mellitus identifies subjects at high and low risk for progression to prediabetes/type 2 diabetes mellitus

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Erstes Gutachten:	Prof. Dr. med. Jochen Seißler
Zweites Gutachten:	Priv. Doz. Dr. med. Stefan Karrasch
Drittes Gutachten:	Prof. Dr. med. Cornelia Then
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Zusammenfassung

Einleitung: Die Heterogenität von Patienten mit Typ-2-Diabetes mellitus (T2DM) und Prädiabetes gewinnt zunehmend an Bedeutung für das therapeutische und langfristige Patientenmanagement. Frauen mit einer Vorgeschichte von Schwangerschaftsdiabetes mellitus (pGDM) haben unabhängig von ihrem Glukosestoffwechsel nach der Geburt ein hohes Risiko, einen Prädiabetes/T2DM zu entwickeln. Eine Stratifizierung dieser Frauen in Risikogruppen könnte die Nachsorge nach GDM verbessern. Diese Studie zielte darauf ab, pGDM-Subtypen zu definieren, die ein hohes oder niedriges Risiko für Prädiabetes/T2DM aufweisen.

Methodik: Wir analysierten 200 Frauen mit pGDM im ersten Jahr nach der Entbindung (Baseline) und erneut nach einer 5-Jahres-Nachbeobachtung (mit 150 verbleibenden Frauen), um die Probandinnen in Personen mit normalem Glukosestoffwechsel (NGM) und solche mit pathologischem Glukosestoffwechsel (PGM) zu unterteilen. Zur Bestimmung der Clusterparameter verwendeten wir anthropometrische Merkmale sowie die Ergebnisse des oralen Glukosetoleranztests (OGTT) zu beiden Zeitpunkten. Wir berechneten verschiedene Indizes der Insulinempfindlichkeit/-resistenz (Matsuda-Indizes, hepatischer Insulinresistenz-Index (HIRI), HOMA-S, HOMA-IR) und Indizes der Betazellfunktion (HOMA-B, insulinogener Index (IGI), oraler Dispositions-Index (DI), oraler Adaptations-Index (AI)) und wählten die prädiktivsten Parameter mittels logistischer Regressionsanalyse aus. Die Cut-off-Werte für die Cluster-Parameter wurden nach einer ROC-Analyse mit Hilfe des Youden-Index bestimmt. Die Signifikanz der identifizierten Cluster wurde anhand der Odds Ratio und eines 95%-Konfidenzintervalls analysiert.

Ergebnisse: In der Baseline-Analyse wiesen 37% der Frauen mit pGDM einen PGM (Prädiabetes/T2DM) auf. PGM war zu Beginn der Studie signifikant mit einem schlechterem Stoffwechselprofil (BMI, Taillen- und Hüftumfang, TGL und HDL), einem höheren Plasmaglukosespiegel zu allen Zeitpunkten des OGTT, höheren Werten der Insulinresistenz (ISI, HOMA-S, HOMA-2-S, HIRI, HOMA-IR, HOMA-2-IR) und einer reduzierten Betazellfunktion (DI und AI) verbunden. Nach

einer Nachbeobachtungszeit von fünf Jahren behielten die Probandinnen, die zu Beginn der Studie eine PGM aufwiesen, zu allen Zeitpunkten des OGTT einen höheren Plasmaglukosespiegel, höhere Werte der Insulinresistenz (ISI, HOMA-S, HOMA-IR) und eine verringerte Betazellfunktion (DI). Nach der auf der ROC-Analyse basierenden Clusterbildung mit Einteilung der Kohorte in der Baseline Untersuchung in BMI-Kategorien (< oder ≥ 25 kg/m²) und Insulinindex-Cut-off-Werten (HOMA-2-IR ≥ 1,7 und AI ≤ 23,1) ergaben sich drei signifikante Cluster für das Risiko der Entwicklung von Prädiabetes/T2DM. Frauen in Cluster 1 (Hochrisikocluster, definiert durch hohen HOMA-2-IR und niedrigen AI) hatten ein 5,6- bzw. 28,0-fach erhöhtes Risiko für PGM im Vergleich zu Cluster 2 (hoher HOMA-2-IR oder niedriger AI) und Cluster 3 (BMI < 25 kg/m² und normaler AI), unabhängig von Alter, BMI, Familienanamnese von T2DM, Parität und Rauchen.

Schlussfolgerung: In unserer Studie wurden drei Cluster für die Risikobewertung bei Frauen nach GDM identifiziert. Mit diesem Clustersystem kann ein besseres Screening-System und eine bessere Vorhersage für Prädiabetes/T2DM bei gesunden Frauen nach GDM erreicht werden.

Abstract

Introduction: Heterogeneity among patients presenting with type 2 diabetes mellitus (T2DM) and prediabetes has become increasingly significant for therapeutic and long-term patient management. Women with a history of gestational diabetes mellitus (pGDM), regardless of their post-delivery glucose metabolism, are at high risk of developing prediabetes/T2DM. Stratifying these women into risk groups could improve follow-up care after GDM. This study aimed to define pGDM subtypes that show high or low risk of prediabetes/T2DM.

Methodology: We analysed 200 pGDM women during the first year after delivery (baseline) and again after a 5-year follow-up (with 150 women remaining) to separate the subjects between having normal glucose metabolism (NGM) or having pathological glucose metabolism (PGM). To determine the clustering parameters, we used anthropometric characteristics as well as oral glucose tolerance test (OGTT) results from both time-points. We calculated various insulin sensitivity/resistance indices (Matsuda indices, hepatic insulin resistance index (HIRI), HOMA-S, HOMA-IR) and beta-cell function indices (HOMA-B, insulinogenic index (IGI), oral disposition index (DI), oral adaptation index (AI)), and selected the most predictive parameters using logistic regression analysis. The cut-off values for the clustering parameters were determined after ROC analysis using the Youden Index. The significance of the identified clusters was analysed using Odds Ratio and a 95% Confidence Interval.

Results: In the baseline analysis, 37% of pGDM women presented with PGM (prediabetes/T2DM). PGM at baseline was significantly associated with a worse metabolic profile (BMI, waist and hip circumference, TGL and HDL), higher plasma glucose at all time points during OGTT, higher levels of insulin resistance (ISI, HOMA-S, HOMA-2-S, HIRI, HOMA-IR, HOMA-2-IR) and reduced beta-cell function (DI and AI). After 5 years of follow-up, the subjects that had PGM at baseline maintained their higher plasma glucose at all time points during OGTT, higher levels of insulin resistance (ISI, HOMA-S, HOMA-IR) and reduced beta-cell function (DI). After the ROC-analysis based clustering

with the division of the cohort at baseline examination into BMI categories (< or ≥ 25 kg/m²) and insulin index cut-off values (HOMA-2-IR ≥ 1.7 and AI ≤ 23.1), three significant clusters emerged for the risk of developing prediabetes/T2DM. Women in cluster 1 (high-risk cluster defined by high HOMA-2-IR and low AI) had a 5.6-fold and 28.0-fold increased risk for PGM compared to cluster 2 (high HOMA-2-IR or low AI) and cluster 3 (BMI < 25 kg/m² and normal AI), regardless of age, BMI, family history of T2DM, parity, and smoking.

Conclusion: Three clusters were defined for the risk assessment in women after GDM. With this clustering system, a better screening system and a better prediction for prediabetes/T2DM in healthy women after GDM can be achieved.

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List of Abbreviations

- **5y** - 5 years
- **AI** - adaptation index
- **AUC** - area under the curve
- **BMI** - body-mass index
- **circ.** - circumference
- **C-Pep.** - C-peptide
- **CRP** - C-reactive protein
- **DI** - disposition index
- **Dia. BP** - diastolic blood pressure
- **FU** - follow-up
- **Gamma-GT** - gamma-glutamyl transferase
- **GDM** - gestational diabetes mellitus
- **GPT** - glutamic-pyruvic transaminase
- **HbA1c** - Glycated haemoglobin A1c
- **HDL** - high-density lipoprotein
- **HIRI** - hepatic insulin resistance index
- **HOMA** - homeostatic assessment model
- **HOMA-B** - HOMA (of) beta-cell secretion
- **HOMA-IR** - HOMA (of) insulin resistance
- **HOMA-S** - HOMA (of insulin) sensitivity
- **HR** - heart rate
- **IFG** - impaired fasting glucose
- **IGI** - insulinogenic index
- **IGT** - impaired glucose tolerance
- **INS** - insulin

- **IR** - insulin resistance
- **ISI** - insulin sensitivity index
- **LDL** - low-density lipoprotein
- **MetS** - metabolic syndrome
- **ML** - machine learning
- **NGM** - normal glucose metabolism
- **OGTT** - oral glucose tolerance test
- **pGDM** - post-GDM
- **PGM** - pathological glucose metabolism
- **ROC** - receiving operating characteristic
- **Sys. BP** - systolic blood pressure
- **T1DM** - type 1 diabetes mellitus
- **T2DM** - type 2 diabetes mellitus
- **TGL** - triglycerides
- **TSH** - thyroid stimulating hormone
- **WHR** - waist to hip ratio

Introduction

1.1 Diabetes Mellitus

Glucose metabolism refers to the process by which the body breaks down carbohydrates from food into glucose, which serves as the primary source of energy for cells [65]. In a healthy individual, glucose concentration is tightly regulated by hormones, mainly insulin and glucagon released from the pancreatic islet cells. Insulin acts as a key that unlocks the cells [70], allowing glucose to enter and be utilised for energy production and energy storage e.g. in the liver, muscle and adipose tissue. The amount of insulin released from beta cells and the sensitivity of the peripheral tissue cells to insulin are major component for glucoregulation to maintain constant glucose levels in the body and avoid hyperglycaemia. Diabetes mellitus is the generic term for chronic metabolic disorder characterised by elevated blood glucose levels (hyperglycaemia) [5], resulting from the body's impaired ability to produce sufficient amounts of insulin, from insulin insensitivity of target tissue cells or an impairment of both in various degrees.

The risk for the development of diabetes is dependent on genetic factors but is mainly influenced by the sum of environmental factors and lifestyle behaviours. Insulin resistance (IR) describes the body's cells becoming insensitive to the effects of insulin, meaning not responding adequately to the hormone. As a result, glucose uptake by the cells reduces, leading to high blood sugar levels. Factors such as obesity, chronic inflammation sedentary lifestyle, and genetics influence IR. Reduced beta cell function can be mediated by genetic predisposition, inflammation and a chronic exhaustion due to a high insulin demand in subjects with increased IR.

1.1.1 Classification

The German classification of diabetes mellitus is following the 2022 recommendations of the German Diabetes Society (Deutsche Diabetes Gesellschaft, DDG) [71].

Type 1 Diabetes Mellitus (T1DM)

Individuals with T1DM present a destruction of insulin producing beta cells, which leads to an absolute insulin deficiency and need for exogenous insulin therapy. The majority of T1DM is caused by autoimmune beta-cell destruction. Some cases are due to infections of the beta cells or are drug-induced (checkpoint-inhibitor-therapy associated diabetes).

Type 2 Diabetes Mellitus (T2DM)

In individuals with T2DM, the mechanisms that lead to impaired glucose metabolism (IGM) ranged from a strong IR with a relative insulin deficit to a complete impaired insulin secretion paired with IR [29]. Most of the time, this type of diabetes is associated with other diseases (hypertonia, obesity, impaired lipid metabolism, arteriosclerosis, COPD, obstructive sleep-apnoea-syndrome, depression and fatty liver disease).

Other Types of Diabetes Mellitus

- Diseases from the exocrine pancreas (i.e. pancreatitis, cystic fibrosis, haemochromatosis, pancreatic carcinoma, post-pancreatectomy)
- Endocrinopathy (i.e. Cushing-Syndrome, Acromegaly, Phaeochromocytoma)
- Drug induced (i.e. glucocorticoids, neuroleptics, alpha-interferon, pentamidine)
- Infection
- Rare forms of autoimmunological diabetes

- Genetic defects of beta cell function (i.e. MODY and other neonatal forms) or of insulin effect
- Other genetic syndromes linked to diabetes.

Gestational Diabetes Mellitus (GDM)

Glucose intolerance with onset or first recognition during pregnancy.

1.1.2 Diagnostic Criteria

The diagnosis of T2DM involves measurement of fasting or occasional venous blood glucose concentration or a metabolic challenge test and considering additional factors such as symptoms, medical history, and risk factors [4]. The following diagnostic criteria correspond to the recommendations of national and international diabetes associations (DDG, IDF, ADA, EASD) and the WHO.

Venous Plasma Glucose Measurement

- Occasional plasma glucose level of 200 milligrams per decilitre (mg/dl) or higher, or
- Fasting Plasma Glucose (FPG): A blood sample is taken after an overnight fast (typically 8 to 12 hours). A fasting plasma glucose level of 126 mg/dl or higher, or
- 75 g Oral Glucose Tolerance Test (OGTT): This test involves measuring blood glucose levels before and 2 hours after consuming 75g glucose in 250-300 ml water. A result of 200 mg/dl or higher indicates diabetes mellitus.

Glycated Haemoglobin A1c (HbA1c) Measurement

- Glycated haemoglobin A1c (HbA1c) Test: This blood test provides an estimate of average blood sugar levels over the past 2-3 months. An HbA1c level of 6.5% or higher indicates

diabetes mellitus.

Additionally, the DDG and American Diabetes Association (ADA)[4] defines prediabetes, or an abnormally high plasma glucose, with slightly lower OGTT results: FBG of 100-125 mg/dl, which is an impaired fasting glucose (IFG), 2h glucose tolerance (GT) of 140-199 mg/dl, which is an impaired glucose tolerance (IGT), and/or HbA1c 5.7-6.4%. Presenting a fasting plasma glucose of below 100 mg/dl does not exclude a manifest diabetes. In our study, we designate IFG, IGT and T2DM as pathological glucose metabolism (PGM), and considered normal fasting and normal OGTT values as normal glucose metabolism (NGM).

1.1.3 Epidemiology in Germany

According to the DDG health report of 2023 [36], in Germany in 2015, there were about 7 million people with documented T2DM and 32,000 children and adolescents, as well as 340,000 adults, with T1DM. Due to increasing prevalence, the number of people with documented T2DM in 2021 is estimated to be around 8.5 million, with an additional undisclosed number of cases, likely at least 2 million [95]. A further increase in diabetes prevalence is expected in the future, affecting both life expectancy and the number of healthy years, especially in younger and middle-aged groups.

The estimated frequency of diabetes is approximately 11-12% of the adult population. Prevalence increases significantly with age, and in 2019, it is estimated that 6.1 (women) and 7.7 (men) per 1000 total population of adults are newly diagnosed with diabetes each year. According to a modelling study, health-related taxes could potentially prevent around 600,000 prevalent cases of T2DM by 2040, but by this time it is estimated that around 11.5 million individuals will be diagnosed with T2DM. While the undisclosed cases of diabetes have decreased in recent years, there are still approximately 1.6 million undetected cases. Regional disparities in diabetes prevalence are linked to socio-economic factors and living conditions. Notably, the

eastern part of Germany maintains a higher diabetes prevalence compared to the western region. People with diabetes are living longer today, but their quality of life may be affected by health impairments. The prevention of health limitations is becoming increasingly important as life expectancy rises. Diabetes-related mortality, particularly in T2DM, is higher primarily due to cardiovascular complications, but the excess-mortality in people with diabetes linked to cardio-vascular risk compared to the general population diminished in the last decade.

In Germany, the average risk of developing type 2 diabetes has decreased, but a significant portion of the population still carries a considered ”high” risk. Prevention courses offered by statutory health insurance funds in the field of nutrition, crucial for individual behavioural prevention of type 2 diabetes, are scarcely utilised. Individuals with a high diabetes risk often underestimate this risk and perceive their own control possibilities as limited. Risk scores, such as the German-validated DRT test, can be utilised for determining and communicating the risk of developing the condition, forming the basis for individual behavioural prevention. Diabetes prevention is possible through dietary intervention, increased physical activity, and, in some cases, medications. Large randomised studies consistently demonstrate that a significant reduction in the risk of diabetes can be achieved, particularly through dietary changes and weight reduction.

In recent years, diabetic research has aimed to narrow down the target audience for specific diabetes prevention efforts, sometimes identifying those who would respond most effectively. However, despite these efforts, the number of cases continues to rise, and there is no trend towards successful prevention. Obesity, lack of physical activity, and poor nutrition are the primary avoidable risk factors for T2DM. Reducing these factors is the core focus of diabetes prevention. Allocating more resources to research effective lifestyle interventions and changing purchasing habits through taxes on unhealthy foods is necessary. It is crucial to elevate income levels to make healthier foods more affordable. Additionally, nutrition counselling deserves greater emphasis in the overall strategy.

1.1.4 Heterogeneity

Currently, the diagnosis of Type 2 diabetes mellitus relies on glucose or HbA1c testing, and once diagnosed, treatment is often standardised without considering individual differences. However, there is growing awareness that people with T2DM vary in factors like obesity, age, beta cell function, insulin sensitivity and genetics, leading to different responses to treatment. Efforts are being made to understand and categorize these variations through methods like clustering, which groups individuals based on specific characteristics. Precision medicine, tailoring treatments to individual characteristics, has been successful in certain types of diabetes, but applying it to T2DM, given its complexity, is a challenge.

One significant advancement in the field is the identification of subtypes within T2DM. Studies such as the Swedish All New Diabetics in Scania (ANDIS) have demonstrated that T2DM can be further categorised into subgroups by different underlying mechanisms [2]. The subgroups were defined based on several characteristics such as age at onset of diabetes, body-mass index (BMI), glycated haemoglobin percentage (HbA1c), homeostatic model assessment (HOMA)-2-B (as an beta-cells insulin secretion index), HOMA-2-IR (as an insulin resistance index), and glutamic acid decarboxylase autoantibodies (GADA) (as an indicator for autoimmune diabetes). Some studies also considered alternative characteristics suited for the analysis. To help make this alternative classification of DM more approachable for general practitioners (GP) and physicians outside of the university hospital context [82], they replaced C-peptide with other measures or calculations [7], added hyperuricemia [22] to the list, or used components of the metabolic syndrome (waist circumference, hypertension, dyslipidaemia with TGL and HDL) [73]. These subtypes include:

- Severe Insulin-Deficient Diabetes (SIDD): This subtype is characterised by a lack of insulin production and typically affects individuals with a younger age of onset. It shares some

similarities with classical T1DM but occurs in individuals who do not have autoantibodies associated with T1DM.

- Severe Insulin-Resistant Diabetes (SIRD): This subtype is associated with severe IR and usually affects individuals with obesity. IR refers to the reduced ability of cells to respond to insulin, leading to elevated blood sugar levels.

- Mild Obesity-Related Diabetes (MORD): This subtype primarily affects individuals with overweight or obesity but do not exhibit severe IR. It is typically diagnosed later in life and is associated with a milder form of the disease.

- Mild Age-Related Diabetes (MARD): This subtype occurs in older individuals and is characterised by a moderate increase in blood sugar levels. It is not strongly associated with obesity or IR.

- Severe Autoimmune-Related Diabetes (SARD): This subtype is characterised by severe insulin deficiency and an autoimmune component. It resembles T1DM but occurs in individuals without the traditional autoantibodies associated with it, typically presenting with rapid onset of severe hyperglycaemia and a need for insulin therapy.

The review of Misra and Wagner et al. [62] explores the evidence for classifying type 2 diabetes into subtypes. It examines both simpler methods, like using clinical features or biomarkers, and more complex approaches involving machine learning (ML) and genetic data. Their goal was to identify areas where further research is needed to develop accurate and cost-effective strategies for classifying T2DM, ultimately leading to better patient outcomes and more efficient healthcare resource allocation.

The text reports of 51 studies, with over 1.7 million participants that used simple classification methods to classify T2DM. Out of the 51 simple classification method studies, 43% analysed

exclusively subjects from non-white European ancestries, and 22% of the studies analysed populations presenting a newly onset T2DM. The studies looked at different aspects, such as glycaemia, clinical characteristics, progression to insulin treatment, and cardiovascular outcomes. Simple classification methods included using lipid profiles, BMI, pancreatic measures, age at diagnosis, OGTT data, and cardiovascular measures. For instance, triglycerides, LDL cholesterol, and HDL cholesterol were used to categorize type 2 diabetes in eight studies, with some focusing on cardiovascular outcomes. Islet autoantibodies were assessed in six studies, showing associations with insulin treatment and clinical characteristics. BMI was used in six studies, but results were inconsistent. The studies had varying levels of evidence quality, with 55% rated as low certainty and none achieving high certainty. Overall, the research aimed to identify effective and precise ways to classify type 2 diabetes subtypes for better patient outcomes and resource allocation.

Other 62 studies, with nearly 800,000 participants, used ML or genetic data approach to classify T2DM. Out of the 62 complex classification method studies, not a single study analysed exclusively participants from non-European ancestries, but more than half of them included these subjects in proportions >20%, and 30% of the studies analysed populations presenting a newly onset T2DM. Most studies were observational, and the primary ML approach was K-means clustering. Some studies used genetic data and Bayesian methods to identify clusters based on clinical and genetic features. The categorised subgroups included replicating known diabetes subtypes and exploring variations. Some studies used C-peptide and lipid traits, while others explored novel subgroups using advanced statistical methods. There were also studies using diverse clinical features, electronic health records, cardiovascular traits, and behavioural traits for classification. Additionally, two sets of papers used genomic data to identify subtypes based on genetic variation and gene expression. However, despite statistical significance, the observed genetic subtypes had limited clinical utility. Half of the studies had cross-sectional designs, and the other half involved prospective follow-up. About 70% of the studies had moderate evidence

certainty.

Interestingly, some variables used in complex ML approaches were also seen in simple approaches, supporting the biological validity of certain clustering variables. One study suggested that a quantitative analysis of single clinical measures might be more predictive of outcomes than the clustering approach. The review concludes by emphasizing the need for further research to determine the clinical benefits of assigning patients to specific clusters. Rigorous studies, including randomised controlled trials, are essential to establish whether knowledge of a patient's cluster membership significantly affects treatment decisions and clinical outcomes beyond current standards of care.

1.2 Gestational Diabetes Mellitus

Gestational Diabetes Mellitus (GDM) is a common pregnancy complication affecting an estimated 1 in 10 pregnancies worldwide, posing risks to both the mother and the baby [61]. Women with GDM have an increased risk of developing preeclampsia, caesarean section, and type 2 diabetes later in life [74]. Babies born to mothers with GDM are at a higher risk of macrosomia (excessive birth weight), birth injuries, hypoglycaemia after birth, and an increased likelihood of developing obesity and T2DM in the future [17].

According to the DDG recommendations [17], starting January 2012, all public insurances covered pregnant women should get a 50 g glucose "screening-test" between their 24 + 0 and 27 + 6 week of pregnancy. The test can be done without a fast and at any time of the day. Subjects with a 1h venous plasma glucose measurement of 15 to 200 mg/dl are considered as positive to the screening, and will need a standardised 75 g OGTT to consolidate the diagnosis. A fasting plasma glucose $\geq$ 92 mg/dl, a one-hour plasma glucose $\geq$ 180 mg/dl, or a two-hour plasma glucose $\geq$ 153 mg/dl are the diagnostic criteria for the diagnosis GDM (IADPSG criteria).

Tight glycaemic control during pregnancy is crucial for reducing the risks associated with GDM. Lifestyle modifications, including a healthy diet and regular physical activity, are often recommended as the first line of treatment [17]. In some cases, insulin therapy may be necessary to maintain optimal blood sugar levels. Recent research emphasizes the importance of postpartum follow-up for women with GDM [52, 69]. In the majority of women, GDM is transient and returns to normal glucose metabolism after delivery (about 70%). 20-30% have disturbed glucose tolerance post-GDM (pGDM). Overall, women with a history of GDM have an up to 10-fold increased risk for future development of type 2 diabetes and represent therefore a unique population to identify early metabolic changes in a normoglycaemic period associated with later development of the disease and provide the opportunity to develop strategies for the prevention of prediabetes and overt T2DM.

Women pGDM should be screened for diabetes within 6 to 12 weeks after delivery and regularly thereafter. Lifestyle modifications, such as healthy eating, physical activity, and weight management, remain important to reduce the risk of developing type 2 diabetes in the future. Ongoing research is exploring various aspects of GDM, including the underlying mechanisms of the condition, genetic factors, and novel therapeutic approaches. Additionally, studies are investigating the long-term health outcomes of women with a history of GDM and their offspring to understand better the potential intergenerational impacts.

1.2.1 Epidemiology of Gestational Diabetes Mellitus

In 2018, a total of 51,318 women who gave birth in German clinics were diagnosed with GDM (7.3%) [76]. This percentage has steadily increased from 4.6% in 2013 to 6.8% in 2018. The prevalence of GDM significantly rises with the age of the mothers at birth: while 2.5% of women under 20 years were affected in 2018, the frequency rises to 15.9% for women aged 45 years and older. Differences in prevalence were also observed between German states. The prevalence of

GDM is below 6% in Bavaria, Hamburg, and Schleswig-Holstein, while it exceeds 9% in Berlin, Rhineland-Palatinate, and Saarland. Several risk factors have been identified, including advanced maternal age, family history of diabetes, pre-pregnancy overweight or obesity, and certain ethnic backgrounds (such as South Asian, Hispanic, or African descent). The increasing prevalence of GDM can be influenced by some of them. Firstly, the average age of mothers at birth and the frequency of obesity, which are risk factors for GDM, have increased [45, 18]. Additionally, in 2012, the guidelines for GDM were revised, and screening became a covered health insurance benefit, leading to increased diagnosis and documentation [60, 90]. Studies based on other data sources report higher estimates for GDM, highlighting the need for studies to improve data quality, such as examining possible gaps in documentation.

1.2.2 High-risk Diabetes Care Parameters in Different Settings

The monitoring and care provided in different settings play a crucial role in identifying and preventing the progression of abnormal glucose metabolism after a GDM [17]. In the outpatient setting, regular check-ups with a general practitioner or a specialised diabetologist are common. These check-ups focus on routine assessments such as blood glucose monitoring, OGTT, HbA1c testing, BMI evaluation, and discussions about lifestyle modifications to minimize the risk of diabetes. The emphasis is on early detection and intervention. On the other hand, in research-focused settings, the check-up routines may vary. They may involve more comprehensive assessments, including advanced laboratory tests, genetic screenings, and specialised investigations to explore the mechanisms and predictors of diabetes development. These research settings aim to gain a deeper understanding of the pathophysiology and risk factors associated with GDM and its progression to diabetes. By tailoring the check-up routines to the specific setting, healthcare providers can effectively monitor women pGDM, identify early signs of abnormal glucose metabolism, and provide appropriate interventions to prevent or delay the onset of prediabetes or T2DM.

Clinical Parameters

There are several clinical parameters that can be used to monitor the risk of diabetes in women pGDM after childbirth. Here are some key parameters:

- Body Mass Index (BMI): BMI is a measure of body fat based on height and weight. Women with a higher BMI, especially those in the overweight or obesity range, are at a greater risk of developing diabetes.

- Waist Circumference: Excess abdominal fat is a known risk factor for diabetes. Measuring waist circumference helps assess central obesity, which is particularly relevant to diabetes risk.

- Blood Pressure: Hypertension (high blood pressure) is often associated with diabetes. Regular monitoring of blood pressure can help identify individuals at risk and manage hypertension, which is crucial for diabetes prevention.

- Physical Examination: A comprehensive physical examination can reveal signs associated with diabetes risk, such as acanthosis nigricans (darkened skin patches) often observed in severe IR.

- Postpartum weight retention: The amount of weight a woman retains after giving birth can be an indicator of increased diabetes risk. Women who retain excessive weight may have a higher likelihood of developing type 2 diabetes.

- Family history: A family history of diabetes, especially in first-degree relatives, can increase the risk of developing diabetes. It is essential to consider the genetic predisposition when assessing the risk in pGDM women.

- Age and Ethnicity: Age and ethnicity are significant risk factors for diabetes. Advanced age and certain ethnicities, such as African Americans, Hispanic/Latino Americans, Native

Americans, and Asian Americans, have a higher predisposition to developing diabetes.

- Symptomatology: Recognizing common diabetes symptoms like frequent urination, excessive thirst, unexplained weight loss, increased hunger, fatigue, and blurred vision can prompt further evaluation and monitoring for diabetes risk.

- Cardiovascular Risk Factors: Diabetes is closely linked to cardiovascular disease. Assessing parameters such as lipid profile, smoking history, and family history of cardiovascular disease can aid in identifying individuals at higher risk for diabetes.

- Lifestyle Factors: Assessing lifestyle factors, including sedentary behaviour, poor dietary habits, and tobacco use, helps determine modifiable risk factors that contribute to the development of diabetes.

Laboratory Parameters

There are several laboratory parameters that can be used to monitor the risk of diabetes in women pGDM after childbirth. Here are some key parameters:

- Fasting Plasma Glucose (FPG): FPG is a simple blood test that measures the amount of glucose in the blood after an overnight fast. Elevated fasting blood glucose levels may indicate impaired glucose metabolism.

- 75 g Oral Glucose Tolerance Test (OGTT): OGTT measures blood glucose levels after fasting and again two hours after consuming a glucose drink. It can identify impaired glucose tolerance or diabetes that may not be detected by a fasting blood glucose test alone.

- Glycated Haemoglobin (HbA1c): HbA1c is a blood test that provides an average blood glucose level over the past two to three months. Slightly elevated HbA1c levels may indicate worsening of glucose control and prediabetes.

- Insulin levels: Insulin levels in relation to glucose can be measured to evaluate IR, a common precursor to T2DM. Elevated fasting or postprandial insulin levels may indicate IR and an increased risk of developing diabetes.

- C-peptide levels: C-peptide is produced during the formation of insulin in the pancreas. Measuring C-peptide levels can provide insight into insulin production and secretion. Abnormal levels may indicate impaired insulin secretion and an increased risk of diabetes.

- Lipid Profile: A lipid profile measures various blood lipids, including total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. Abnormal lipid levels, such as elevated triglycerides or reduced HDL cholesterol, are associated with an increased risk of diabetes.

- High-Sensitivity C-Reactive Protein (hs-CRP): hs-CRP is a marker of subclinical inflammation. Elevated levels of hs-CRP have been linked to IR and an increased risk of developing type 2 diabetes.

- Kidney Function Tests: Diabetes can affect kidney function, so tests such as serum creatinine and estimated glomerular filtration rate (eGFR) are important to assess renal health and identify any signs of diabetic nephropathy.

- Liver Function Tests: Diabetes is associated with an increased risk of fatty liver disease. Ultrasound examination, Liver function tests, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), can help evaluate liver health.

Monitoring these parameters at regular intervals following GDM can help identify women who are at a higher risk of developing diabetes and facilitate early intervention, such as lifestyle modifications, close monitoring, and potential pharmacological interventions, to prevent or delay the onset of T2DM. It is recommended that pGDM women undergo regular monitoring (e.g. annual OGTT) after delivery to detect and manage any potential progression towards diabetes.

1.2.3 Factors Influencing the Risk of Diabetes Mellitus

Obesity and Metabolic Syndrome

In Germany, just about two third of men (60%) and half the women (47%) present overweight, and 19% of the adult population presents obesity [84].

As definition of metabolic syndrome (MetS), we used in the present study the 2005 revision of the National Cholesterol Education Program (NCEP) Adult Treatment Panel 3 (ATP 3) definition of it [38]. Subjects presenting three or more characteristics from the list below were diagnosed a metabolic syndrome.

- Waist circumference $\geq$ 88 cm
- Blood pressure $\geq$ 130/85 mmHg
- Triglycerides $\geq$ 150 mg/dl
- HDL-Cholesterol < 50 mg/dl
- Pathological glucose metabolism: presenting either an IFG, or an IGT, or both.

Metabolic syndrome serves as a collection of risk factors that predispose individuals to increased risk for the development of T2DM. These mechanisms include:

- Obesity and inflammation: The central obesity described in the definition of metabolic syndrome in associated with chronic low-grade inflammation, which in turn contribute to IR and impairs glucose metabolism.
- Hypertension: The damages caused by the high blood pressure in blood vessels and the impaired blood flow affect the delivery of nutrients and oxygen to tissues, including insulin-sensitive tissues. This can further contribute to IR.

- Dyslipidaemia: Common features of metabolic syndrome, such as elevated triglyceride levels and low HDL cholesterol levels, can contribute to IR and impaired glucose metabolism.
- Insulin resistance: Many people with metabolic syndrome experience IR as cells become less responsive to insulin's effects, for reasons named above. This is a key underlying factor in the development of T2DM.
- Pancreatic dysfunction: The increased demand for insulin due to IR can strain the pancreatic beta cells over time, and eventually leads to decreased insulin secretion as well. All of it leading to the development of T2DM.

Selected Adipokines Associated with Diabetes Mellitus/Insulin Resistance

Adipose tissue is a very active endocrine organ secreting hundreds of polypetides that regulate adipocytes, pancreas, liver, muscle and other organs [49]. Some adipokines have anti-inflammatory effects (e.g. adiponectin, omentin-1), others are categorised as pro-inflammatory (e.g. leptin, resistin) which exacerbate IR and increase risk for diabetes. Moreover, hyperleptinemia, hyperresistinemia were observed during the first trimester of women that develop GDM, and could serve as predictive marker for GDM [6]. In the present study adiponectin, leptin and resistin were selected for analysis in the PPS-Diab cohort.

Adiponectin is a 30 kDa protein hormone mainly produced by white adipose tissue and, to a lesser extent, by other tissues such as the liver and placenta, which has anti-diabetic, anti-inflammatory, and anti-atherogenic properties [87]. Several studies also reported adiponectin to correlate negatively with T2DM, and proper healthy nutrition has proven to significantly increase its circulating levels. It promotes insulin sensitivity in the brain and the muscle, as well as controls glucose and lipid metabolism in the liver [54]. As a known indicator of T2DM in adults, adiponectin showed association with future abnormal glucose tolerance in both genders, especially its low levels as a predictor of T2DM in women [9]. Another study on rodents presented that a genetic

connection between adiponectin and T2DM could be observed as well, found in the expression of different adiponectin expressing alleles, thus making it a T2DM susceptibility gene. These findings emphasise that adiponectin may participate in the pathogenesis of T2DM, by regulating blood glucose and lipid metabolism [91]. In another study specifically analysing pregnant women with GDM, mean adiponectin levels in GDM subjects was significantly lower than in controls and the risk of GDM decreased in women with increasing adiponectin levels. The increase of circulating adiponectin during the first two trimesters of a pregnancy decreased the risk of GDM in women. Therefore, adiponectin has been recognised as a potential early biomarker for screening or predicting GDM [30].

Resistin is also an adipokine and an influential factor, interfering with insulin and glucose metabolism. Even though this pro-inflammatory adipokine is exclusively expressed in adipose tissue and adipocytes in rodents, human resistin is mainly produced by macrophages and monocytes. Subjects with obesity tend to present an increased circulating resisting level, since it is associated with body fat storage [94]. The transcription of the resistin gene is associated with inflammation, IR, and overall T2DM development. Increased resistin gene expression lead to the inhibition of insulin effect, therefore increases IR. Obesity plays a role in the increased secretion of resistin [20]. During pregnancy, the resistin and CRP levels were significantly higher in GDM women than in healthy women, underlining the possibility that resistin plays a role in glucose metabolism. The same study concluded that not only IR, but more complex pathophysiology can lead to GDM [92].

Leptin, sometimes called “satiety hormone”, is a 167 amino acids long digestive hormone [64]. This hormone is mostly known for its hunger and fat reserves regulation in the body and is predominantly secreted by the white adipose tissue and enterocytes. The hypothalamus, an important site for the regulation of energy homeostasis and neuroendocrine function, expresses strongly the long form of the leptin receptor, which activates various signal transduction pathways

[50]. The regulation of energy homeostasis, of both food intake and glucose homeostasis, and of immune responses are some of them [93, 41, 66]. The regulation of glucose metabolism by leptin is achieved by direct and centrally mediated actions on various tissues. For example, leptin directly decreases insulin signalling, synthesis and secretion in white adipose tissues, in the liver and in the pancreas. It also improves gluconeogenesis in the liver and glucose uptake in skeletal muscles and brown adipose tissues through the centrally mediated actions [16]. The observations have been made with mice models. In a study based with the same population as the one used for our research, a significant correlation was found between the beta cell function decrease or the insulin sensitivity decrease in women with both pGDM and an IGT and a higher risk in developing a T2DM [79]. This association was observed with plasma leptin as the most important predictive factor, together with BMI, triglycerides, and waist circumference, whereas the association with ectopic fat (liver, muscle and visceral fat), using a study with MR-imaging and spectrographic analysis in a subgroup analysis, did not show a BMI-independent association to low insulin sensitivity [27].

The PPS-Diab Cohort

The prospective Study Prediction, Prevention and Subclassification of Type 2 Diabetes (PPS-Diab) aimed to follow-up subjects from a very early, normoglycemic state to prediabetes and overt diabetes. To do this, women after GDM and women with a normal OGTT during pregnancy were selected who were deeply phenotyped using metabolic tests, biomarker measurements and MRI imaging. In the PPS-Diab study it was described that:

- pGDM women have significantly higher risk for prediabetes but glucose tolerance is dynamic and a single post-partum OGTT is insufficient for accurate long-term risk prediction [39]
- GDM history is related to lower physical fitness and higher leptin levels [32]
- Individuals with 10,000 steps/day showed lower insulin resistance, independent of BMI [32]
- Glucose tolerance correlated with BMI, blood pressure, visceral fat, and depression [23]
- Functional measurements are more effective than morphologic for assessing muscular contribution to insulin sensitivity [99]
- Sleep quality influences glucose tolerance and insulin sensitivity and perceived stress is a risk factor for impaired sleep quality [24]
- Exercise positively impacts insulin sensitivity, and triggers hormone release for stress response in women with overweight [33]
- Hormonal axes (leptin, hGH/IGF-1, fetuin-A, glucagon) are linked to insulin resistance [88, 35, 51, 75]
- There is a three-way association between liver fat, glucagon-alanine index, and insulin resistance [31]

- Serum amino acids are associated with metabolic syndrome and specific amino acids are weakly linked to glucose tolerance [34]
- The microbiome is associated with T2DM risk (prevotellaceae-dominated microbiome) [28]

5-year follow-up investigation of women with previous GDM and controls now allows to investigate whether it is possible to identify some subtypes of pGDM subjects at very high or low risk for progression to prediabetes or overt T2DM. This would have important impact on the long-term primary care of these patients after delivery and guideline recommendations.

Aim and Objectives

The present study aims to investigate the subclassification of women with a history of GDM into categories that assess the risk of developing impaired glucose tolerance and T2DM. By following these patients over 5 years, we seek to identify a cluster of phenotype variables and biomarkers that allow us to identify subtypes at high and low risk for rapid progression to prediabetes/T2DM.

3.1 Primary objectives

The primary objectives of our study are as follows:

A. Is it possible to divide subjects with previous GDM into specific subgroups by clinical criteria?

B. Can we define clusters based on parameters for glucose metabolism during OGTT? Can we organise two clustering methods, one for outpatient care in the setting of routine care vs. research-oriented university care?

C. What additional biomarkers can enhance the clustering analysis of our cohort?

3.2 Secondary objectives

The secondary objectives of this study are:

a. What are the differences in glucose tolerance at baseline and after 5 years of follow-up in our cohort?

b. Which parameter or index is most appropriate to describe insulin resistance, beta-cell function and insulin secretion in our cohort?

c. What are the cut-off values that distinguish between different categories of insulin resistance, beta cell function and insulin secretion related to progression to dysglycaemia?

By addressing these objectives, this thesis endeavours to contribute to a deeper understanding of the heterogeneity among individuals with previous GDM. Subclassification may contribute to novel strategies for post GDM management and provide valuable insights into the parameters and predictors relevant to glucose metabolism, which are prerequisites for pathogenetically defined treatments.

Materials and methods

4.1 Study population

The participants in this analysis were taken from the prospective, monocentre observational study Prediction, Prevention and Subclassification of Type 2 Diabetes (PPS-Diab). The group under investigation consisted of two cohorts: women who experienced gestational diabetes mellitus (GDM) during their most recent pregnancy (referred to as pGDM), and women who had a normal 75-g oral glucose tolerance test (OGTT) or a normal 50-g screening OGTT (with plasma glucose levels < 135 mg/dL) after the 23rd week of gestation (serving as control subjects).

The diagnosis of GDM was established through a 75-g OGTT conducted after the 23rd week of gestation. The criteria for defining GDM were set at plasma glucose levels of fasting 92 mg/dl, 1 hour 180 mg/dl, 2 hours 153 mg/dl, following the recommendations outlined by the International Association of the Diabetes and Pregnancy Study (IADPSG)[81]. A single participant was found to have diabetes during pregnancy according to IADPSG standards (fasting plasma glucose $\geq$ 126 mg/dL). Because her blood sugar levels returned to normal after pregnancy she was not excluded from the analysis.

The exclusion criteria for this study encompassed alcohol or substance abuse, as well as chronic conditions necessitating medication (except for hypothyroidism (n=52), bronchial asthma (n=8), hypertension (n=4), gastro-oesophageal reflux (n=2) and history of pulmonary embolism resulting in rivaroxaban prophylaxis (n=1)). Written informed consent was obtained from all study participants. The study protocol was approved by the ethics review committee of the Ludwig-Maximilians-Universität.

The baseline visit of the PPS-Diab Study took place within 3 to 16 months after delivery. There was no difference in time to index pregnancy in pGDM women and controls. Subsequently, the 5-year follow-up session was scheduled for a timeframe spanning 58 to 66 months after childbirth. In cases where there was an overlap with an additional pregnancy or the early postpartum phase (within 6 months postpartum) during this period, or due to personal reasons or intermittent illness (observed in 47 instances; median [Q1 : Q3] = 70 [68 : 74] months postpartum), this follow-up visit was rescheduled. During the span between the baseline and the five-year follow-up, participants belonging to the pGDM group attended annual in-person appointments involving an OGTT, while those in the control group underwent yearly phone interviews. Study participation ended in woman diagnosed with overt diabetes mellitus. For the remaining participants, the PPS-Diab study is still ongoing.

4.2 Methods description

4.2.1 Medical history

Medical history was collected using questionnaires. The data provided by the participants themselves underwent thorough scrutiny by a study physician. In cases where there were any discrepancies or uncertainties, necessary adjustments were made after discussing the matter with the participant.

4.2.2 Anthropometric measurements

Body weight was measured using a bioelectrical impedance analysis (BIA) scale (Tanita BC-418, Tanita Corporation, Tokyo, Japan). Body-mass index (BMI) was calculated as weight in kilograms divided by height squared in metres [67]. We measured hip and waist circumference and height to the nearest centimetre using a tape and calculated waist-to-hip ratio as waist

circumference divided by hip circumference, both in centimetres. Systolic and diastolic blood pressure readings were obtained from all subjects in a sitting position (both arms, repeated measurements, average from the ”higher” arm recorded). A detailed description of the visits has been described previously [24].

4.2.3 Laboratory measurements

After an overnight fast, a five-point 75-g OGTT was conducted with blood samples drawn at regular 30-minute intervals from 0 min until 120 min after the ingestion of glucose. Blood was collected without stasis and immediately processed. Plasma and serum samples were stored at -80°C until assay. We used the hexokinase-glucose-6-phosphate dehydrogenase method with collection tubes containing inhibitors of glycolysis to measure plasma glucose (Glucose HK Gen. 3, Roche Diagnostics, Mannheim, Germany).

Plasma insulin and C-peptide concentrations were determined using DiaSorin LIAISON® Insulin and C-peptide assays, and plasma HbA1c with high-performance liquid chromatography (VARIANT II TURBO HbA1c Kit, Bio-Rad Laboratories, Hercules, USA). C-peptide is released from the beta cells of the pancreas in a one-to-one ratio with insulin [58]. C-peptide is cleared from the circulation at a slower (negligible hepatic clearance) and more constant rate compared to insulin. It is widely used to assess beta cell function. C-peptide measurements were available from only 128 subjects.

Blood lipids (high-density lipoprotein (HDL)-cholesterol and triglycerides) were quantified by enzymatic calorimetric test (Roche Diagnostics, Mannheim Germany). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation [26].

Plasma leptin was measured by ELISA ”Dual Range” (Merck Millipore, Darmstadt, Germany), plasma adiponectin was measured by radioimmunoassay (RIA) (Merck Millipore, Darmstadt,

Germany), and plasma resistin was measured by Quantikine ELISA (R&D Systems, Wiesbaden-Nordenstadt, Germany).

4.2.4 Fasting and OGTT based indices of glucose clearance, insulin resistance or sensitivity and insulin secretion

Several parameters obtained under fasting conditions and during OGTT were used to estimate insulin resistance and insulin secretion.

The AUC (Area Under the Curve) of an OGTT is a quantitative measure that summarises the overall response of blood glucose levels to a glucose load over the specific period of the test. The AUC of the OGTT curve represents the total area under the plotted graph of blood glucose levels over time. This value provides valuable information about how efficiently the body is handling glucose. A higher AUC typically indicates poorer glucose control and may suggest underlying metabolic issues. The AUC of plasma glucose during the 75 g OGTT, in mg.h/dl, was calculated using the trapezoidal approximation [83]. We used the same formula for the AUC of plasma insulin (in µIU.h/dl) and plasma C-peptide (in ng.h/ml). (PG AUC: Area under the curve of plasma glucose, and PG levels at x min as PG(x))

$$\text{PG AUC (mg.h/dl)} = \frac{\text{PG}(0) + 2 \cdot \text{PG}(30) + 2 \cdot \text{PG}(60) + 2 \cdot \text{PG}(90) + \text{PG}(120)}{4} \qquad (1)$$

Indices of insulin sensitivity/insulin resistance

The Insulin Sensitivity Index (ISI) is an indicator of the effectiveness of insulin in terms of promoting glucose uptake and utilisation. A higher ISI indicates better insulin sensitivity. Conversely, a lower index suggests reduced insulin sensitivity / higher insulin resistance and an increased risk of type 2 diabetes. We calculated the whole-body ISI using the Matsuda ISI

composite formula described in the publication of Matsuda et al, in 1999 [59, 14]. (FPG: fasting plasma glucose, FPI: fasting plasma insulin)

$$\text{ISI composite (Matsuda Index)} = \frac{10{,}000}{\sqrt{(\text{FPG} \cdot \text{FPI})(\text{mean glucose} \cdot \text{mean insulin during OGTT})}} \tag{2}$$

The Hepatic Insulin Resistance Index (HIRI) is a parameter used to evaluate the degree of insulin resistance specifically in the liver. The index reflects that after a glucose load endogenous hepatic glucose production is strongly inhibited in healthy subjects and, therefore, the rise of glucose and insulin in the first phase after a glucose load display a strong association with hepatic insulin resistance. A higher HIRI value indicates reduced liver sensitivity to insulin. We calculated the Hepatic Insulin Resistance Index (HIRI) using the glucose and insulin AUC during the first 30 min of the OGTT [1]. (INS AUC: Area under the curve of plasma insulin)

$$\text{HIRI} = \frac{\text{PG AUC(30)} \cdot \text{INS AUC(30)}}{10{,}000} \tag{3}$$

There are two models of Homeostasis Model Assessment (HOMA) for insulin sensitivity (S) and insulin resistance (IR) based on fasting glucose and fasting plasma insulin levels (HOMA-S, HOMA-IR) or on fasting glucose and fasting plasma C-peptide levels (HOMA-2S, HOMA-2-IR). In this study, HOMA parameters were calculated with the HOMA Calculator of Oxford University, version 2.2.3 (from the website http://www.dtu.ox.ac.uk/homacalculator, HOMA Calculator, Oxford University, Oxford, United Kingdom). HOMA-IR is the inverse of HOMA-S. We used fasting plasma C-peptide instead of fasting plasma insulin for HOMA-2-IR calculations.

Only clinically realistic values that would be seen in a fasting subject were used, because HOMA is a steady-state model. These are:

- Plasma glucose 63.06 to 450.45 mg/dl (3.5 to 25.0 mmol/L)
- Plasma insulin 3.33 to 66.67 µIU/ml (20 to 400 pmol/L)
- Plasma C-peptide 0.6 to 10.5 ng/ml (0.2 to 3.5 nmol/L)

Therefore, subjects with plasma glucose, insulin, or C-peptide values outside these limits were excluded for determining HOMA (at baseline n=12 (6%); 12 subjects had fasting insulin < 3.33 µIU/ml, and at 5 years follow-up n=9 (6%); 9 subjects had fasting insulin < 3.33 µIU/ml).

Indices of insulin secretion and beta cell function

Fasting plasma glucose, fasting plasma insulin, and fasting plasma C-peptide levels were used to calculate HOMA-B (HOMA Calculator of Oxford University), a parameter to estimate beta cell function.

The oral Disposition Index (DI), in mg/dl/IU, provides an integrated measure of beta cell function. DI assesses insulin secretion in relation to prevailing insulin sensitivity. A high DI indicates a good response of the beta cells (insulin secretion) to glucose load at a given insulin sensitivity. When beta cell function decreases, their capacity to compensate for insulin resistance is reduced, resulting in a lower DI. We calculated the Disposition Index (DI) from the OGTT, with $\triangle$I (30') being the rise in plasma insulin during the first 30 min of the OGTT [48].

$$\mathrm{DI} = \mathrm{ISI} \cdot \triangle \mathrm{I}(30) \tag{4}$$

$$\triangle \mathrm{I} = \mathrm{insulin}(30) - \mathrm{insulin}(0) \tag{5}$$

The Oral Adaptation Index (AI), in ng/dl/IU, is similar to the disposition index, a parameter for beta cell function (insulin secretion) adjusted for insulin sensitivity, where instead of insulin, C-peptide levels are measured at 0 and 30 min during an OGTT. C-peptide is only minimally

degraded by the liver (first-pass effect), and therefore, AI can serve as a marker of prehepatic beta cell function in relation to insulin sensitivity. A higher AI indicates that beta cells are efficient in increasing their response to a glucose load for defined insulin resistance. A low AI can serve as a marker for reduced beta cell compensation. We calculated the Adaptation Index (AI) from the OGTT, with $\triangle$C (30') being the rise in plasma C-peptide during the first 30 min of the OGTT [3].

$$\text{AI} = \text{ISI} \cdot \triangle\text{C}(30) \tag{6}$$

$$\triangle\text{C} = \text{C-peptide}(30) - \text{C-peptide}(0) \tag{7}$$

The Insulinogenic Index (IGI) measures beta-cell insulin secretion, taking into account the change in insulin relative to the change in glucose during the first 30 minutes of an OGTT. It provides insights into the ability of the beta-cells to respond to the increase in blood glucose levels. A higher IGI suggests a more efficient insulin release in response to an increase in blood glucose, and a lower IGI may indicate impaired beta-cell function. The IGI was calculated from the OGTT, with $\triangle$I (30') and $\triangle$G (30') being the rise in insulin and plasma glucose during the first 30 min of the OGTT, respectively.

$$\text{IGI} = \frac{\triangle\text{I}}{\triangle\text{G}} \tag{8}$$

$$\triangle\text{I} = \text{insulin}(30) - \text{insulin}(0) \tag{9}$$

$$\triangle\text{G} = \text{glucose}(30) - \text{glucose}(0) \tag{10}$$

4.3 Statistical analysis

All statistical calculations were performed using the program SPSS (IBM Statistics) version 29.0 for Microsoft Windows (SPSS Inc., Chicago, IL, USA). Variables are reported as mean ± SD

and/or median [1st quartile : 3rd quartile]. The Kolmogorov-Smirnov test was used to test for normality. Categorical variables are presented as frequency and percentage. P-values were considered statistically significant when <0.05. Significance levels are expressed as *<0.05, **< 0.01, and ***<0.001. The t-test was used to compare normally distributed measures. The Mann-Whitney-U test was used to determine the p-values for non-normally distributed measures. Nominal variables and frequencies were analysed by the Chi-Squared-Test. The Kruskal-Wallis test was used to compare variables when divided into more than two groups. To compare differences between values at baseline and at 5 years follow-up, the Wilcoxon-test was used. Relationships between continuous variables were assessed using Spearman's correlation coefficient.

To determine the predictive strength of our models, we used logistic regression analysis, measuring the Nagelkerkes R Squared (R^2). The Nagelkerkes R^2 is a measure of goodness of fit in a binary regression analysis. The Nagelkerkes R^2 ranges from 0 to 1, where values closer to 1 indicate a better fit of the model. A value of 0.2 or less indicates a weak relationship between the predictors and the dependent variable or outcome. A value of 0.2 to 0.4 indicates a moderate relationship and a value of 0.4 or higher indicates a strong relationship [37].

We used the Receiver Operating Characteristic (ROC) analysis to evaluate the performance of our binary classification models with glucose tolerance. The ROC curve illustrates the trade-off between the true positive rate (sensitivity) and the false positive rate (1-specificity) as we vary the classification threshold [104].

To select the optimal threshold and decide which cut-off values of each glucose metabolism index answer best our objectives, we used the Youden Index [100], defined as:

$$\text{Youden Index} = \text{Sensitivity} - (1 + \text{Specificity}) \tag{11}$$

The threshold that maximizes the Youden Index corresponds to a point on the ROC curve that is closest to the ideal top-left corner (100% sensitivity and 100% specificity). We also balanced sensitivity and specificity in a single metric to find the threshold that achieves a good balance between correctly identifying positive cases (sensitivity) and correctly excluding negative cases (specificity). The positive predictive value of our tests was calculated using the formula [63]:

$$\text{PPV} = \frac{\text{Sensitivity} \cdot \text{Prevalence}}{\text{Sensitivity} \cdot \text{Prevalence} + (1 - \text{Specificity}) \cdot (1 - \text{Prevalence})} \tag{12}$$

Results

5.1 Baseline characteristics

200 pGDM women were included in the PPS-Diab study (baseline visit 3-16 months after delivery). Table 1 describes the baseline characteristics of the cohort. The subjects were compared regarding their glucose metabolism status at baseline. 126 (63.0%) of the subjects were classified as having a normal glucose metabolism and 74 (37.0%) of the subjects were defined as having a pathological glucose metabolism, which was either an impaired fasting glucose (IFG: n=31), an impaired glucose tolerance (IGT: n=24), both IFG and IGT (n=12) or T2DM (n=7). The OGTT results during the pregnancy were not significantly different at fasting and 120 min between subjects with normal and pathological glucose metabolism after delivery. Only the 60 min glucose concentrations were slightly decreased (171 ± 29 vs. 181 ± 31 mg/dl). However, treatment of GDM during pregnancy differed between both groups. Insulin-treated subjects had more frequently a pathological glucose metabolism at the first visit after delivery compared to diet-treated subjects (66.2% vs. 50.0%) ($p < 0.05$).

TABLE 1. Baseline characteristics of pGDM women (n=200) divided by glucose metabolism status at baseline visit 1

	NGM	PGM	
No. of subjects (n=200)	126 (63.0%)	74 (37.0%)	*p-value*
Clinical characteristics: mean ± SD			
Age [years]	35 ±4	36 ±5	0.258
BMI [kg/m²]	25.1 ±5.8	28.2 ±7.0	<0.001
Waist circ. [cm]	81±11	87 ±13	0.001
Hip circ. [cm]	100 ±13	105 ±13	0.010
WHR	0.81 ±0.06	0.82 ±0.06	0.087
Sys. BP [mmHg]	118 ±10	121 ±13	0.043
Dia. BP [mmHg]	74 ±8	76 ±10	0.208
HR [/min]	74 ±9	78 ±9	0.004
Medical history: n (%)			
GDM Therapy:			0.026
Insulin	63 (50.0%)	49 (66.2%)	
Diet	63 (50.0%)	25 (33.8%)	
Metabolic Syndrome:			<0.001
Yes	4 (3.2%)	29 (39.2%)	
No	122 (96.8%)	45 (60.8%)	
Pregnancy OGTT [mg/dl]:			
0 min	89 ±12	93 ±14	0.075
60 min	171 ±29	181 ±31	0.052
120 min	139 ±34	144 ±33	0.286
Clinical chemistry: mean ± SD			
HbA1c [%]	5.3 ±0.3	5.5 ±0.4	<0.001
CRP [mg/dl]	0.33 ±0.23	0.40 ±0.39	0.221
TGL [mg/dl]	72 ±30	103 ±56	<0.001
Cholesterol [mg/gl]	181 ±30	179 ±33	0.623
LDL-Ch [mg/dl]	105 ±28	106 ±28	0.775
HDL-Ch [mg/dl]	65 ±15	56 ±13	<0.001
GPT [U/l]	17 ±9	22 ±22	0.022
Gamma-GT [U/l]	16 ±8	20 ±13	0.002
Creatinine [mg/dl]	0.69 ±0.11	0.67 ±0.12	0.159
TSH [µU/ml]	2.3 ±4.4	1.9 ±1.0	0.455
Ferritin [µg/l]	37 ±25	42 ±29	0.175
Adipokine: mean ± SD			
Leptin [ng/ml]	10.8 ±7.1	16.3 ±7.7	0.002
Adiponectin [µg/ml]	11.6 ±7.1	10.0 ±5.4	0.265
Resistin [ng/ml]	10.6 ±8.8	8.9 ±4.0	0.314

Abbreviations: NGM, normal glucose metabolism; PGM, pathological glucose metabolism; No., number; SD, standard deviation; BMI, body mass index; circ., circumference; WHR, waist-to-hip ratio; Sys. BP, systolic blood pressure; Dia. BP, diastolic blood pressure; HR, heart rate; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test; HbA1c, glycated haemoglobin concentration; CRP, C-reactive protein; TGL, triglycerides; LDL-Ch, low-density lipoprotein cholesterol; HDL-Ch, high-density lipoprotein cholesterol; GPT, glutamate-pyruvate-transaminase; gamma-GT, gamma-glutamyl-transferase; TSH, thyroid-stimulating-hormone

TABLE 2. (A) Baseline OGTT results and indices of insulin sensitivity, insulin resistance and beta-cell function of pGDM women (n=200) divided by glucose metabolism status

No. of subjects (n=200)	NGM 126 (63.0%)	PGM 74 (37.0%)	*p-value*
Glucose tolerance at baseline:			<0.001
NGM	126 (100.0%)	0 (0%)	
IFG	0 (0%)	31 (41.9%)	
IGT	0 (0%)	24 (32.4%)	
IFG+IGT	0 (0%)	12 (16.2%)	
T2D	0 (0%)	7 (9.5%)	
Glucose during OGTT [mg/dl]:			
0 min	90 ±5	102 ±9	<0.001
30 min	151 ±26	172 ±27	<0.001
60 min	139 ±32	174 ±40	<0.001
90 min	117 ±26	154 ±46	<0.001
120 min	108 ±19	143 ±38	<0.001
△glucose 0-30 min [mg/dl]	61.0 ±25.5	69.4 ±27.5	0.029
AUC glucose [mg.h/dl]	253.1 ±41.2	311.0 ±60.1	<0.001
Insulin during OGTT [µIU/ml]:			
0 min	8.0 ±5.8	11.6 ±6.1	<0.001
30 min	63.7 ±39.9	68.6 ±38.2	0.393
60 min	72.3 ±47.3	91.0 ±54.7	0.012
90 min	59.1 ±44.7	87.2 ±63.8	<0.001
120 min	52.3 ±37.0	90.1 ±67.6	<0.001
△insulin 0-30 min [µIU/ml]	55.7 ±36.0	57.1 ±35.3	0.799
AUC insulin [µIU.h/ml]	112.7 ±69.6	148.8 ±88.3	0.002
C-peptide during OGTT* [ng/ml]:			
0 min	1.84 ±0.51	2.6 ±0.8	<0.001
30 min	6.7 ±1.9	7.4 ±2.0	0.038
60 min	8.9 ±2.4	10.7 ±2.9	<0.001
90 min	8.8 ±2.4	11.2 ±3.3	<0.001
120 min	8.5 ±2.3	11.3 ±4.2	<0.001
△C-peptide 0-30 min [ng/ml]	4.9 ±1.7	4.8 ±1.6	0.913
AUC C-peptide [ng.h/ml]	14.8 ±3.5	18.2 ±4.9	<0.001

Abbreviations: NGM, normal glucose metabolism; PGM, pathological glucose metabolism; No., number; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes mellitus; OGTT, oral glucose tolerance test; AUC, area under the curve. *C-Peptide was measured in n=128 subjects.

TABLE 2. (B) Baseline OGTT results and indices of insulin sensitivity, insulin resistance and beta-cell function of pGDM women (n=200) divided by glucose metabolism status

	NGM	PGM	
No. of subjects (n=200)	126 (63.0%)	74 (37.0%)	*p-value*
OGTT-based indices at baseline:			
Insulin sensitivity:			
ISI	5.8 ±2.7	3.7 ±2.3	
	5.5 [3.7 : 7.5]	3.3 [2.2 : 4.6]	<0.001
HOMA-S	139.0 ±61.9	90.0 ±45.2	
	137.9 [88.7 : 191.1]	76.6 [57.9 : 118.5]	<0.001
HOMA-2-S	80.2 ±23.2	55.8 ±19.2	<0.001
	78.6 [63.5 : 91.4]	50.3 [44.0 : 62.4]	<0.001
Insulin resistance:			
HIRI	0.11 ±0.07	0.14 ±0.08	
	0.09 [0.07 : 0.14]	0.12 [0.08 : 0.17]	0.012
HOMA-IR	0.9 ±0.6	1.4 ±0.7	
	0.7 [0.5 : 1.1]	1.3 [0.8 : 1.7]	<0.001
HOMA-2-IR	1.4 ±0.4	2.0 ±0.6	
	1.3 [1.1 : 1.6]	2.0 [1.6 : 2.3]	<0.001
Beta-cell function:			
HOMA-B	87.3 ±35.6	90.1 ±28.1	0.394
	78.8 [64.9 : 100.1]	83.5 [69.8 : 111.3]	0.206
HOMA-2-B	115.4 ±21.3	120.9 ±26.7	0.375
	112.7 [101.5 : 128.0]	121.5 [101.6 : 137.4]	
DI	262.2 ±101.9	176.3 ±107.0	
	246.5 [179.8 : 322.1]	158.0 [111.4 : 203.7]	<0.001
AI	26.6 ±10.9	16.9 ±12.0	
	24.7 [18.3 : 33.7]	13.6 [9.7 : 19.3]	<0.001
IGI	1.1 ±0.8	0.9 ±0.6	
	0.8 [0.6 : 1.3]	0.8 [0.4 : 1.2]	0.073

Abbreviations: NGM, normal glucose metabolism; PGM, pathological glucose metabolism; No., number; OGTT, oral glucose tolerance test; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; HIRI, hepatic insulin resistance index; -IR, for insulin resistance; -B, for beta-cell secretion; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

The subjects having a normal glucose metabolism had a significantly lower BMI (25.1 ± 5.8 vs. 28.2 ± 7.0 kg/m²), a smaller waist circumference (81 ± 11 vs. 87 ± 13 cm), a smaller hip circumference (100 ± 13 vs. 105 ± 13 cm), a lower systolic blood pressure (118 ± 10 vs. 121 ± 13 mmHg) and a lower heart rate (74 ± 9 vs. 78 ± 9 /min). Analysis of subjects according to metabolic syndrome (MetS) revealed that only 3.2% in the group with normal glucose metabolism had MetS versus 39.2% in the pathological glucose metabolism group ($p < 0.001$).

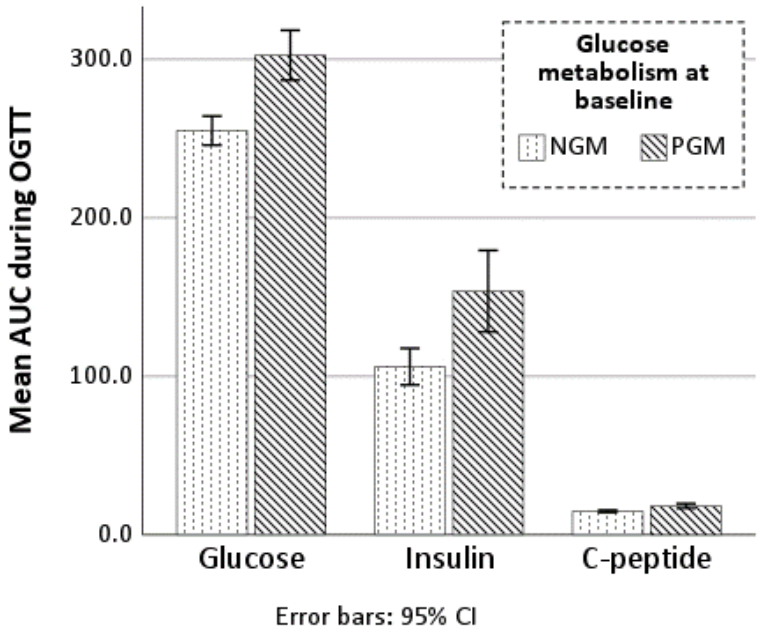


FIGURE 1. (A) Mean AUC of plasma glucose (mg.h/dl), plasma insulin (µIU.h/ml) and plasma C-peptide (ng.h/ml) of pGDM women during 75 g OGTT at baseline, according to glucose metabolism status (NGM vs. PGM)

Regarding the clinical chemistry results, HbA1c was significantly different in both subgroups, with 5.3% for NGM subjects, and 5.5% for PGM subjects ($p < 0.001$). TGL and HDL-cholesterol were significantly different whereas cholesterol and LDL-cholesterol did not present any difference in the two subgroups. GPT and gamma GT presented significant differences, but not CRP, creatinine or TSH. The only adipokine presenting differences between the two groups was leptin (10.8 ± 7.1 vs. 16.3 ± 7.7 ng/ml) (Table 1).

Metabolic testing of pGDM subjects by OGTT detected highly significant differences in 0-120 min glucose concentrations and insulin and C-peptide plasma levels (0 min, 60 min, 90 min, 120 min) between both groups (Table 2A). All areas under the curves (AUC) were significantly different as well (Figure 1A and B). At baseline, all indices of insulin sensitivity and insulin resistance (ISI, HOMA-S, HOMA-2-S, HIRI, HOMA-IR and HOMA-2-IR) were significantly higher in post GDM women with normal glucose tolerance (NGM). Parameters of insulin secretion such as HOMA-B,

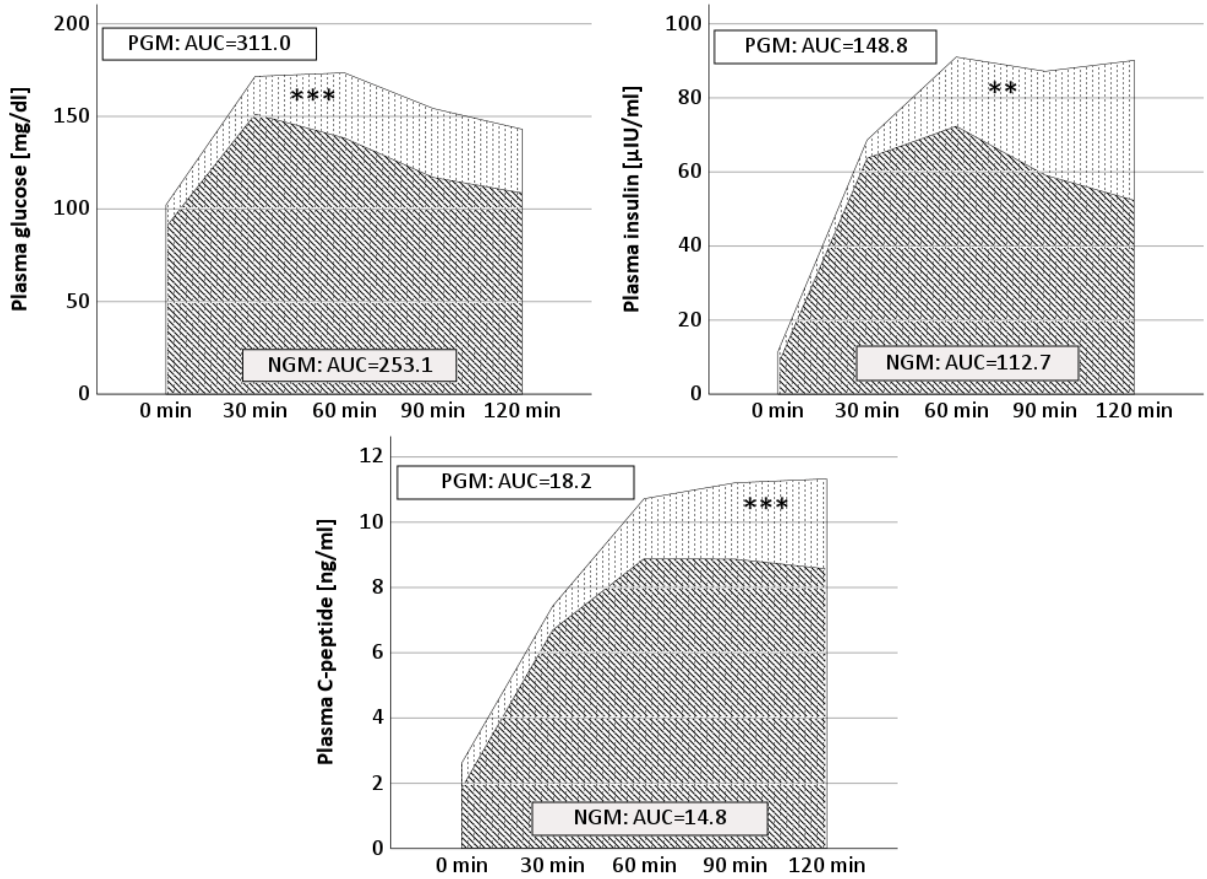


FIGURE 1. (B) AUC of plasma glucose (up left, in mg.h/dl), plasma insulin (up right, in µIU.h/ml) and plasma C-peptide (down middle, in ng.h/ml) of pGDM women during 75 g OGTT at baseline, divided by glucose metabolism status (NGM vs. PGM)

HOMA-2-B and the insulinogenic index (IGI) were not different between NGM and pathological glucose tolerance (PGM). When DI or AI were analysed, both parameters indicating beta cell function in relation to insulin sensitivity, better beta cell function was observed in subjects with NGM as compared to PGM ($p < 0.001$) (Table 2B).

5.2 Clustering in different subtypes

Age at disease onset, sex, baseline BMI, diabetes-specific autoantibodies (antibodies to glutamic acid decarboxylase: GADA), HbA1c, HOMA-2B and HOMA2-IR were used in previous

studies to cluster patients with T2DM or prediabetes into five to six different subtypes with different patient characteristics and risk for diabetes-associated complications or different risk for diabetes development, respectively. Our cohort of subjects with a previous GDM is homogeneous with respect to age (all are young to middle-aged), sex and GADA (autoantibody positive individuals are prone to type 1 diabetes and were excluded). Thus, GADA, age and sex cannot be used for subtyping in the PPS-Diab study. To classify pGDM women, we first analysed BMI and metabolic syndrome (MetS) and then added the different indices for insulin sensitivity/resistance and insulin secretion. The analysis focused first on the whole cohort of women pGDM, and then on women with normoglycaemia at baseline visit to identify clusters predicting rapid conversion from normoglycaemia to pathological glucose metabolism and T2DM.

5.2.1 Clustering according to metabolic syndrome and BMI

As performed in previous studies, we wondered whether BMI or metabolic syndrome helps to characterise pGDM women at risk for progression to disturbed glucose homeostasis. Table 3 and Figure 2 show that 93.9% of the subjects with MetS at baseline also presented a BMI ≥ 25 kg/m². From these data we conclude that it is feasible to use BMI < / ≥ 25kg/m² to divide our study population and must not include additional parameters of MetS.

TABLE 3. Distribution of the subjects depending on BMI and metabolic syndrome

	No MetS	MetS	Total
BMI < 25 kg/m²	111 (66.1%)	2 (6.1%)	113 (55.8%)
BMI ≥ 25 kg/m²	57 (33.9%)	31 (**93.9**%)	88 (44.2%)
Total	168 (83.5%)	33 (16.5%)	201 (100.0%)

Abbreviations: MetS, metabolic syndrome; BMI, body-mass index

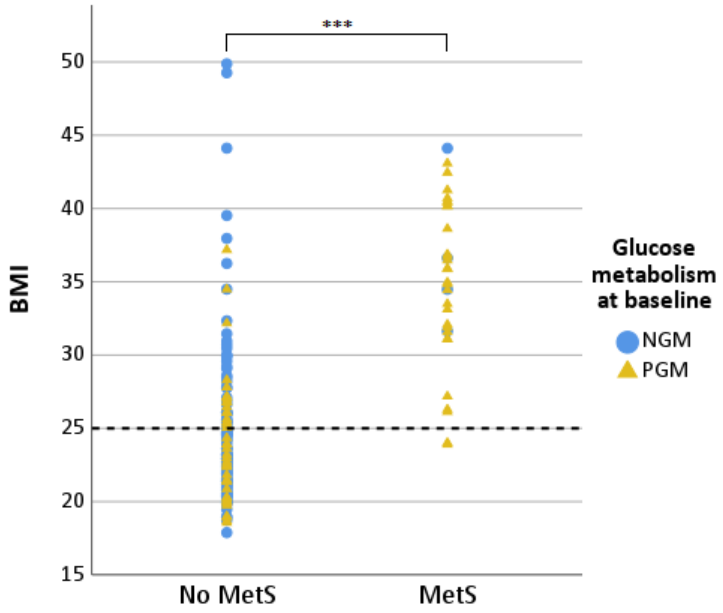


FIGURE 2. Correlation between metabolic syndrome and BMI in subjects with normal or pathological glucose metabolism at baseline

5.2.2 Indices of glucose metabolism at baseline

Figure 3A and B depict the correlation between indices for insulin sensitivity/resistance and insulin secretion and BMI in subjects with NGM or PGM at baseline. In both cohorts there was a significant positive correlation between BMI and HIRI, HOMA-IR, HOMA-2-IR, HOMA-B, HOMA-2-B and IGI. A significant negative correlation was found between BMI and ISI, HOMA-S, HOMA-2-B, HOMA-2-S, DI and AI in subjects with NGM and PGM (Figure 3A and B). Thus, using the 25 kg/m² BMI cut-off no clear division of the two cohorts was possible at baseline suggesting that additional markers are required for subtyping.

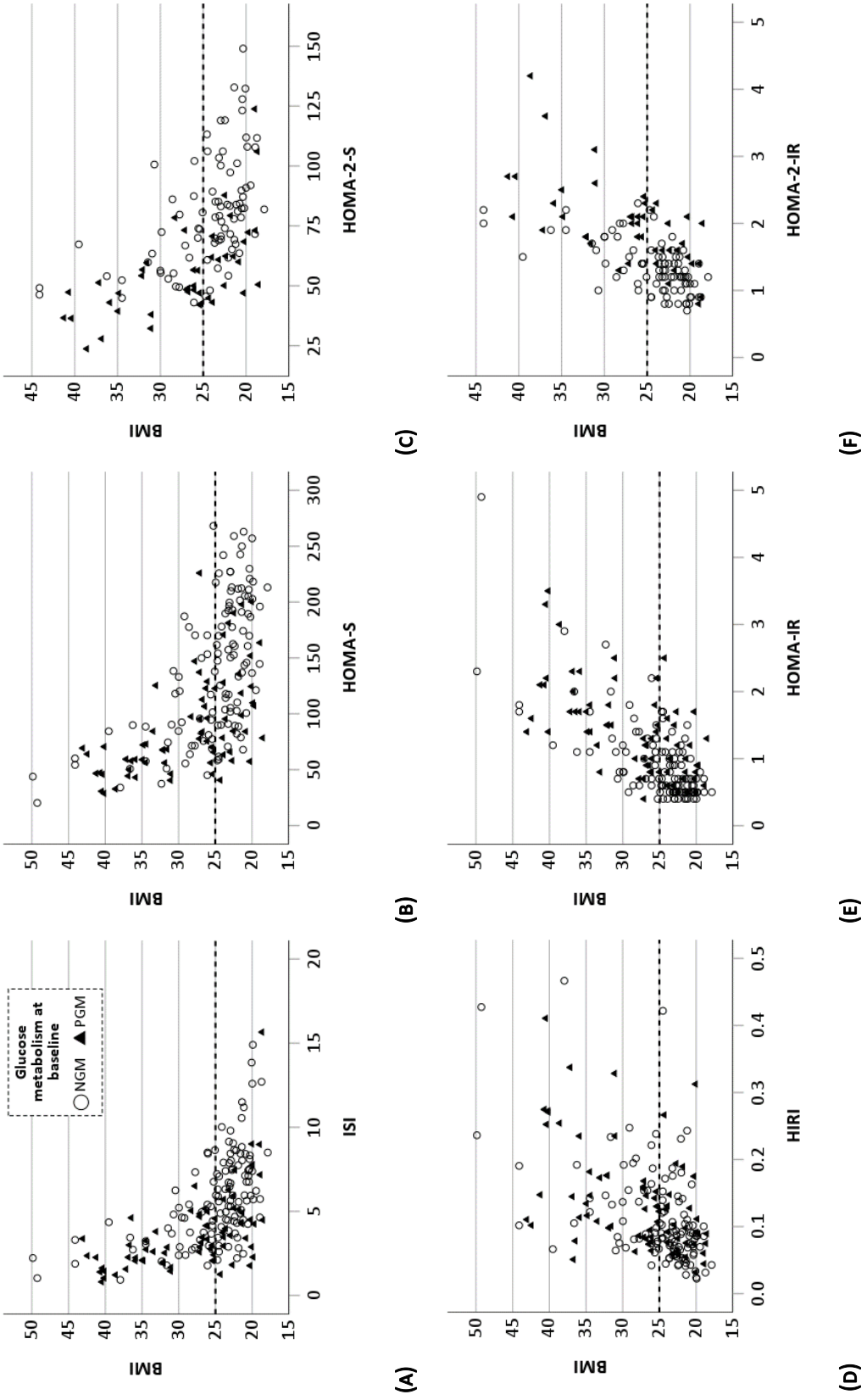


FIGURE 3. (A) Correlation between BMI and insulin sensitivity/insulin resistance indices in the PPS-Diab cohort at baseline

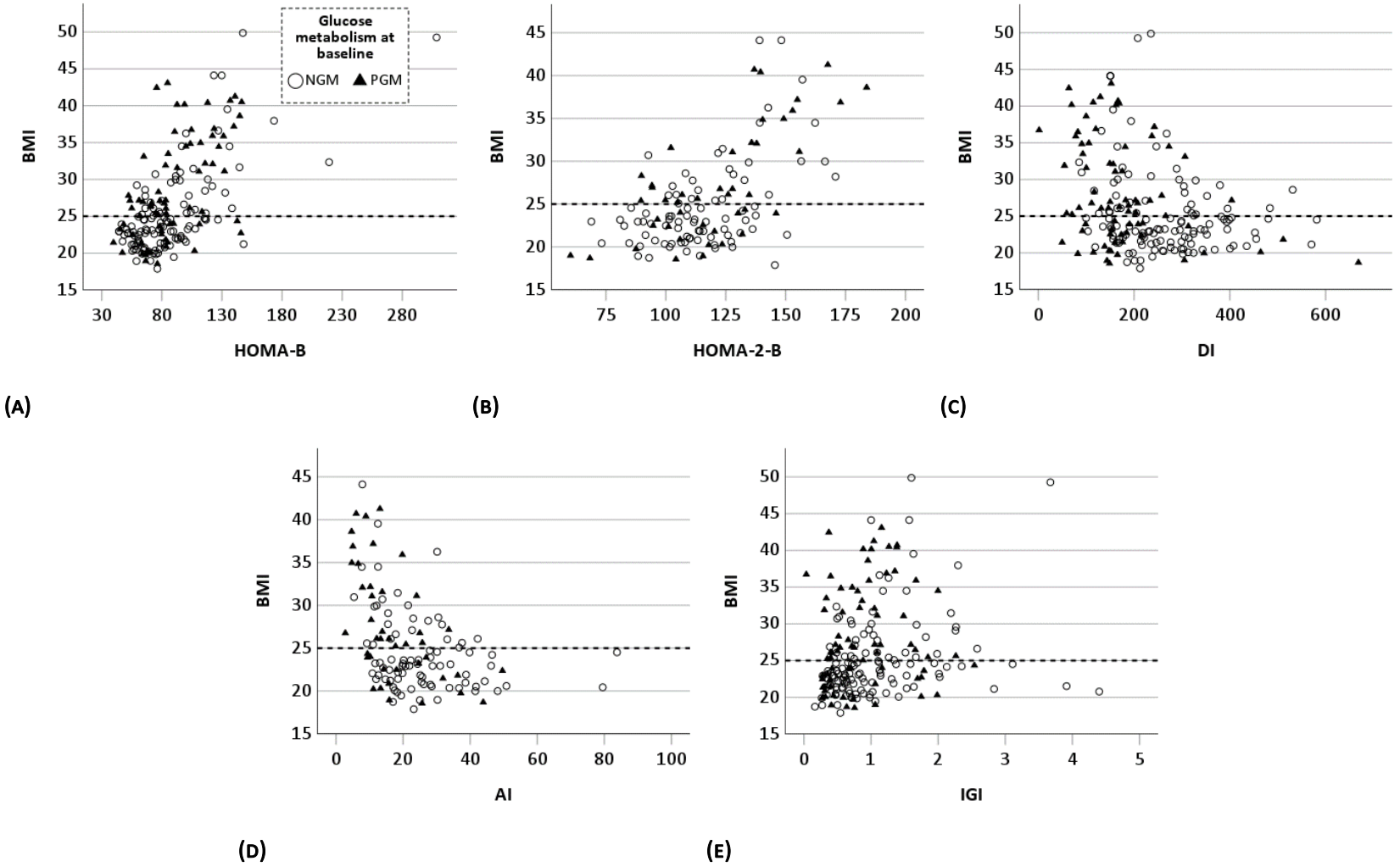


FIGURE 3. (B) Correlation between BMI and insulin secretion/beta-cell function indices in the PPS-Diab cohort at baseline

Distribution of insulin indices for insulin sensitivity/resistance, indices for insulin secretion depending on the BMI in the PPS-Diab cohort at baseline.

5.3 Glucose metabolism during follow-up

A high proportion of women with GDM develop normal glucose tolerance after delivery. An aim of the present study was to differentiate between subjects showing progression from NGM to pathological glucose metabolism during follow-up from non-progressors. During follow-up of 5 years, 12 women developed T2DM (study endpoint) but were kept in the analysis of this study, 50 subjects withdrew consent, were lost during follow-up or either had an external OGTT with no plasma samples or only had a telephone interview.

At 5-year visit, 96 (64%) subjects were normoglycaemic, 26 (17%) had IFG, 14 (9%) suffered from IGT, 11 (7%) had IFG and IGT and cumulative 12 (8%) had developed T2DM (total of 159, including 9 subjects that developed T2DM during the 5 years span). 79% (n=76) of the normoglycaemic subjects at baseline visit remained normoglycaemic, 10% developed an IFG, 7% an IGT, 2% a mixture of both and 2% developed T2DM (n=2). In the subgroup with pathological glucose metabolism at the first visit after delivery 20% reversed to normal glucose metabolism at 5-year follow-up, 31% had an IFG, 13% an IGT, 17% a mixture of both, and 19% (n=10) T2DM. Stratification according to BMI revealed that 12 of 65 (18%) women with a BMI < 25 kg/m2 and 8 of 31 (26%) women with a BMI ≥ 25 kg/m² developed PGM at 5 years of follow-up.

TABLE 4. 5-year follow-up OGTT results of pGDM women (n=150) divided by glucose tolerance status at baseline

No. of subjects (n=150)	NGM 96 (64.0%)	PGM 54 (36.0%)	*p-value*
Glucose tolerance at 5y. FU:			< 0.001
NGM	76 (79.2%)	11 (20.3%)	
IFG	9 (9.4%)	17 (31.5%)	
IGT	7 (7.2%)	7 (13.0%)	
IFG+IGT	2 (2.1%)	9 (16.7%)	
T2D	2 (2.1%)	10 (18.5%)	
Glucose during OGTT [mg/dl]			
0 min	93 ±9	102 ±9	<0.001
30 min	154 ±28	166 ±27	0.006
60 min	144 ±39	165 ±44	0.007
90 min	122 ±34	149 ±47	0.002
120 min	113 ±27	130 ±37	0.014
AUC glucose [mg.h/dl]	260.1 ±52.2	299.1 ±63.3	<0.001
Insulin during OGTT [µIU/ml]			
0 min	7.8 ±4.2	12.0 ±7.1	<0.001
30 min	64.5 ±33.9	74.4 ±47.5	0.602
60 min	79.0 ±46.0	107.6 ±78.7	0.055
90 min	65.7 ±44.3	109.3 ±96.9	0.005
120 min	57.6 ±40.1	97.5 ±90.2	0.011
AUC insulin [µIU.h/ml]	120.3 ±62.3	173.0 ±129.3	0.053
OGTT-based indices at 5y FU:			
Insulin sensitivity:			
ISI	5.4 ±2.6	3.8 ±2.4	<0.001
	5.1 [3.4 : 7.0]	3.7 [1.8 : 4.8]	0.004
HOMA-S	133.4 ±59.3	95.7 ±54.9	
	120.5 [82.9 : 177.0]	94.1 [50.2 : 126.8]	0.008
Insulin resistance:			
HIRI	0.11 ±0.06	0.15 ±0.10	
	0.09 [0.06 : 0.15]	0.11 [0.07 : 0.23]	0.127
HOMA-IR	0.9 ±0.5	1.4 ±0.8	
	0.8 [0.6 : 1.2]	1.1 [0.8 : 2.0]	0.017
Beta cell function:			
HOMA-B	82.6 ±26.2	90.1 ±33.4	
	77.0 [64.8 : 93.3]	83.8 [63.7 : 116.6]	0.070
DI	265.2 ±153.7	172.0 ±72.1	
	238.9 [185.2 : 286.9]	171.3 [117.7 : 215.5]	<0.001
IGI	1.1 ±0.7	0.7 ±2.9	
	0.9 [0.6 : 1.4]	0.7 [0.5 : 1.5]	0.501

Abbreviations: NGM, normal glucose metabolism; PGM, pathological glucose metabolism; No., number; 5y FU, 5-year follow-up; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes mellitus; OGTT, oral glucose tolerance test; AUC, area under the curve; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; HIRI, hepatic insulin resistance index; -IR, for insulin resistance; -B, for beta-cell secretion; DI, disposition index; IGI, insulinogenic index

Table 4 summarises OGTT results at 5-year follow-up. Glucose levels were significantly higher at all time-points in the group that presented PGM at baseline. Plasma insulin was also significantly higher at 0-, 90- and 120-min during OGTT. The indices for insulin sensitivity (ISI and HOMA-S) calculated at 5-year follow-up were significantly higher, therefore better, in the group of pGDM subjects that presented normal glucose tolerance at baseline, even though 20 out of 96 (21%) of them developed PGM. Further analysis revealed that among indices for insulin resistance (HIRI and HOMA-IR), only HOMA-IR were significantly higher in the group of subjects that presented a pathological glucose metabolism at baseline. Within the indices for beta cell function (HOMA-B, DI and IGI), only DI was significantly higher for the subjects with NGM at baseline.

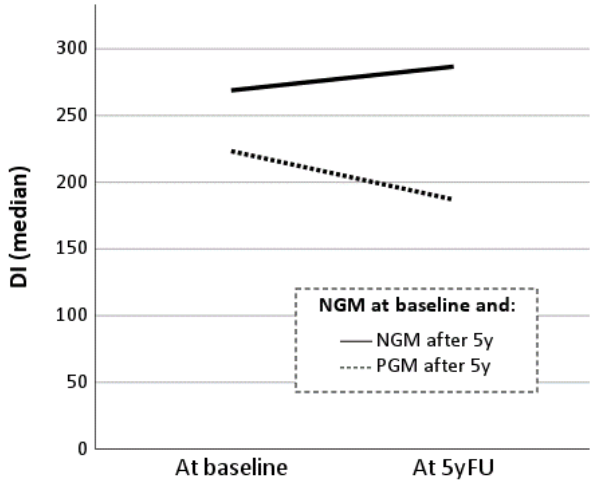


FIGURE 4. Development of disposition index (DI) over time in individuals developing PGM at 5 years (n=20) as compared to subjects remaining in a normoglycaemic state (n=76)

As illustrated in Figure 4, disposition index reduced significantly from 222.5 to 173.7 in women who developed PGM during 5-year follow-up ($p < 0.05$) as compared to women remaining normoglycaemic (DI baseline 254.3; 5 years follow-up 248.0, $p=0.751$) indicating that an impairment of beta cell secretion in response to glucose relative to insulin sensitivity precedes the development of prediabetes and overt T2DM.

5.4 Indices of glucose metabolism combined with BMI status

Table 5 describes the indices for glucose metabolism at baseline in subjects with a normal (NGM) or pathological glucose metabolism (PGM) at 5-year visit within the subgroups of subjects with BMI < and ≥ 25 kg/m². The two indices with the best discrimination throughout both groups were the disposition index (DI) and adaptation index (AI) indicating insulin secretion in relation to insulin sensitivity. The DI was significantly higher in NGM (258.4 [190.5 : 357.2]) compared to PGM (167.0 [150.7 : 225.6]) in the normal weight group (p=0.004), and less pronounced, but still significantly higher in the overweight/obesity group (175.9 [156.2 :304.0] vs. 146.6 [90.9 : 207.5], p=0.014). Similar results were obtained using the AI (30.9 [22.2 : 36.3]vs. 20.3 [15.9 : 22.5] in the normal weight group, p < 0.001; 16.8 [13.3 : 22.2] vs. 11.5 [9.4 : 17.5] in the overweight/obesity group, p=0.012). Very specific to the normal weight group, we observed that indices for insulin sensitivity (ISI and HOMA-2-S) were significantly different between women who remained normoglycaemic and subjects who developed pathological glucose tolerance. ISI was higher for the NGM women (6.7 [4.6 : 7.7] vs. 4.9 [4.0 : 6.3], p=0.042), as well as HOMA-2-S (83.1 [69.8 : 101.1] vs. 70.8 [57.3 : 85.1], p=0.044).

Interestingly, these significant differences were not observed in the overweight/obesity group. The homeostatic assessment of beta cell secretion (HOMA-B and HOMA-2-B), as well as the one for insulin resistance (HOMA-IR and HOMA-2-IR), was not significantly different in the normal weight nor the overweight/obesity groups. The insulinogenic index (IGI) was the only index which was significantly different in pGDM women with overweight/obesity but not in pGDM women with normal weight, with 1.1 [0.9 : 1.6] vs. 0.9 [0.5 : 1.2] (p=0.016).

TABLE 5. Indices for insulin sensitivity, insulin resistance and beta-cell function at baseline in women with NGM or PGM at 5-years stratified by BMI at baseline

	BMI <25 kg/m² (n=88)			BMI ≥25 kg/m² (n=62)		
	NGM 5y	PGM 5y	*p-value*	NGM 5y	PGM 5y	*p-value*
Total number (n=150)	60 (68.2%)	28 (31.8%)	0.003	27 (43.5%)	35 (56.5%)	0.003
AUC OGTT	257.4 ±46.3	287.6 ±61.0	0.037	254.6 ±41.0	315.0 ±58.7	<0.001
AUC OGTT (5yFU)	244.7 ±39.7	302.7 ±58.3	<0.001	248.4 ±45.2	324.9 ±55.8	<0.001
AUC INS	96.3 ±46.6	109.1 ±79.5	0.986	143.3 ±61.3	158.5 ±74.0	0.390
AUC INS (5yFU)	102.4 ±53.4	18.3 ±73.7	0.562	153.1 ±69.9	206.9 ±134.8	0.321
AUC C-pep.	14.7 ±3.4	14.4 ±3.8	0.723	16.8 ±4.6	19.0 ±4.5	0.043
△PG 0-30 min	62.4 ±25.8	71.0 ±34.1	0.522	55.6 ±24.7	69.0 ±23.5	0.038
△INS 0-30 min	48.4 ±30.4	44.5 ±34.0	0.084	65.5 ±29.3	59.6 ±35.4	0.271
△C-pep.* 0-30 min	4.8 ±1.6	4.0 ±1.3	0.026	5.4 ±1.6	5.2 ±1.8	0.833
Insulin sensitivity at baseline:						
ISI	6.6 ±2.7 6.7 [4.6 : 7.7]	5.4 ±2.6 4.9 [4.0 : 6.3]	0.042	3.9 ±1.9 3.7 [2.4 : 4.8]	3.0 ±1.3 2.9 [2.1 : 3.5]	0.154
HOMA-S	163.1 ±536.1 169.8 [117.8 : 211.1]	133.3 ±53.6 130.7 [84.2 : 174.2]	0.180	84.0 ±38.5 75.1 [55.6 : 94.5]	77.7 ±31.6 68.3 [57.6 : 95.7]	0.849
HOMA-2-S	86.1 ±22.4 83.1 [69.8 : 101.1]	74.7 ±24.9 70.8 [57.3 : 85.1]	0.027 0.044	61.6 ±18.9 56.4 [49.6 : 73.8]	52.3 ±14.0 49.1 [45.9 : 58.2]	0.055 0.125
Insulin resistance at baseline:						
HIRI	0.09 ±0.06 0.08 [0.06 : 0.10]	0.10 ±0.07 0.08 [0.06 : 0.11]	0.921	0.14 ±0.07 0.4 [0.08 : 0.16]	0.15 ±0.07 0.13 [0.08 : 0.19]	0.923
HOMA-IR	0.7 ±0.3 0.6 [0.5 : 0.8]	0.9 ±0.5 0.8 [0.6 : 1.2]	0.187	1.4 ±0.6 1.3 [1.1 : 1.8]	1.5 ±0.6 1.5 [1.0 : 1.7]	0.963
HOMA-2-IR	1.2 ±0.3 1.2 [1.0 : 1.4]	1.5 ±0.4 1.4 [1.2 : 1.7]	0.097	1.8 ±0.5 1.8 [1.4 : 2.0]	2.1 ±0.6 2.0 [1.8 : 2.2]	0.264
Beta cell function at baseline:						
HOMA-B	74.8 ±18.5 73.2 [60.7 : 86.7]	72.8 ±22.5 66.6 [58.9 : 79.8]	0.848	112.1 ±32.0 114.7 [91.4 : 132.1]	97.4 ±24.1 95.1 [77.2 : 116.0]	0.188
HOMA-2-B	109.9 ±19.1 110.1 [99.5 : 120.6]	106.2 ±18.3 105.1 [94.7 : 115.5]	0.358 0.478	131.3 ±22.9 127.4 [112.2 : 152.0]	128.8 ±24.5 131.2 [105.9 :141.6]	0.633 0.916
DI	283.8 ±122.6 258.4 [190.5 : 357.2]	193.6 ±83.6 167.0 [150.7 : 225.6]	0.004	222.6 ±96.3 175.9 [156.2 :304.0]	160.5 ±104.1 146.6 [90.9 : 207.5]	0.014
AI	30.6 ±12.0 30.9 [22.2 : 36.3]	20.5 ±6.8 20.3 [15.9 : 22.5]	<0.001	20.6 ±11.4 16.8 [13.3 : 22.2]	14.5 ±7.7 11.5 [9.4 : 17.5]	0.012
IGI	0.9 ±0.7 0.7 [0.5 : 1.1]	0.7 ±0.6 0.5 [0.3 : 1.0]	0.072	1.3 ±0.6 1.1 [0.9 : 1.6]	0.9 ±0.5 0.9 [0.5 : 1.2]	0.016

Abbreviations: BMI, body-mass index; NGM, normal glucose metabolism; PGM, pathological glucose metabolism; 5y, 5 years; AUC, area under the curve; OGTT, oral glucose tolerance test; INS, insulin; C-pep., C-peptide; PG, plasma glucose; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; HIRI, hepatic insulin resistance index; -IR, for insulin resistance; -B, for beta-cell secretion; DI, disposition index; AI, adaptation index; IGI, insulinogenic index. *C-peptide was measured for n=128 subjects.

5.5 Logistic regression analysis - prediction of PGM at 5 years in the whole cohort

To determine the indices of glucose metabolism that predict glucose metabolism at a 5-year follow-up, we conducted a bivariate logistic regression analysis. Table 6 shows the results of the binary regression analysis for the dependent variable glucose metabolism status after 5 years of follow-up (NGM vs. PGM) in the whole cohort. In the category for insulin secretion indices, neither HOMA-B nor HOMA-2-B were statistically significant predictors for glucose metabolism, with or without age adjustment and BMI category (< 25 kg/m² vs. ≥ 25kg/m²). The insulin sensitivity indices ISI (R^2=0.15, $p < 0.01$) and HOMA-S (R^2=0.15, $p < 0.01$), the insulin resistance index HOMA-2-IR (R^2=0.17, $p < 0.05$), as well as the DI (R^2=0.24, $p < 0.001$) and AI (R^2=0.29, $p < 0.001$) were statistically significant predictors for the glucose status in the whole cohort after 5-year follow-up in the crude model and after adjustment for age and BMI status. The best predictive marker for disturbed glucose tolerance at 5 years was the AI (R^2 = 0.38).

5.5.1 Logistic regression analysis: prediction of incident PGM at 5 years in women with NGM at baseline

Table 7 shows the results of the logistic regression analysis in the group of subjects that presented a normal glucose metabolism (NGM) at baseline visit after delivery. There was no association between any of the insulin indices with PGM and this didn't change after adjustment for age and BMI. The only index that was significantly associated with development of pathological glucose metabolism was the adaptation index (AI) with an Odds-Ratio of 0.92 (CI 0.85-0.99), $p < 0.05$ and a Nagelkerkes R^2=0.15.

TABLE 6. Logistic regression analysis of glucose tolerance after 5 years follow-up, whole cohort analysis

Regression analysis depending on clustering factors: BMI status at baseline, insulin indices at baseline. Significance levels are expressed as *p <0.05, **<0.01, and ***<0.001. Glucose metabolism status: NGM vs. PGM, BMI status: 2 categories < or ≥ 25 kg/m² (binary categorical variable)

Odds Ratio (confidence interval) + R^2 (Nagelkerkes)				
Dependent variable	**Parameter**	**Crude model**	**Adjusted for age**	**Adjusted for age and BMI status**
Glucose metabolism status after 5y FU	**- with insulin sensitivity indices**			
	ISI	0.76 (0.65-0.88)*** R^2: 0.13	0.76 (0.65-0.88)*** R^2: 0.14	0.79 (0.67-0.95)* R^2: 0.14
	HOMA-S	0.99 (0.98-1.0)*** R^2: 0.12	0.99 (0.98-1.0)*** R^2: 0.12	0.99 (0.98-1.0)* R^2: 0.13
	HOMA-2-S	0.97 (0.95-0.99)*** R^2: 0.15	0.97 (0.95-0.99)*** R^2: 0.17	0.97 (0.95-0.99)** R^2: 0.18
	- with insulin resistance indices			
	HIRI	61.1 (0.47-7925.0) R^2: 0.03	62.3 (0.46-8382.3) R^2: 0.03	5.7 (0.03-1028.3) R^2: 0.09
	HOMA-IR	2.4 (1.3-4.5)** R^2: 0.08	2.5 (1.3-4.6)** R^2: 0.08	1.8 (0.86-3.7) R^2: 0.11
	HOMA-2-IR	4.1 (1.9-9.0)*** R^2: 0.16	4.2 (1.9-9.2)*** R^2: 0.18	3.4 (1.4-8.4)** R^2: 0.18
	- with beta cell function indices			
	HOMA-B	1.0 (0.99-1.0) R^2: 0.00	1.0 (0.99-1.0) R^2: 0.00	0.99 (0.97-1.0) R^2: 0.11
	HOMA-2-B	1.00(0.99-1.0) R^2: 0.00	1.0 (0.99-1.0) R^2: 0.02	0.99 (0.98-1.01) R^2.0.11
	DI	0.99 (0.99-1.0)*** R^2: 0.21	0.99 (0.99-1.0)*** R^2: 0.21	0.99 (0.99-1.0)*** R^2: 0.23
	AI	0.90 (0.86-0.94)*** R^2: 0.27	0.90 (0.86-0.94)*** R^2: 0.28	0.90 (0.86-0.95)*** R^2: 0.29
	IGI	0.49 (0.27-0.89)* R^2: 0.06	0.49 (0.27-0.91)* R^2: 0.06	0.35 (0.17-0.73)** R^2: 0.17

Abbreviations: 5y FU, 5-year follow-up; BMI, body-mass index; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; HIRI, hepatic insulin resistance index; -IR, for insulin resistance; -B, for beta-cell secretion; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

TABLE 7. Logistic regression analysis of glucose tolerance after 5 years follow-up, analysis of subjects with NGM at baseline

Regression analysis depending on clustering factors: BMI status at baseline, insulin indices at baseline. Significance levels are expressed as *p <0.05, **<0.01, and ***<0.001. Glucose metabolism status: NGM vs. PGM, BMI status: 2 categories < or ≥ 25 kg/m² (binary categorical variable)

Odds Ratio (confidence interval) + R^2 (Nagelkerkes)				
Dependent variable	**Parameter**	**Crude model**	**Adjusted for age**	**Adjusted for age and BMI status**
Glucose metabolism status after 5y FU	**- with insulin sensitivity indices**			
	ISI	0.92 (0.75-1.1) R^2: 0.01	0.91 (0.74-1.1) R^2: 0.02	0.94 (0.74-1.2) R^2: 0.03
	HOMA-S	1.0 (0.99-1.0) R^2: 0.01	1.0 (0.99-1.0) R^2: 0.02	1.0 (0.99-1.0) R^2: 0.03
	HOMA-2-S	0.99 (0.96-1.0) R^2: 0.02	0.99 (0.96-1.0) R^2: 0.04	0.99 (0.96-1.0) R^2: 0.04
	- with insulin resistance indices			
	HIRI	24.6 (0.01-51301.3) R^2: 0.01	22.9 (0.01-48899.9) R^2: 0.02	8.4 (0.00-28143.1) R^2: 0.03
	HOMA-IR	1.1 (0.37-3.2) R^2: 0.00	1.1 (0.38-3.3) R^2: 0.01	0.73 (0.19-2.8) R^2: 0.03
	HOMA-2-IR	2.2 (0.58-8.2) R^2: 0.02	2.5 (0.64-9.7) R^2: 0.04	2.2 (0.46-10.7) R^2: 0.04
	- with beta cell function indices			
	HOMA-B	1.0 (0.98-1.0) R^2: 0.00	1.0 (0.98-1.0) R^2: 0.01	0.99 (0.97-1.0) R^2: 0.04
	HOMA-2-B	0.93 (0.98-1.0) R^2: 0.00	1.0 (0.98-1.0) R^2: 0.01	1.0 (0.97-1.0) R^2: 0.03
	DI	1.0 (0.99-1.0) R^2: 0.03	1.0 (0.991-1.0) R^2: 0.04	1.0 (0.99-1.0) R^2: 0.05
	AI	0.93 (0.87-0.99)* R^2: 0.13	0.92 (0.86-0.98)* R^2: 0.15	0.92 (0.85-0.99)* R^2: 0.15
	IGI	0.39 (0.13-1.2) R^2: 0.06	0.41 (0.13-1.2) R^2: 0.06	0.29 (0.08-1.1) R^2: 0.10

Abbreviations: 5y FU, 5-year follow-up; BMI, body-mass index; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; HIRI, hepatic insulin resistance index; -IR, for insulin resistance; -B, for beta-cell secretion; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

5.6 Determination of cut-offs for identification of high-risk pGDM women

5.6.1 ROC-curve analysis

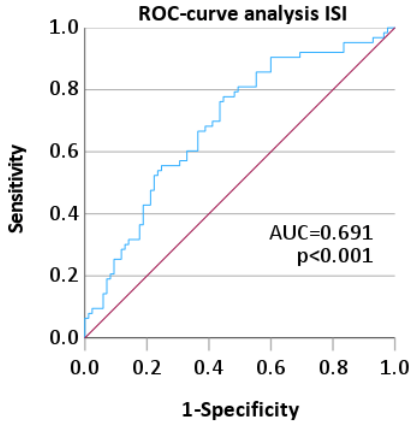
We conducted a Receiver Operating Characteristics analysis to determine the predictive strength of the different insulin sensitivity/insulin resistance and beta cell function indices. A value of 1 would indicate prediction with 100% sensitivity and 100% specificity, a value of 0.5 would indicate a right positive sensitivity and specificity of 50% which indicates that the values are randomly distributed and of no value for prediction.

Figure 5A, 5B and 5C illustrate ROC-curves and ROC Area under the ROC-Curve (ROC-AUCs). The best ROC-Curve was presented by adaptation index (AI), with a result of 0.761 and a p-value < 0.001, followed by DI with a ROC-AUC of 0.743 ($p < 0.001$). The ROC-AUCs of ISI, HOMA-S and HOMA-2-IR were very similar with 0.697, 0.683 and 0.693, respectively ($p < 0.001$).

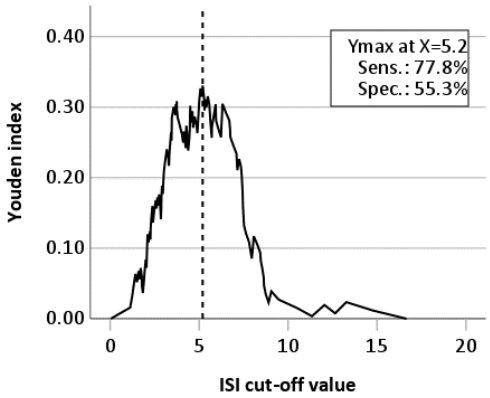
5.6.2 Youden index

To find the best predictive cut-off value of each insulin index, we calculated the Youden index for all point of the ROC curves (Fig. 5A, 5B and 5C) and used the maximum value as the optimal cut-off point for PGM prediction. The index with the overall best results was the adaptation index (AI), with an AUC of 0.761, a Youden Index of 0.431 for a sensitivity of 86% and a specificity of 57% at the cut-off value of ≤ 23.1. Second best was the disposition index (DI) at a cut-off value of ≤ 168.66, presented a Youden index of 0.418, a sensitivity of 64% and a specificity of 80%.

The other three indices ISI, HOMA-S and HOMA-2-IR, representing insulin resistance or insulin sensitivity, presented similar AUC results, and similar Youden indices. The cut-off values



(A)



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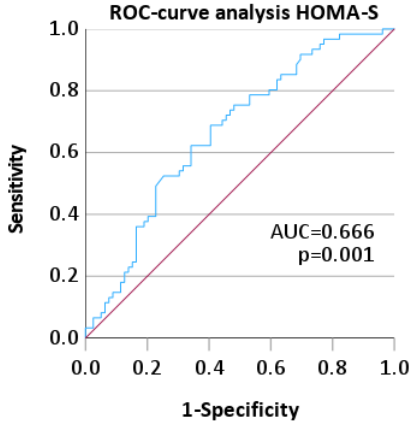

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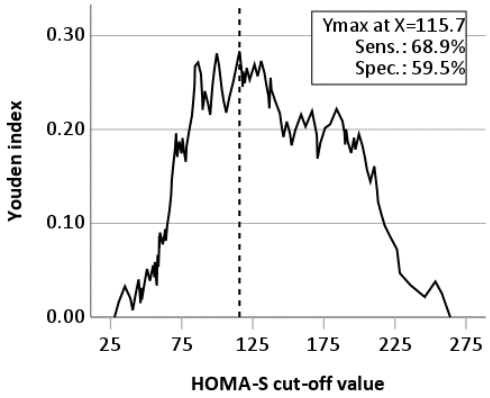

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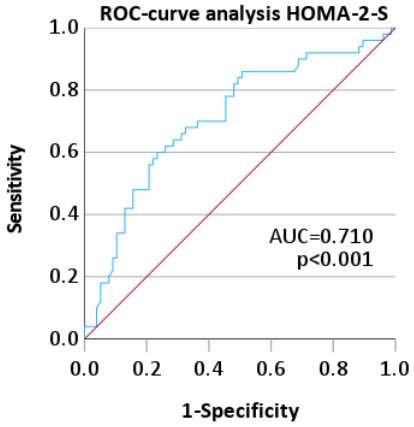

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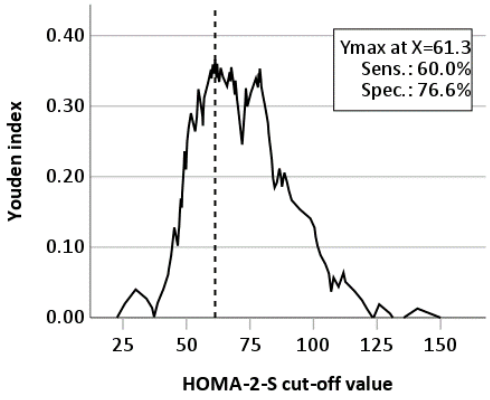
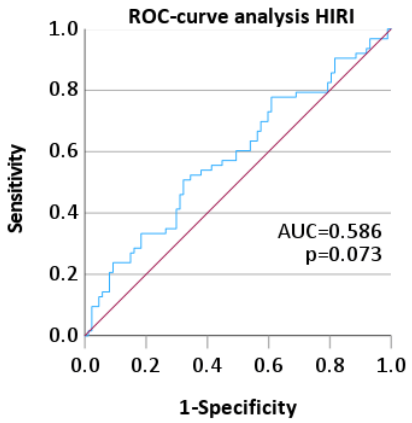


(F)

FIGURE 5. (A) ROC-curves (left) and Youden index graphs (right) for insulin sensitivity (ISI, HOMA-S, HOMA-2-S) indices for prediction of prediabetes/T2DM (whole cohort analysis)



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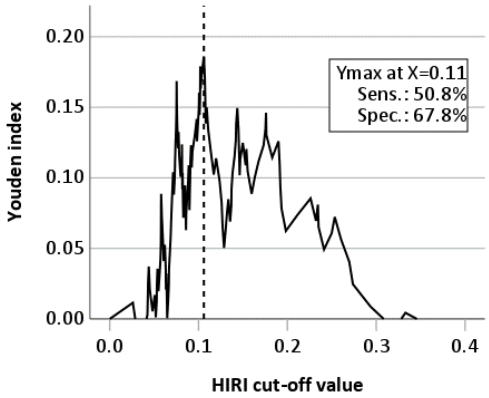

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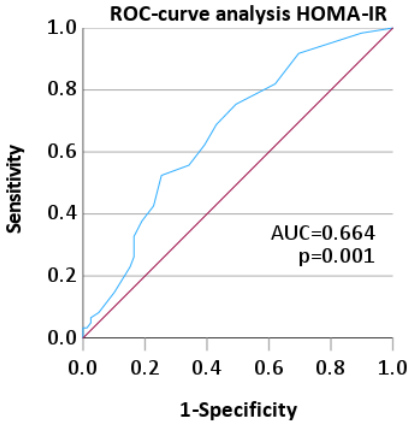

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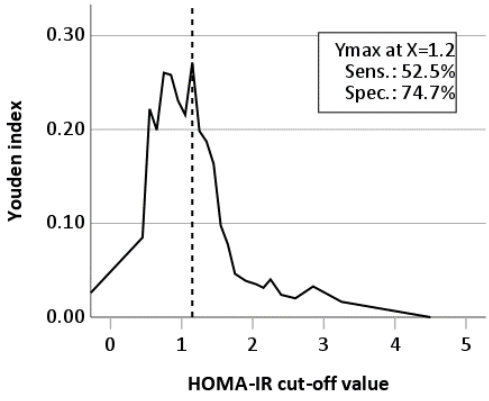

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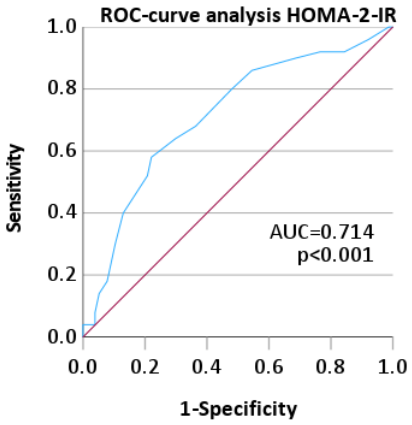

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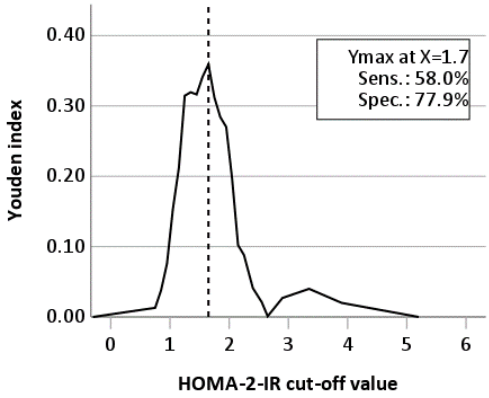
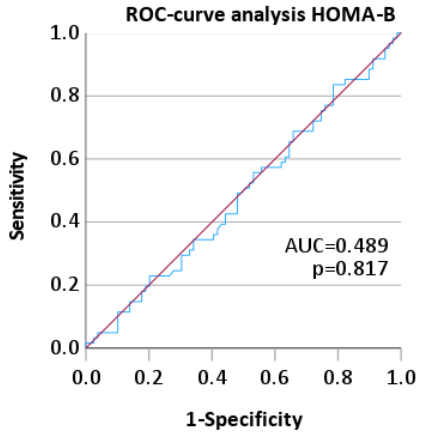


(F)

FIGURE 5. (B) ROC-curves (left) and Youden index graphs (right) for insulin resistance (HIRI, HOMA-IR, HOMA-2-IR) indices for prediction of prediabetes/T2DM (whole cohort analysis)



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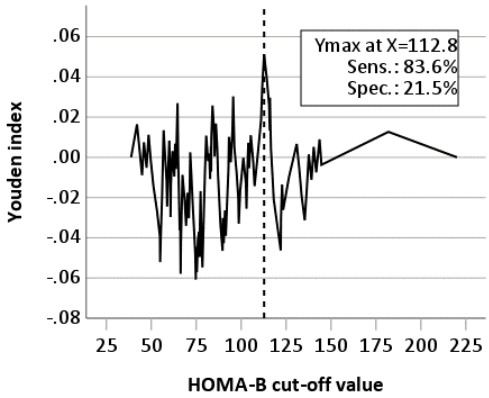

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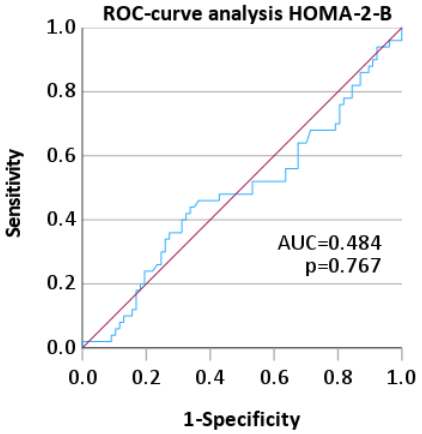

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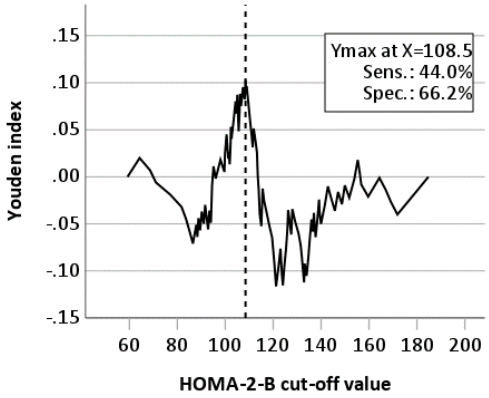
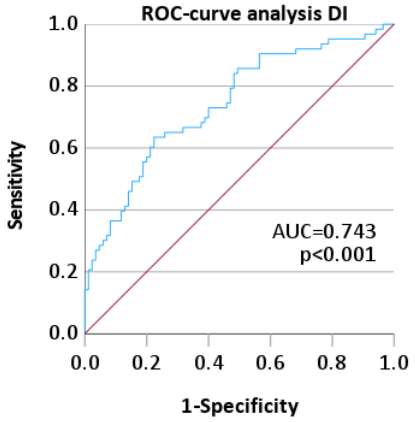


(D)

FIGURE 5. (C) ROC-curves (left) and Youden index graphs (right) for beta-cell function (HOMA-B, HOMA-2-B, DI, AI, IGI) indices for prediction of prediabetes/T2DM (whole cohort analysis)



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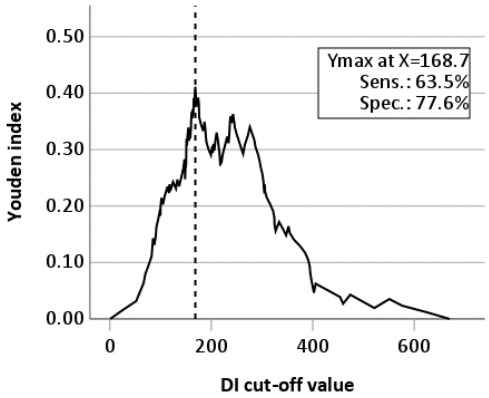

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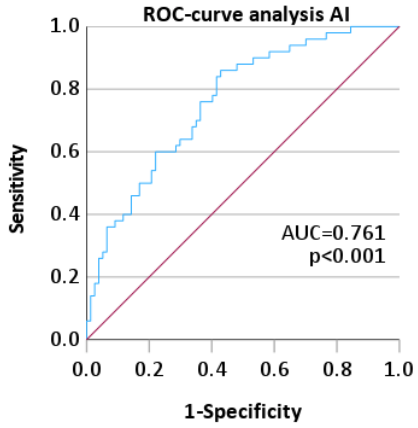

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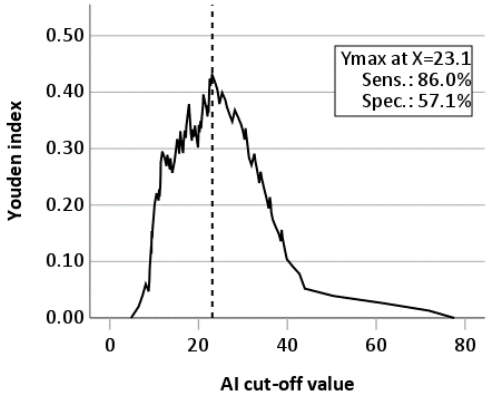

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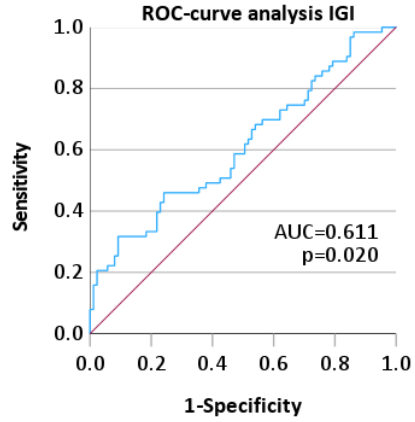

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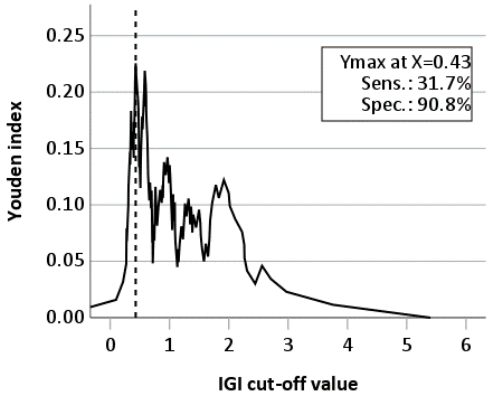


(F)

FIGURE 5. (D) ROC-curves (left) and Youden index graphs (right) for beta-cell function (HOMA-B, HOMA-2-B, DI, AI, IGI) indices for prediction of prediabetes/T2DM (whole cohort analysis)

were ≤ 5.2 for the ISI, ≤ 100.2 for the HOMA-S and ≥ 1.7 for the HOMA-2-IR. Table 8 summarises the cut-off values and sensitivity-specificity pairs of different indices predict disturbed glucose homeostasis at 5-year follow up after GDM.

TABLE 8. Cut-off value for prediction of prediabetes/T2DM of each index according to the highest Youden Index (results for the whole cohort)

Sensitivity, specificity and PPV refers to the Youden index. Cut-offs using above/below median or first/third quartile are given for comparison.

Index	ROC -AUC	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	Youden Index	Cut-off median	Cut-off quartile
ISI	0.691	**≤ 5.2**	77.8	55.3	55.8	0.331	**≤ 5.5**	**≤ 3.7**
HOMA-S	0.666	**≤ 115.7**	68.9	59.5	55.2	0.284	**≤ 137.9**	**≤ 88.7**
HOMA-2-S	0.710	**≤ 61.3**	60.0	76.6	65.0	0.366	**≤ 78.6**	**≤ 63.5**
HOMA-2-IR	0.714	**≥ 1.7**	58.0	77.9	65.5	0.287	**≥ 1.3**	**≥ 1.6**
DI	0.743	**≤ 168.7**	63.5	77.6	67.3	0.411	**≤ 246.5**	**≤ 179.8**
AI	0.761	**≤ 23.1**	86.0	57.1	59.2	0.431	**≤ 24.7**	**≤ 18.3**
IGI	0.611	**≤ 0.43**	31.7	90.8	71.4	0.225	**≤ 0.82**	**≤ 0.57**

Abbreviations: ROC, receiver operating characteristics; AUC, area under the curve; PPV, positive predictive value; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; -IR, for insulin resistance; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

5.7 Identification or characterisation of variables for clustering within the pGDM cohort

According to our data on the association of ISI, HOMA-S, HOMA-2-S, HOMA-IR and HOMA-2-IR with PGM in pGDM women with BMI < 25 kg/m2 but not in subjects with overweight/obesity (Table 5) and a significant association of IGI with PGM in the overweight/obesity group only, we hypothesised that there may be different pathogenetic mechanisms of diabetes development in these two groups. Therefore, we separate subjects into two BMI groups and used indices for insulin sensitivity/resistance and beta cell function measured at baseline that were highly significant different in NGM and PGM subjects.

Clustering of the cohorts were performed using cut-offs for indices determined by ROC-plot analysis (Table 8), and by indices median and quartiles of the pGDM subjects with a normal glucose tolerance (Table 2A, below median of NGM subjects: ISI 5.5, HOMA-S 137.9, HOMA-2-S 78.6, DI 246.5, AI 22.6, IGI 0.82; above median of NGM subjects: HOMA-2-IR 1.3), within 1st quartile: ISI 3.7, HOMA-S 88.7, HOMA-2-S 63.5, DI 179.8, AI 16.2, IGI 0.57; within 4th quartile: HOMA-2-IR 1.6).

When the cohort was stratified by BMI and ROC curves/Youden index cut-offs is shown in Table 9A. This results in a division of our study population into four similar-sized groups. Major differences between NGM and PGM at 5-year follow-up were found for variables of insulin sensitivity (ISI [22.6% vs 48.5%], HOMA-S [25.5% vs. 50.0%], HOMA-2-S [22.2% vs. 67.7%] and HOMA-2-IR [23.1% vs. 70.0%]) and beta cell function (DI [21.0% vs. 62.5%], AI [16.7% vs. 51.9%] and IGI [23.5% vs. 60.0%]) in subjects with normal weight and for DI (38.5% vs. 69.4%) and IGI (50.0% vs. 100.0%) in pGDM women with overweight/obesity. The insulinogenic index (IGI) showed significant differences between the subjects with normal weight and the ones with overweight/obesity at 5 years, when comparing the proportion of PGM in the lower IGI groups (23.5% vs. 50.0%), and also when comparing the higher IGI groups (60.0% vs. 100.0%).

Subdividing the cohort according to BMI and below median cut-off is shown in Table 9B. This resulted in in four similar-sized groups. Major differences between NGM and PGM at 5-year follow-up were found for variables of insulin sensitivity (ISI [21.6% vs. 48.6%], HOMA-2-S [17.5% vs. 42.9%] and HOMA-2-IR [16.2% vs. 42.1%]) and beta cell function (DI [13.9% vs. 46.0%] and AI [12.5% vs. 48.6%]) in subjects with normal weight and for DI (28.6% vs. 64.6%) in pGDM women with overweight/obesity. The insulinogenic index (IGI) showed significant differences between the lean and subjects with overweight/obesity at 5 years, when comparing the proportion of PGM in the lower IGI groups (34.5% vs. 75.0%).

TABLE 9. (A) Manual clustering of the whole cohort using ROC-plot and BMI cut-off values

Distribution of the subjects after clustering using the cut-off value from ROC curve analysis (Youden-Index). Subject developing T2DM during follow-up are included. *indicates the significant differences when comparing with BMI status. Significance levels are expressed as *<0.05, **<0.01, and ***<0.001

	BMI <25 kg/m²			BMI ≥ 25 kg/m²		
ISI status (cut-off ≤ 5.2)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/197	9 (13.6%)	19 (44.2%)	<0.001	1 (9.1%)	45 (58.4%)	0.002
PGM at 5y FU n[%], n=63/148	12 (22.6%)	16 (48.5%)	0.013	2 (25.0%)	33 (61.1%)	0.055
DI status (cut-off ≤ 168.7)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/197	13 (15.9%)**	15 (55.6%)	<0.001	17 (38.6%)**	29 (65.9%)	0.010
PGM at 5y FU n[%], n=63/148	13 (21.0%)	15 (62.5%)	<0.001	10 (38.5%)	25 (69.4%)	0.015
AI status (cut-off ≤ 22.1)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=44/124	4 (8.3%)***	14 (51.9%)	<0.001	2 (20.0%)***	24 (55.8%)	0.041
PGM at 5y FU n[%], n=50/124	8 (16.7%)***	14 (51.9%)	0.001	4 (40.0%)***	24 (57.1%)	0.328
HOMA-S status (cut-off ≤ 115.7)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=70/186	11 (16.4%)	13 (40.6%)	0.009	7 (35.0%)	39 (58.2%)	0.068
PGM at 5y FU n[%], n=61/140	14 (25.5%)	12 (50.0%)	0.033	5 (45.5%)	30 (60.0%)	0.377
HOMA-2-S status (cut-off ≤ 61.3)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=44/128	11 (17.5%)	7 (58.3%)	0.002	2 (12.5%)	24 (64.9%)	<0.001
PGM at 5y FU n[%], n=50/127	14 (22.2%)	8 (67.7%)	0.002	6 (37.5%)	22 (61.1%)	0.115
HOMA-2-IR status (cut-off ≥ 1.7)	**Lower**	**Above**	*p-value*	**Lower**	**Above**	*p-value*
PGM at baseline n[%], n=44/128	12 (18.5%)	6 (60.0%)	0.004	2 (12.5%)	24 (64.9%)	<0.001
PGM at 5y FU n[%], n=50/127	15 (23.1%)	7 (70.0%)	0.002	6 (37.5%)	22 (61.1%)	0.115
IGI status (cut-off ≤ 0.43)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/199	16 (18.4%)***	12 (50.0%)*	0.002	37 (47.4%)***	9 (90.0%)*	0.011
PGM at 5y FU n[%], n=63/150	16 (23.5%)**	12 (60.0%)*	0.002	27 (50.0%)**	8 (100.0%)*	0.008

Abbreviations: ROC, receiver operating characteristics; BMI, body-mass index; PGM, pathological glucose metabolism; 5y FU, 5-year follow-up; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; -IR, for insulin resistance; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

TABLE 9. (B) Manual clustering of the whole cohort using median and BMI cut-off values

Distribution of the subjects after clustering using the cut-off value below median. Subject developing T2DM during follow-up are included. *indicates the significant differences when comparing with BMI status. Significance levels are expressed as *<0.05, **<0.01, and ***<0.001

	BMI <25 kg/m²			BMI ≥ 25 kg/m²		
ISI status (cut-off ≤ 5.5)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/197	9 (14.1%)	19 (42.2%)	<0.001	1 (14.3%)	45 (55.6%)	0.036
PGM at 5y FU n[%], n=63/148	11 (21.6%)	17 (48.6%)	0.009	1 (25.0%)	34 (58.6%)	0.190
DI status (cut-off ≤ 246.5)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/197	6 (11.8%)	22 (37.9%)**	0.002	5 (22.7%)	41 (62.1%)**	0.001
PGM at 5y FU n[%], n=63/148	5 (13.9%)	23 (46.0%)	0.002	4 (28.6%)	31 (64.6%)	0.017
AI status (cut-off ≤ 24.7)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=44/124	4 (10.0%)***	14 (40.0%)	0.002	1 (14.3%)***	25 (54.3%)	0.048
PGM at 5y FU n[%], n=50/124	5 (12.5%)***	17 (48.6%)	<0.001	2 (28.6%)***	26 (57.9%)	0.149
HOMA-S status (cut-off ≤ 137.9)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=70/186	7 (12.5%)	17 (39.5%)	0.002	2 (20.0%)	44 (57.1%)	0.027
PGM at 5y FU n[%], n=50/140	12 (26.7%)	14 (41.2%)	0.174	1 (25.0%)	34 (59.6%)	0.176
HOMA-2-S status (cut-off ≤ 78.6)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=44/128	4 (10.0%)	14 (40.0%)	0.002	0 (0.0%)	26 (55.3%)	0.011
PGM at 5y FU n[%], n=50/127	7 (17.5%)	15 (42.9%)	0.016	1 (16.7%)	27 (58.7%)	0.052
HOMA-2-IR status (cut-off ≥ 1.3)	**Lower**	**Above**	*p-value*	**Lower**	**Above**	*p-value*
PGM at baseline n[%], n=44/128	3 (8.1%)	15 (39.5%)	0.001	0 (0.0%)	26 (54.2%)	0.021
PGM at 5y FU n[%], n=50/127	6 (16.2%)	16 (42.1%)	0.014	1 (20.0%)	27 (57.4%)	0.110
IGI status (cut-off ≤ 0.82)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/199	9 (21.4%)**	19 (27.5%)**	0.472	26 (47.3%)**	20 (60.6%)**	0.225
PGM at 5y FU n[%], n=63/150	8 (26.7%)	20 (34.5%)***	0.456	17 (44.7%)	18 (75.0%)***	0.019

Abbreviations: ROC, receiver operating characteristics; BMI, body-mass index; PGM, pathological glucose metabolism; 5y FU, 5-year follow-up; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; -IR, for insulin resistance; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

TABLE 9. (C) Manual clustering of the whole cohort using lowest or highest quartile and BMI cut-off values

Distribution of the subjects after clustering using the cut-off value first quartile (ISI, DI, AI, HOMA-S, HOMA-2-S, IGI) or fourth quartile (HOMA-2-IR). Subject developing T2DM during follow-up are included. *indicates the significant differences when comparing with BMI status. Significance levels are expressed as *<0.05, **<0.01, and ***<0.001

	BMI <25 kg/m²			BMI ≥ 25 kg/m²		
ISI status (cut-off ≤ 3.7)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/197	19 (21.1%)	9 (47.4%)	0.017	11 (35.5%)	35 (61.4%)	0.020
PGM at 5y FU n[%], n=63/148	22 (30.1%)	6 (46.2%)	0.256	7 (35.0%)	28 (66.7%)	0.019
DI status (cut-off ≤ 179.8)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/197	11 (14.1%)**	17 (54.8%)	<0.001	15 (37.5%)**	31 (64.6%)	<0.001
PGM at 5y FU n[%], n=63/148	12 (20.7%)*	16 (57.1%)	<0.001	10 (43.5%)*	25 (64.1%)	0.114
AI status (cut-off ≤ 18.3)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=44/124	10 (16.4%)***	8 (57.1%)**	0.001	3 (20.0%)***	23 (60.5%)**	0.008
PGM at 5y FU n[%], n=50/124	14 (23.0%)***	8 (57.1%)**	0.011	6 (40.0%)***	22 (59.5%)**	0.202
HOMA-S status (cut-off ≤ 88.7)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=70/186	15 (18.3%)*	9 (52.9%)	0.002	12 (38.7%)*	34 (60.7%)	0.049
PGM at 5y FU n[%], n=61/140	18 (26.9%)*	8 (66.7%)	0.007	11 (57.9%)*	24 (57.1%)	0.956
HOMA-2-S status (cut-off ≤ 63.5)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=44/128	8 (13.6%)	10 (62.5%)	<0.001	2 (14.3%)	24 (61.5%)	<0.001
PGM at 5y FU n[%], n=50/127	13 (22.0%)	9 (56.3%)	0.008	5 (35.7%)	23 (60.5%)	0.111
HOMA-2-IR status (cut-off ≥ 1.6)	**Lower**	**Above**	*p-value*	**Lower**	**Above**	*p-value*
PGM at baseline n[%], n=44/128	8 (13.8%)	10 (58.8%)	<0.001	2 (14.3%)	24 (61.5%)	0.002
PGM at 5y FU n[%], n=50/127	13 (22.4%)	9 (52.9%)	0.015	5 (35.7%)	23 (60.5%)	0.111
IGI status (cut-off ≤ 0.57)	**Above**	**Lower**	*p-value*	**Above**	**Lower**	*p-value*
PGM at baseline n[%], n=74/199	15 (21.1%)**	13 (32.5%)**	0.185	31 (46.3%)**	15 (71.4%)**	0.044
PGM at 5y FU n[%], n=63/150	13 (24.1%)*	15 (44.1%)**	0.049	22 (46.8%)*	13 (86.7%)**	0.007

Abbreviations: ROC, receiver operating characteristics; BMI, body-mass index; PGM, pathological glucose metabolism; 5y FU, 5-year follow-up; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; -IR, for insulin resistance; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

Stratifying the cohort according to BMI and within 1st or 4th quartile is shown in Table 9C. Again, four similar-sized groups were present. As shown in Table 8 cut-offs using quartiles were very similar to Youden Index for HOMA-2-S, HOMA-2-IR, DI and IGI. By quartile cut-offs, major differences between NGM and PGM at 5-year follow-up were found for variables of insulin sensitivity (HOMA-S [26.9% vs. 66.7%], HOMA-2-S [22.0% vs. 56.3%] and HOMA-2-IR [22.4% vs. 52.9%]) and beta cell function (DI [20.7% vs. 57.1%], AI [23.0% vs. 57.1%] and IGI [24.1% vs. 44.1%]) in subjects with normal weight and for ISI (35.0% vs. 66.7%) and IGI (46.8% vs. 86.7%) in pGDM women with overweight/obesity.

TABLE 10. Discrimination between pGDM women having PGM at 5 years follow-up using different insulin sensitivity and beta cell function indices

Percentages of subjects with PGM below cut-off values for abnormal beta cell function and insulin resistance indices were subtracted by percentages above cut-offs. Different cut-offs derived from ROC analysis, below median and lowest quartiles (ISI, DI. AI, IGI) /highest quartiles (HOMA-2-IR).

	Youden index		Median		Quartile	
Indices	**Lean**	**Overweight**	**Lean**	**Overweight**	**Lean**	**Overweight**
ISI	25.9%	36.1%	27%	33.6%	16.1%	31.7%
DI	41.5%	30.9%	32.1%	36.0%	36.4%	20.6%
AI	35.2%	17.1%	36.1%	29.2%	34.1%	20.6%
HOMA-2-IR	46.9%	23.6%	25.9%	37.4%	30.5%	24.8%
IGI	36.5%	50.0%	7.8%	30.3%	20.0%	39.9%

Abbreviations: pGDM, post-gestational diabetes mellitus; PGM, pathological glucose metabolism; ROC, receiver operating characteristics; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; -IR, for insulin resistance; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

In summary, indices obtained from 75 g OGTT revealed those with the best abilities to discriminate glucose metabolism status in the cross-sectional study at baseline and at 5 years is the ROC AUC analysis. As shown in Table 10, HOMA-2-IR ranked highest, followed by DI and IGI in the population with normal weight. In contrast, in individuals with overweight/obesity IGI was found to possess the best discriminator abilities followed by DI and ISI.

Next, we analysed whether the addition of the adipokine leptin, which was found to be significantly associated with PGM at baseline (Table 1), can serve as an additional predictor for progression to PGM in our cohort. In ROC-AUC analysis the optimal cut-off for leptin to identify PGM at 5 years in women after GDM was 9.7 ng/ml. Leptin plasma levels were strongly associated with BMI (Spearman: 0.812, $p < 0.001$) and did not represent an independent predictor of prediabetes.

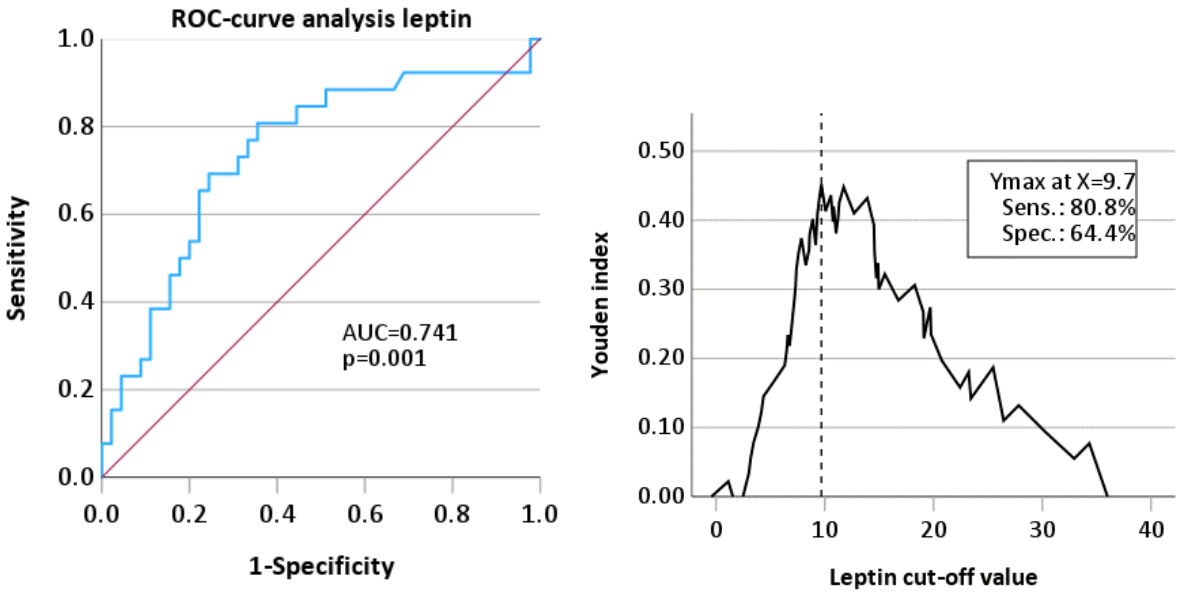


FIGURE 6. ROC-curve (left) and Youden index graph (right) for leptin plasma levels (whole cohort analysis)

In a binary regression analysis of indices for beta cell function and insulin sensitivity leptin was adjusted for age and BMI category (see Table 11). It was observed that DI and AI kept their significance (both $p < 0.01$) with an Odds-Ratio of 0.99 (CI 0.98-1.0) for DI, and 0.87 (CI 0.79-0.97) for AI. Use of AI and DI presented a better prediction model to the 5-year follow-up glucose metabolism of our subjects after adjustment for leptin (Nagelkerkes R^2 of 0.40 and 0.44 compared to 0.29 and 0.23, respectively).

TABLE 11. Binary regression analysis with leptin

Regression analysis depending on clustering factors: BMI status, indices. Significance levels are expressed as *<0.05, **<0.01, and ***<0.001. Glucose metabolism status: NGM vs. PGM, BMI status: 2 categories < or ≥ 25 kg/m² (binary categorical variable)

Odds Ratio (CI) + R^2 (Nagelkerkes)			
Dependent variable	**Parameter**	**Adjusted for age and BMI status**	**Adjusted for age, BMI status and leptin**
Glucose metabolism status after 5y FU	**Age [years]**	1.0 (0.95-1.1)	1.1 (0.96-1.3)
	BMI status	2.9 (1.5-5.7)**	2.1 (0.42-10.8)
	Leptin [ng/ml]		1.1 (1.0-1.2)
		R^2: 0.09	R^2: 0.24
	- Insulin sensitivity indices		
	ISI	0.79 (0.67-0.95)*	1.0 (0.78-1.4)
		R^2: 0.14	R^2: 0.24
	HOMA-S	0.99 (0.98-1.0)*	1.0 (0.98-1.0)
		R^2: 0.13	R^2: 0.26
	HOMA-2-S	0.97 (0.95-0.99)**	1.0 (0.96-1.0)
		R^2: 0.18	R^2: 0.25
	- Insulin resistance indices		
	HIRI	5.7 (0.03-1028.3)	0.00 (0.00-2.3)
		R^2: 0.09	R^2: 0.30
	HOMA-IR	1.8 (0.86-3.7)	1.6 (0.50-4.9)
		R^2: 0.11	R^2: 0.27
	HOMA-2-IR	3.4 (1.4-8.4)**	1.8 (0.56-5.6)
		R^2: 0.18	R^2: 0.26
	- Beta cell function indices		
	HOMA-B	0.99 (0.97-1.0)	0.98 (0.96-1.0)
		R^2: 0.11	R^2: 0.28
	HOMA-2-B	0.99 (0.98-1.0)	0.99 (0.96-1.0)
		R^2.0.11	R^2: 0.26
	DI	0.99 (0.99-1.0)***	0.99 (0.98-1.0)**
		R^2: 0.23	R^2: 0.44
	AI	0.90 (0.86-0.95)***	0.87 (0.79-0.97)**
		R^2: 0.29	R^2: 0.40
	IGI	0.35 (0.17-0.73)**	0.12 (0.02-0.65)*
		R^2: 0.17	R^2: 0.40

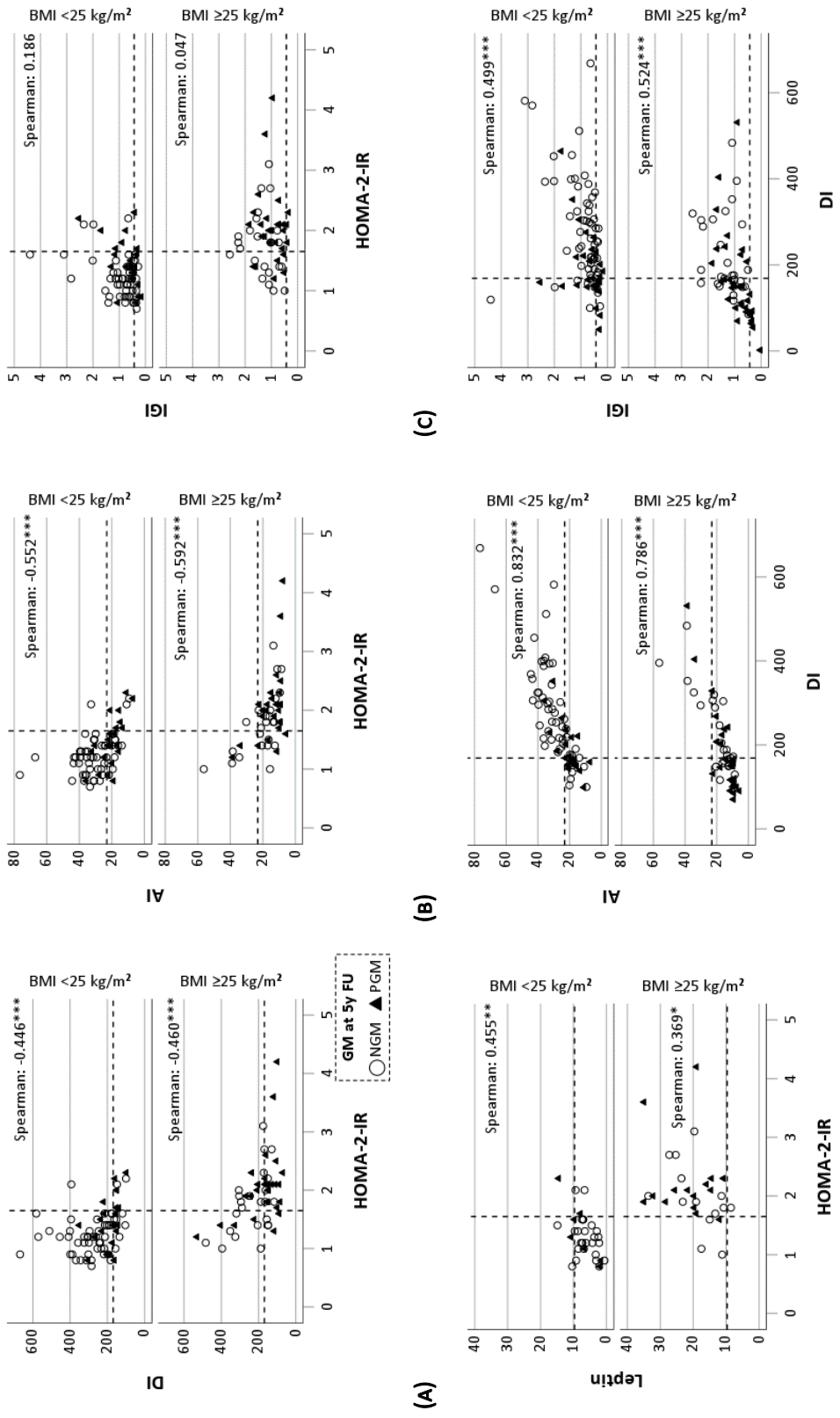
Abbreviations: CI, confidence interval; 5y FU, 5-year follow-up; BMI, body-mass index; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; HIRI, hepatic insulin resistance index; -IR, for insulin resistance; -B, for beta-cell secretion; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

The other three indices that were significantly associated with PGM after adjusting for age and BMI status (ISI, HOMA-S, HOMA-2-S and HOMA-2-IR), all indices for insulin sensitivity or resistance, lost their significance when adjusting for leptin as well, indicating that circulating leptin levels are strongly related to insulin resistance.

5.8 Clustering of pGDM women

We observed in the present study that BMI, markers for pancreatic beta cell function (DI, AI, IGI) and insulin resistance (HOMA-2-IR, leptin) are associated with risk to have PGM 5 years after GDM. Next, we ask the question on the usefulness to combine individual variables to define clusters associated with low or high risk for pathological glucose metabolism. Figure 7 illustrates the separation and discrimination between different indices and the Spearman-Rank correlation coefficients in subjects divided by BMI < 25 und ≥ 25 kg/m².

Figure 7 demonstrates that HOMA-2-IR and AI and HOMA-2-IR and DI differentiated between PGM and NGM in subjects with by BMI < 25 and ≥ 25 kg/m2. As expected, there was a high positive correlation between AI and DI ($p < 0.001$). Leptin was highly associated with insulin resistance (high HOMA-2-IR in subjects with normal weight ($p < 0.01$) and with overweight/obesity ($p < 0.05$)). IGI was not associated with HOMA-2-IR but with leptin in subjects with overweight/obesity ($p < 0.01$).



Significance levels are expressed as *<0.05, **<0.01, and ***<0.001

FIGURE 7. Correlation of indices for sensitivity, beta cell function and leptin and glucose metabolism at 5 years divided by BMI status at baseline

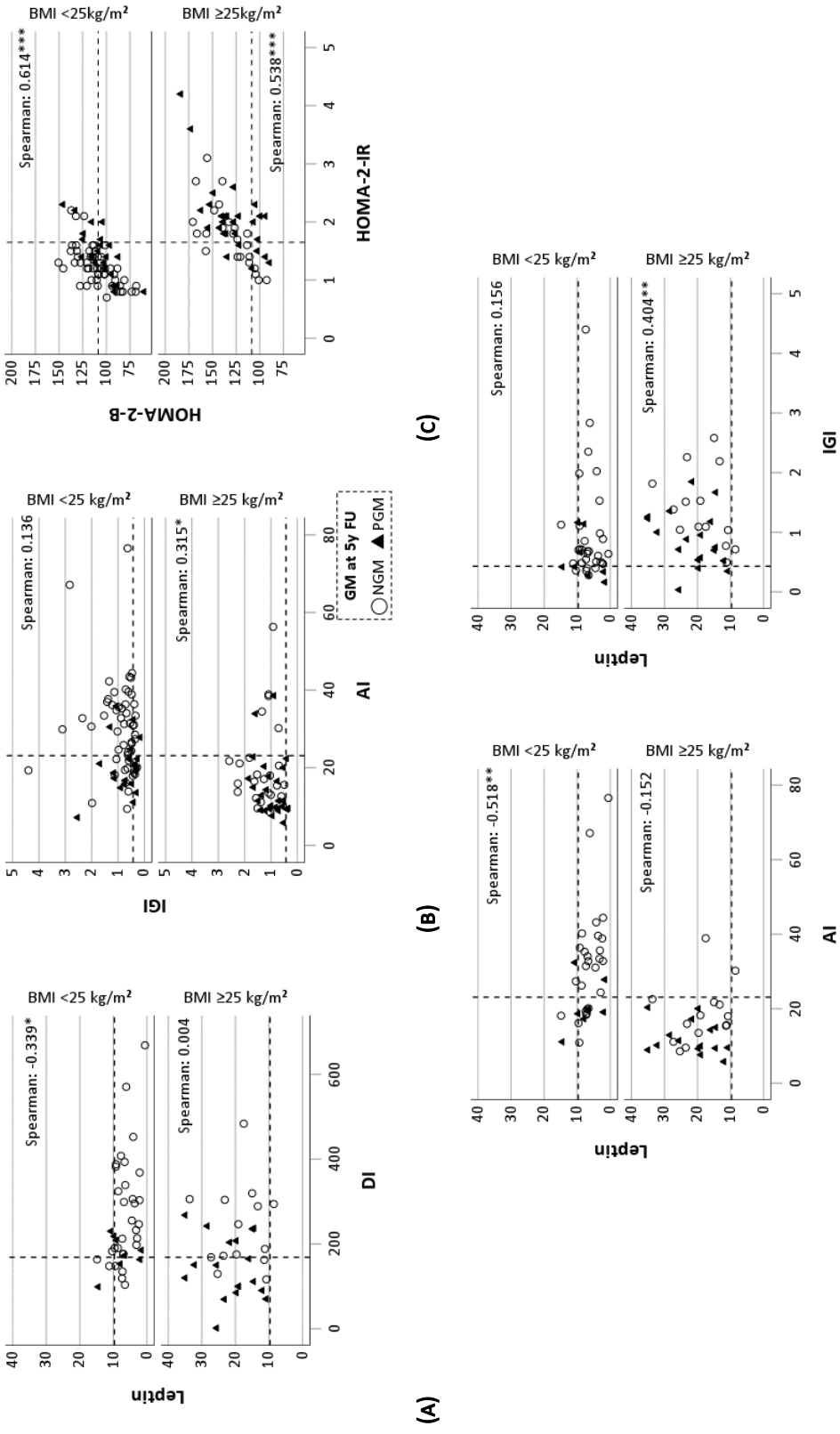


FIGURE 7. Correlation of indices for sensitivity, beta cell function and leptin and glucose metabolism at 5 years divided by BMI status at baseline

From these data we selected AI and HOMA-2-IR to deconstruct heterogeneity within pGDM women, because both are based on measurement of glucose and C-peptide and IGI (based on glucose and insulin at 0 and 30 min) to perform clustering and calculated positive predictive values. Possible clusters were defined as:

- **Cluster A:** overweight/obesity, beta-cell dysfunction (low AI), insulin resistance (increased HOMA-2-IR)
- **Cluster A-2:** overweight/obesity, beta-cell dysfunction (low IGI), insulin resistance (increased HOMA-2-IR)
- **Cluster B:** overweight/obesity, no insulin resistance (normal HOMA-2-IR) and no decreased beta cell function (normal AI)
- **Cluster B-2:** overweight/obesity, no insulin resistance (normal HOMA-2-IR) and no decreased beta cell function (normal IGI)
- **Cluster C:** overweight/obesity and not in cluster A or B (mixed population with either normal HOMA-2-IR and low AI or IGI or increased HOMA-2-IR and normal AI or IGI)
- **Cluster D:** normal weight and decreased beta cell function (AI low), no increased insulin resistance
- **Cluster D-2:** normal weight and decreased beta cell function (low IGI), no increased insulin resistance
- **Cluster E:** normal weight, decreased beta cell function (AI low) and insulin resistance (increased HOMA-2-IR)
- **Cluster E-2:** normal weight, decreased beta cell function (low IGI) and insulin resistance (increased HOMA-2-IR)

- **Cluster F:** normal weight and not in cluster D or E (mixed population with normal AI and either normal or increased HOMA-2-IR)

Table 12A summarises the number of individuals in the different clusters. As expected, only few subjects displayed a relative healthy metabolism and fulfilled the criteria of clusters B (5.5%) or B2 (12.5%). Low IGI was related to prediabetes/diabetes development in 100%. However, low IGI was detected in only a minority (3 women with overweight/obesity and 2 women with normal weight) indicating that this parameter is not well suited for clustering of our cohort. The majority of women with overweight/obesity were grouped in cluster A (63.5%) whereas in the normal weight group subjects predominantly present a mixed phenotype (cluster F: 64.0%). Analysis of prediabetes/diabetes at 5 years follow-up demonstrates that within the clusters the highest percentage of PGM was detected in cluster E (77.8%) followed by cluster A (63.6%) and cluster B (42.9%) (Table 12B).

TABLE 12. (A) Numbers of subjects subclassified into different clusters

Cluster definition is described at page 77. C-peptide was measured for a total of 127 subjects

BMI	Clusters	HOMA-2-IR and AI	HOMA-2-IR and IGI
≥ 25kg/m²	Cluster A/A2	33	3
	Cluster B/B2	7	16
	Cluster C	12	33
	Total overweight	52	52
<25kg/m²	Cluster D/D2	18	15
	Cluster E/E2	9	2
	Cluster F	48	58
	Total normal weight	75	75
Total:		127	127

Abbreviations: BMI, body-mass index; HOMA, homeostatic assessment; -IR, for insulin resistance; AI, adaptation index; IGI, insulinogenic index

TABLE 12. (B) Percentage of subjects in each cluster presenting PGM at baseline or PGM at 5 years follow-up (n=127)

BMI	Clusters	PGM at baseline	PGM at 5yFU	Total	Clusters	PGM baseline	PGM 5yFU	Total
≥ 25kg/m²	Cluster A	22 (66.7%)	21 (63.6%)	33	Cluster A2	3 (100.0%)	3 (100.0%)	3
	Cluster B	1 (14.3%)	3 (42.9%)	7	Cluster B2	2 (12.5%)	6 (37.5%)	16
	Cluster C	2 (16.7%)	4 (33.3%)	12				
<25kg/m²	Cluster D	8 (44.4%)	7 (38.9%)	18	Cluster D2	5 (33.3%)	7 (46.7%)	15
	Cluster E	6 (66.7%)	7 (77.8%)	9	Cluster E2	2 (100.0%)	2 (100.0%)	2
	Cluster F	4 (8.3%)	8 (16.7%)	48				

Abbreviations: BMI, body-mass index; PGM, pathological glucose metabolism; 5y FU, 5-year follow-up

Next, we analysed prediction of the development of prediabetes/T2DM within individual clusters. The highest risk for development of prediabetes was found in cluster A and cluster E representing individuals with a combination of low AI and increased insulin resistance with and without overweight/obesity (Table 13). The other groups including clusters B, C and D had an intermediate risk (PPV of 42.9%, 33.3% and 38.9% respectively) whereas cluster F represents individuals with normal weight with a relatively low 5-year prediabetes/diabetes risk (16.7%).

TABLE 13. Risk for having prediabetes/T2DM 5 years after GDM in clusters A-F

Sensitivity, specificity and positive predictive value of proposed clusters

BMI	Clusters	Sens.	Spec.	PPV	Clusters	Sens.	Spec.	PPV
≥ 25kg/m²	Cluster A	75.0%	50.0%	63.6%	Cluster A2	10.7%	100.0%	100.0%
	Cluster B	10.7%	83.3%	42.9%	Cluster B-2	10.7%	75.0%	33.3%
	Cluster C	14.3%	66.7%	33.3%				
<25kg/m²	Cluster D	31.8%	79.2%	38.9%	Cluster D-2	31.8%	84.9%	46.7%
	Cluster E	31.8%	96.2%	77.8%	Cluster E-2	9.1%	100.0%	100.0%
	Cluster F	36.4%	24.5%	16.7%				

Abbreviations: BMI, body-mass index; PPV, positive predictive value

TABLE 14. Risk for having prediabetes/T2DM 5 years after GDM in clusters derived by HOMA-2-IR and HOMA-2-B variables

Sensitivity, specificity and positive predictive value of different clusters

BMI	Clusters	N	Sensitivity	Specificity	PPV
≥ 25kg/m²	Cluster A - HOMA-2-B	5	17.9%	100.0%	100%
	Cluster B - HOMA-2-B	8	7.1%	75.0%	25.0%
	Cluster C - HOMA-2-B	39	75.0%	25.0%	53.8%
<25kg/m²	Cluster D - HOMA-2-B	32	50.0%	60.4%	34.4%
	Cluster E - HOMA-2-B	3	9.1%	98.1%	66.7%
	Cluster F - HOMA-2-B	40	40.9%	41.5%	22.5%

Abbreviations: BMI, body-mass index; PPV, positive predictive value; HOMA, homeostatic assessment; -B, for beta-cell secretion

Since the determination of AI is too complicated for clinical routine practice, we asked the question whether it is possible to replace the AI index by HOMA-2-B. Therefore, we re-calculated sensitivity, specificity and PPV in clusters A-F using HOMA-2-B (Youden index cut-off 108.5) as parameter of beta cell function assessed by fasting glucose and C-peptide measurement. As shown in Table 14, HOMA-2-B displayed a high specificity and PPV but a very low sensitivity in clusters A and E. HOMA-2-B cluster C was associated with a high sensitivity (75%) and PPV (53.8%) but a low specificity (25.0%). The others cluster based on HOMA-2-B were associated with an intermediate risk (PPV 25.0-34.4%). Overall, these data indicate that the use of HOMA-2-B as the index for beta-cell function is much less correlated with development of prediabetes/T2DM as compared to AI and therefore may not represent a valuable parameter for clustering in pGDM women.

From these data we identified three clinically feasible clusters based on two research variables (AI, HOMA-2-IR) and clinical phenotype (BMI), which we labelled pGDM cluster 1, 2, and 3.

- **Cluster 1 (high risk):** pGDM women with decreased beta-cell function (low AI) and high

insulin resistance (increased HOMA-2-IR) irrespective of body weight (cluster A and E together)

- **Cluster 2 (intermediate risk):** pGDM women not in cluster 1 or 3 (women with normal weight with or without insulin resistance and with low beta-cell function, women with overweight/obesity with high insulin resistance or low beta-cell function) (cluster B, C and D together)

- **Cluster 3 (low risk):** pGDM, women with normal weight with normal beta-cell function (normal AI) (cluster F)

As illustrated in Figure 8, each cluster consists of about a third of our cohort. Subjects in pGDM cluster 1 had the highest 5-year risk for prediabetes/T2DM (66.7%) which was much higher as compared to the total population (42.0%) and women stratified according to BMI in normal weight (31.8%) or overweight/obesity (56.5%) (Table 5 and 15). pGDM cluster 2 represents a mixed phenotype with moderate beta cell dysfunction or insulin resistance and a 5-year risk of 37.8%. pGDM cluster 3 includes subjects with relatively healthy clinical phenotype and a preserved beta cell function and thus possessed the lowest prediabetes/T2DM risk (16.7%).

TABLE 15. Association of pGDM clusters 1-3 with prediabetes/T2D outcome

Prevalence of prediabetes/T2D 5 years after GDM; sensitivity, specificity and PPV for prediabetes/T2D in pGDM clusters 1, 2, and 3.

	N	PGM baseline	PGM 5y FU	Sensitivity	Specificity	PPV
pGDM cluster 1	42	66.7%	66.7%	56.0%	81.6%	85.9%
pGDM cluster 2	37	29.7%	37.8%	28.0%	70.1%	36.3%
pGDM cluster 3	48	8.3%	16.7%	16.0%	48.1%	5.8%

Abbreviations: pGDM, post-gestational diabetes mellitus; PGM, pathological glucose mtabolism; 5y FU, 5-year follow-up; PPV, positive predictive value

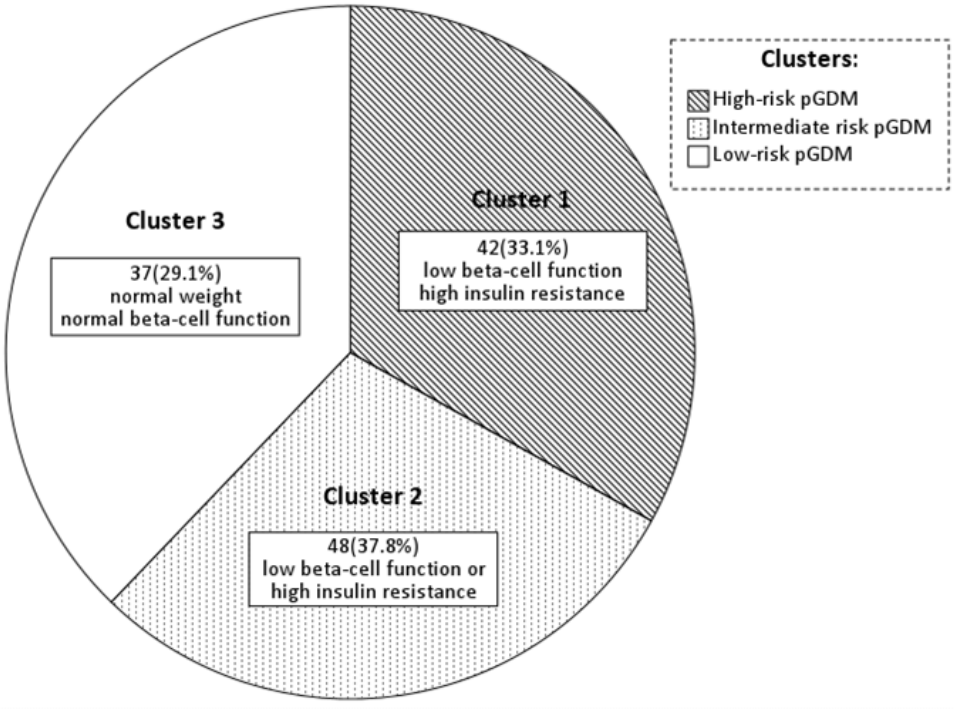


FIGURE 8. Cluster distribution of pGDM subjects

TABLE 16. Association between pGDM clusters and development of prediabetes/T2D at 5-year follow-up

Regression analysis comparing the association of cluster 1, cluster 2 and cluster 3 with prediabetes/T2D. Cluster 1 subjects being compared to subjects in cluster 2 and 3.

		Odds Ratio (CI)			
Dependent variable	**Cluster**	**Crude model**	**Adjusted for age**	**Adjusted for age and BMI**	**Adjusted for age, BMI, first degree relative with T2D, parity, and smoking**
Glucose metabolism status after 5y FU	**Cluster 1**	5.7 (2.6-12.8)***	5.7 (2.5-12.8)***	5.4 (2.0-14.6)***	5.6 (2.0-15.6)**
	Cluster 2	0.91 (0.42-2.0)	0.94 (0.42-2.1)	0.99 (0.44-2.2)	1.0 (0.45-2.3)
	Cluster 3	0.18 (0.07-0.42)***	0.17 (0.07-0.42)***	0.20 (0.07-0.54)**	0.20 (0.07-0.55)**

Abbreviations: CI, confidence interval; BMI, body-mass index; T2D, type 2 diabetes mellitus; 5y FU, 5-year follow-up

Next, we also analysed risk for prediabetes/T2DM in a logistic regression model, illustrated in Table 16. We observed that cluster 1 (high-risk pGDM) was significantly linked to the high risk of developing prediabetes/T2DM. Cluster 1 presented a positive odds ratio of 5.6 ($p < 0.01$) after adjusting for age, BMI, first degree relative with T2DM, parity and smoking, whereas cluster 3 presented an odds ratio of 0.20 ($p < 0.01$) after the same adjustments as compared to cluster 2. These results showed that subjects belonging to cluster 1 had a highly significant higher risk to develop prediabetes/T2DM after 5 years follow-up. In contrast, subjects in cluster 3 had a low risk to develop prediabetes/T2DM. (Table 16)

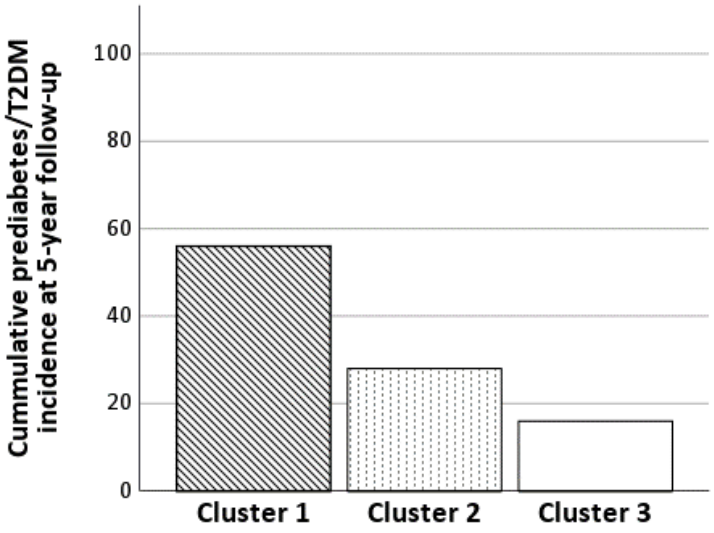


FIGURE 9. Cumulative incidence of prediabetes/T2DM during 5-year follow-up in study population stratified into pGDM clusters

5.9 Clinical phenotype of pGDM clusters

We analysed the association of the three pGDM clusters with clinical parameters including age, waist circumference, blood pressure and laboratory measures such as HbA1c, plasma triglycerides, HDL-cholesterol, LDL-cholesterol, hepatobiliary enzymes, serum creatinine, CRP,

adipokines (Table 17) and variables of glucose metabolism (Table 18). The three clusters were significantly different in systolic blood pressure, waist circumference, triglycerides, LDL-cholesterol, hepatic enzymes and leptin plasma levels. Cluster 1 had a high proportion (n=33, 63.5%) of women with overweight/obesity and was characterised by significantly higher BMI and waist circumference, higher triglycerides and LDL-cholesterol levels, elevated hepatic enzyme levels and very high leptin plasma concentrations as compared to cluster 2 and 3. Numerous glucose, insulin and C-peptide based variables were also significant different between cluster 1 and the two other clusters (Table 18). Cluster 2 exhibit increased BMI and waist circumference but the lowest triglyceride, LDL-cholesterol and CRP levels compared to cluster 3. As expected, pGDM cluster 3 possessed the healthiest phenotype. Interestingly HbA1c and CRP were not different between clusters 2 and 3.

TABLE 17. Clinical characteristics of PPS-Diab participants at baseline in pGDM clusters

*p-values of cluster 1 compared with cluster 2, #p-values compared with cluster 3, §p-values of cluster 2 compared to cluster 3

	Cluster 1	Cluster 2	Cluster 3	*p-values*		
No. of subjects (n=127)	42 (33.1%)	48 (37.8%)	37 (29.1%)	*	#	§
Clinical characteristics: mean ± SD						
Age [years]	36 ±4	36 ±4	36 ±4	0.410	0.480	0.961
BMI [kg/m²]	30.4 ±6.6	25.2 ±4.0	21.5 ±1.7	<0.001	<0.001	<0.001
Waist circ. [cm]	91 ±12	81 ±8	74 ±5	<0.001	<0.001	<0.001
Sys. BP [mmHg]	124 ±10	117 ±9	115 ±10	0.001	<0.001	0.526
Dia. BP [mmHg]	77 ±9	73 ±6	72 ±7	0.032	0.047	0.780
Clinical chemistry: mean ± SD						
HbA1c [%]	5.4 ±0.4	5.3 ±0.3	5.3 ±0.3	0.055	0.002	0.282
CRP [mg/dl]	0.47 ±0.38	0.23 ±0.11	0.30 ±0.21	0.026	0.139	0.853
TGL [mg/dl]	114 ±61	61 ±21	65 ±25	<0.001	<0.001	0.555
LDL-Ch [mg/dl]	116 ±31	98 ±23	103 ±31	0.001	0.033	0.316
HDL-Ch [mg/dl]	55 ±14	66 ±16	68 ±13	<0.001	<0.001	0.710
GPT [U/l]	23 ±27	18 ±12	15 ±4	0.133	0.002	0.075
Gamma-GT [U/l]	24 ±15	16 ±7	13 ±3	<0.001	<0.001	0.031
Creatinine [mg/dl]	0.66 ±0.11	0.69 ±0.12	0.70 ±0.10	0.190	0.028	0.627
Adipokine: mean ± SD						
Leptin [ng/ml]	19.9 ±7.7	11.0 ±7.2	5.4 ±3.1	<0.001	<0.001	0.002
Adiponectin [µg/ml]	8.8 ±3.9	11.0 ±4.9	11.6 ±6.4	0.114	0.085	0.682
Resistin [ng/ml]	8.7 ±3.2	9.4 ±4.5	9.4 ±4.3	0.796	1.000	0.966

Abbreviations: No., number; SD, standard deviation; BMI, body mass index; circ., circumference; Sys. BP, systolic blood pressure; Dia. BP, diastolic blood pressure; HbA1c, glycated haemoglobin concentration; CRP, C-reactive protein; TGL, triglycerides; LDL-Ch, low-density lipoprotein cholesterol; HDL-Ch, high-density lipoprotein cholesterol; GPT, glutamate-pyruvate-transaminase; gamma-GT, gamma-glutamyl-transferase

The present study demonstrated considerable heterogeneity in the clinical characteristics of pGDM women. Our findings indicate that it is feasible to subtype women after GDM in our newly defined clusters to differentiate subjects with high diabetes risk, who should be monitored in short intervals after delivery, from low-risk individuals, who can be controlled by standard care. This strategy enables improved personalised medical care in pGDM women to prevent future complications related to prediabetes and undiagnosed T2DM.

TABLE 18. Comparison of glucose metabolism indices and OGTT results at baseline between pGDM clusters

*p values compared with cluster 2, #p values compared with cluster 3, §p values of cluster 2 compared to cluster 3

	Cluster 1	Cluster 2	Cluster 3	*p-values*		
No. of subjects (n=127)	42 (33.1%)	48 (37.8%)	37 (29.1%)	*	#	§
PGM, baseline	28 (66.7%)	11 (22.9%)	4 (10.8%)	0.001	<0.001	0.010
PGM, 5y FU	28 (66.7%)	14 (29.2%)	8 (21.6%)	0.010	<0.001	0.027
Glucose tolerance at baseline:						
AUC glucose [mg.h/dl]	291.1 ±54.3	268.1 ±45.5	255.3 ±46.3	0.054	0.002	0.185
AUC insulin [µIU.h/ml]	180.3 ±80.6	103.4 ±45.7	86.4 ±32.3	<0.001	<0.001	0.033
AUC C-peptide [ng.h/ml]	19.3 ±4.4	14.6 ±3.5	14.2 ±2.9	<0.001	<0.001	0.405
OGTT-based indices at baseline:						
Insulin sensitivity:						
ISI	2.4 [1.1 : 3.3]	4.8 [4.1 : 5.6]	7.4 [5.6 : 8.5]	<0.001	<0.001	<0.001
HOMA-S	64.8 [55.6 : 78.4]	117.5 [95.5 : 145.2]	189.6 [145.2 : 212.5]	<0.001	<0.001	<0.001
HOMA-2-S	48.7 [43.1 : 54.2]	72.3 [63.6 : 80.1]	84.1 [77.4 : 107.0]	<0.001	<0.001	<0.001
Insulin resistance:						
HIRI	0.14 [0.11 : 0.22]	0.08 [0.06 : 0.13]	0.07 [0.06 : 0.10]	<0.001	<0.001	0.405
HOMA-IR	1.6 [1.3 : 1.8]	0.9 [0.7 : 1.0]	0.5 [0.5 : 0.7]	<0.001	<0.001	<0.001
HOMA-2-IR	2.1 [1.8 : 2.3]	1.4 [1.2 : 1.6]	1.2 [0.9 . 1.3]	<0.001	<0.001	<0.001
Beta cell function:						
HOMA-B	111.8 [93.1 : 123.3]	81.8 [66.6 : 95.2]	65.6 [58.6 : 77.3]	<0.001	<0.001	<0.001
HOMA-2-B	134.4 [123.5 : 175.4]	113.3 [101.4 : 123.5]	107.2 [91.2 : 116.7]	<0.001	<0.001	0.049
DI	151.9 [120.1 : 175.4]	173.3 [150.9 : 294.2]	290.5 [231.4 : 362.8]	0.011	<0.001	<0.001
AI	11.8 [9.6 : 15.7]	19.4 [16.7 : 22.4]	32.6 [27.0 : 36.7]	<0.001	<0.001	<0.001
IGI	1.07 [0.75 : 1.51]	0.70 [0.49 : 1.13]	0.66 [0.49 : 1.06]	0.010	0.003	0.880

Abbreviations: No., number of; PGM, pathological glucose metabolism; 5y FU, 5-year follow-up; AUC, area under the curve; OGTT, oral glucose tolerance test; ISI, insulin sensitivity index; HOMA, homeostatic assessment; -S, for insulin sensitivity; HIRI, hepatic insulin resistance index; -IR, for insulin resistance; -B, for beta-cell secretion; DI, disposition index; AI, adaptation index; IGI, insulinogenic index

Discussion

In the present study, we analysed heterogeneity of prediabetes phenotypes and their relationship to OGTT based parameters in a cohort of young women after a history of GDM. First, we found significant differences in parameters of metabolic syndrome and the type and severity of insulin resistance and pancreatic beta cell dysfunction. Second, we established a simple approach for subtype classification and defined three criteria, BMI, HOMA-IR and oral AI, to discriminate between women at very high from those at low risk for prediabetes/type 2 diabetes during follow-up. Importantly, normoglycaemic cases at baseline who progressed to prediabetes were predominantly characterised by low AI which suggests that an insufficient compensation of beta cell secretion to a given insulin resistance may be the dominant pathogenetic mechanism for rapid progression to dysglycaemia in young women.

The PPS-Diab study is a prospective postpartum cohort study recruiting women after GDM to investigate clinical and metabolic alterations in young individuals from a normoglycaemic state to prediabetes and subsequently to overt type 2 diabetes [80]. As reported from prediabetic patients from the general population [89, 11, 86], it should be emphasised that the rate of diabetes development and the relative contributions of insulin resistance and insulin secretory defect is very heterogeneous in women after GDM. Some subjects develop diabetes soon and others having the disease evolved more slowly over a long period, even when they are in the same category of glucose regulation. Recent cohort studies suggested that type 2 diabetes includes distinct subtypes determined by age at diagnosis, BMI, HbA1c, GAD autoantibodies, beta cell function (HOMA-2-B), and insulin resistance (HOMA-2-IR) calculated from fasting glucose and insulin or C-peptide [2, 102, 79, 55, 22, 85]. Studies in individuals at risk for T2DM provided additional evidence that similar subphenotypes also exists in the prediabetic state and can be used to identify subjects at different risk for complications [98].

On the basis of established clustering algorithms for type 2 diabetes mellitus, we first estimated differences in anthropometric data, basic routine laboratory measurements and OGTT based measures in pGDM women in different categories of glucose tolerance. Prevalence of IFG and/or IGT at baseline visit (31.3%) and at 5-year follow-up visit (34.0%) were consistent with previous results in postpartum studies [40, 42, 77].

In a previous study in Denmark the incidence of prediabetes in women with a history of gestational diabetes mellitus (pGDM) was reported to be 38% during a median follow-up of 7.8 years[46]. When the analysis was restricted to women of Caucasian ethnicity, the prevalence slightly increased to 40%. A Finnish population comprising 489 women with pGDM described that 53% had prediabetes at the 7.5-year follow-up. Specifically, 37% had impaired fasting glucose (IFG), 4% had impaired glucose tolerance (IGT), and 6% had both IFG and IGT [40]. Interestingly, this study concluded that the development of hyperglycaemia might be primarily due to a failure to compensate for decreased insulin sensitivity (measured using the insulin sensitivity index) because of a progressive decline in insulin secretion (measured using the disposition index) over the long term. Huvinen et al. conducted a study comparing 348 women based on whether or not they were obese (defined as having a BMI greater than 30 kg/m²) before pregnancy [42]. After a follow-up period of 5 years, 15.2% of the women who were not obese including 100% of the women having had pGDM presented with IFG and/or IGT. The differences in prediabetes prevalence observed in these various studies can likely be attributed to the differing follow-up durations and variations in the size and characteristics of the study populations. Additionally, other factors such as genetic background, lifestyle, and healthcare practices could also contribute to these discrepancies.

In our cross-sectional analysis we found that pGDM women having NGM at baseline visit displayed significantly fewer components of metabolic syndrome and had decreased insulin resistance measures as well as better beta cell function in relation to prevailing insulin sensitivity

compared to subjects with PGM.

Some previous studies analysed glucose metabolism among women with GDM during pregnancy. Adverse obstetrical and neonatal outcomes such as preeclampsia, caesarian section delivery, weight at birth, preterm birth, admission to neonatal intensive care were reported in women with increased insulin resistance independent on BMI [72, 44] and in women with combined high insulin resistance and beta cell dysfunction [56]. Powe and coworkers described that women with GDM and decreased insulin sensitivity (below the 25th percentile) had higher BMI and larger infants, whereas women with primarily insulin secretion defects showed similar results to those with normal glucose tolerance during pregnancy [72]. In another study, women with GDM were divided based on higher insulin resistance or lower insulin secretion (above or below the median) [44]. The findings echoed those of Powe's study; women with higher BMIs and adverse outcomes for their babies after pregnancy were predominantly in the group with insulin resistance. In contrast, those with only insulin secretion defects or a combination of both were similar to the controls without GDM.

By dividing women with GDM into those with insulin sensitivity (ISI above the median of women without GDM) and those with high insulin resistance it was demonstrated that women with GDM and high insulin resistance had a more adverse metabolic profile, met frequently the criteria of metabolic syndrome and had a significantly higher risk of adverse pregnancy outcomes. Conversely, insulin-sensitive women with GDM were more likely to have a first-degree family history of diabetes and a history of GDM but had lower BMI compared to the control group without GDM [8]. Thus, these studies identified a subgroup of women with GDM and insulin resistance who were associated with higher metabolic risk profiles and adverse pregnancy outcomes. Conversely, women with GDM and isolated insulin secretion defects and/or preserved insulin sensitivity had outcomes similar to those without GDM. Interestingly, the study by Liu et al. presented different results in a Chinese population. The group of women with GDM and

combined insulin resistance and secretion defects had the highest risk for adverse pregnancy outcomes and metabolic risk profiles, compared to those with isolated insulin resistance or secretion defect [56].

While we know that in general women after GDM are at significant risk for future dysglycaemia, we incompletely understand why some women have persistent pathological glucose tolerance whereas others convert to normal glucose tolerance and then progress to type 2 diabetes or remain normoglycemic many years after index pregnancy. Most prospective studies used GDM as a common risk factor and considered GDM as one disease. Observational studies in women after a history of GDM reported an incidence of type 2 diabetes ranging from 2 to 48% at 5 years from delivery [12, 19, 43, 10]. In the present study 33% of women had prediabetes at 5-year follow-up and cumulative 8% progressed to diabetes. This prevalence was along the lines previously described in the literature using the IADPSG criteria for the diagnosis of GDM (type 2 diabetes incidence about 8% after 5 years and 19% after more than 5 years) [47]. In agreement with previous studies, we observed a significant association between components of metabolic syndrome, insulin therapy during pregnancy, AUC-glucose, AUC-insulin, AUC-C-peptide and measures of insulin resistance with PGM after delivery [42, 77, 8].

Several studies analysed glucose metabolism in women after a history of GDM. In a Belgian cohort, the prevalence of IFG, IGT or both were significantly higher in the pGDM group than in control subjects, but there was no difference of glucose metabolism in insulin resistant versus insulin sensitive subgroups [8]. Huvinen and co-workers divided post-GDM women according to their BMI pre-pregnancy (</≥30 kg/m²) and reported that the association between lifestyle and glycaemic health varied depending on the presence of obesity in women [42]. Despite the healthier lifestyle of the women without obesity, the prevalence of prediabetes/T2DM after 5 years follow-up was similar in both groups. Having a healthier diet and being more physically active only improved the glycaemic health of women with obesity, after adjustment for age,

education and GDM history. A Canadian population of women with GDM presented higher AUC of glucose and insulin during OGTT in both groups of women with insulin resistance or with insulins secretion defect. Nevertheless, the different groups determined during the pregnancy did not show a significant difference of risk for prediabetes/type 2 diabetes at 1 years or 5 years after delivery [77].

In the present study, PGM at 5-years was associated with markers indicating lower insulin sensitivity (HOMA-2S; ISI) /higher insulin resistance (HOMA-2-IR) and parameters related to impaired beta cell function (DI, AI) in the total cohort in the crude model and after adjustment for age and BMI. We also analysed potential differences in baseline insulin sensitivity and insulin secretion indices between normoglycemic subjects remaining normoglycemic during follow-up from those who progressed from NGT to PGM or diabetes. Interestingly, the AI was a significant independent predictor of incident dysglycaemia in this subgroup, whereas other parameters for insulin resistance or beta cell secretion were not significantly different. Homeostasis model assessment (HOMA) had been widely used to predict risks in first degree relatives of patients with type 2 diabetes and subjects with metabolic syndrome. HOMA-2 and HOMA-2-IR are simple measures of insulin resistance using fasting glucose, fasting insulin, or fasting C-peptide which indicate increased risk for prediabetes and type 2 diabets [89, 86, 21]. HOMA-B and HOMA-2-B are widely used insulin secretion indices, which were negatively associated with diabetes development [89, 86, 21]. Some studies reported that high insulin resistance many years before diabetes is the most important pathogenetic factor [89, 86], others described beta cell dysfunction as the major driver towards type 2 diabetes [11, 25]. Prospective studies combining measures for insulin sensitivity and beta cell function found a decline in insulin sensitivity and a beta cell dysfunction characterised by insulin levels which do not fully compensate for the increased glucose levels at a very early prediabetic state followed by a failure to further adapt beta cell function for the progressive decline in insulin sensitivity months to few years before diabetes

manifestation [48, 68].

We here observed in young pGDM women that inadequate insulin secretion in relation to increased secretory demand was associated with highest diabetes risk. These features are in agreement with several studies demonstrating that indices combining measures of insulin sensitivity with determinants of beta cell function such as DI or AI are better predictors for progression from normoglycemia to prediabetes and prediabetes to overt T2DM than either parameter of insulin resistance or beta cell secretion alone. DI was shown to indicate poor beta cell function and identify subjects who progressed to overt type 2 diabetes over a 10-year follow-up period [97]. In the Insulin Resistance Atherosclerosis Study, DI significantly predicts conversion to type 2 diabetes in middle aged individuals (age 40-69 years) independent from stages of glucose tolerance, ethnic groups, family history of diabetes, and obesity [57]. Den Biggelaar et al. reported that several indices of beta cell function (0-30 min) based on glucose, insulin or C-peptide measurements ranked highest in prediabetes and type 2 diabetes prediction and had significant better abilities to discriminate who will develop prediabetes/type 2 diabetes in a longitudinal 7-years follow-up as compared to HOMA and Matsuda indices [15].

In the present study, we also analysed the association of plasma leptin, a potential additional biomarker of insulin resistance, with PGM at baseline and after a 5-year follow-up. At baseline, leptin was associated with PGM and presented a positive significant correlation with HOMA-2-IR in both BMI categories. Leptin also exhibited a predictive effect for PGM after the 5-year follow-up and enhanced the predictive strength of insulin secretion indices DI, AI, and IGI in the logistic regression analysis. After separating our study population into pGDM risk clusters, leptin at baseline was also significantly different between the three clusters. In the high-risk pGDM cluster 1, leptin levels were three to four times higher than in the low-risk pGDM cluster 3. This difference can be linked to the existing correlation between leptin and BMI [53], but more importantly, it may be explained by the possible connection with insulin resistance and the HOMA-2-IR used to

define the clusters. Our findings suggest that leptin may be an important additional biomarker for predicting long-term PGM outcomes and underscore the potential of leptin as a valuable tool in assessing insulin resistance and related metabolic conditions. Further longitudinal studies are warranted to validate these findings and explore the underlying mechanisms connecting leptin, insulin resistance and PGM.

Some previous studies have examined progression to diabetes in GDM women postpartum in relation to OGTT based metabolic indices. A similar glucose tolerance was reported at 14 weeks after delivery in insulin resistant and insulin sensitive subjects [8]. Retnakaran and coworkers reported on predominantly insulin-deficient (e.g. Stumvoll 1st phase index below 25 percentile) and predominant insulin-resistant subtypes (e.g. Matsuda-ISI below 25th percentile) at 3-months and 12-months postpartum, but detected no differences in development of prediabetes or overt type 2 diabetes [77]. In a prolonged follow-up investigation of 302 women decreased beta cell function 3 months after delivery (DI and IGI/HOMA-IR) but not insulin sensitivity (ISI, HOMA-IR) and further longitudinal decrease in beta cell function were predominantly associated with progression to prediabetes/ type 2 diabetes at 5-years [78]. We confirmed the association of prediabetes/type 2 diabetes development with DI and AI in our study, but also observed a significant association with increased insulin resistance as assessed by ISI or HOMA-2IR. Differences of our results may be related to population differences (ethnic minority background in 28.5% in the Canadian study), the limited sample size in both studies and differences in the OGTT testing (100g versus 75g OGTT).

Previously, we have shown in the PPS-Diab study that 45% of women displayed no components of metabolic syndrome but had increased muscle, hepatic and adipose tissue insulin resistance [80]. The present data on a significant association of ISI and HOMA-2-S with PGM in pGDM women with BMI <25 kg/m2 but not in subjects with overweight and a negative relation of IGI with PGM only in the overweight group further supports our hypothesis that there may be

different pathogenetic mechanisms of diabetes development in these two groups. Stratifying our cohort according to BMI and age revealed that insulin resistance measures were significantly associated with progression only in subjects with normal weight but not in overweight women. A similar phenotype of normal weight individuals with high insulin resistance and low insulin secretion was reported in 15-18% of incident type 2 diabetes subjects in the RADIEL and the Whitehall II studies [80, 43]. The pathophysiologic mechanisms in these non-obese people are thus far unknown and should be examined in more detail in future studies.

In the current study, we asked the question how to improve risk assessment in young women after history of GDM at baseline visit after delivery. We aimed to classify subtypes by a simple approach which can be used in clinical practice including BMI, insulin sensitivity/insulin secretion parameters established for subphenotyping of patients with type 2 diabetes and tested additional determinants from OGTT. Because the number of participants was limited in our study, it was not possible to use cluster algorithm analysis to identify subtypes. Cluster-analyses are created for large population-based patient groups. A previous study reported that adequate cluster analyses can only be applied with relatively small sample sizes of n = 20-30 per subgroup when a large subgroup separation is expected [13]. Clearly, well separated, non-overlapping clusters were not expected in the present approach. Alternatively, we performed ROC analysis and calculated manual cut-off points for metabolic indices by Youden index to discriminate progressors from non-progressors at 5-years follow-up in women with normal weight and with overweight. From these analyses we defined three clusters, each comprising about one third of the pGDM subjects. Subjects in the high-risk pGDM cluster 1, characterised by high HOMA-IR ≥ 1.7 and low AI ≤23.1 irrespective of BMI, developed dysglycemia in 66.7% and had a 5.6-fold and 28.0-fold increased risk as compared to pGDM cluster 2 and cluster 3, respectively. Thus, we present here novel risk variables associated with prediabetes/type 2 diabetes development with a high positive predictive value (85.9%), when compared with the performance of classic risk

factors.

Until now, only few studies performed subphenotyping in subjects at risk for type 2 diabetes using clinical parameters and/or measures of insulin resistence and beta cell function. Wagner and co-workers described six distinct phenotype clusters in prediabetic individuals from the prospective TUEF/TULIP study and the Whitehall II study defined by OGTT variables, MRI-measured body fat distribution, MRI-based liver fat content and genetic risk. Highest diabetes risk (6.62-fold) was observed in cluster 5 defined by obesity, very low insulin sensitivity, low insulin secretion, and very high liver fat followed by cluster 3 defined by overweight, low insulin sensitivity, low insulin secretion, and increased genetic type 2 diabetes risk (3.45-fold increased diabetes risk) [98]. In a study involving over 55,000 Chinese individuals aged 40 years or older with prediabetes, 12 variables were identified including waist-to-hip ratio, BMI, HbA1c, fasting glucose, 2-hour postprandial glucose, HOMA-B, HOMA-IR, blood lipids (high-density lipoprotein cholesterol and triglycerides), and liver enzymes ALT, AST, and gamma-GT to define six clusters with varying levels of T2DM risk [103]. The odds ratio of diabetes development in comparison to the reference cluster 1 was 4.93 in the high-risk cluster 6. During the three-year follow-up, the study described trends for migration into four of Ahlqvist's type 2 diabetes subgroups: prediabetes clusters 4 and 5 mostly developed mild obesity-related diabetes and severe insulin-resistant diabetes; cluster 1, 2, and 3 predominantly progressed to mild age-related diabetes and severe insulin-deficient diabetes [103]. These studies underscore the complexity and heterogeneity of prediabetes and the necessity for multifactorial approaches in identifying and managing individuals at risk for developing T2DM.

Our subtyping criteria based on clinical and OGTT criteria allows to allocate women into groups with different risk which is very important for the development of tailored prevention and intervention strategies. We propose that OGTT based varables for insulin resistence and insulin secretion should be included in assessment of diabetes riks in post-GDM women. We

acknowledge that this approach increases costs because it is time-consuming and needs blood sampling during oGTT to measure insulin and C-peptide to calculate metabolic indices. However, we describe a reliable way to identify subjects who should be monitored in short intervals after delivery, whereas low-risk individuals may be controlled by standard care. This strategy enables improved personalised medical care in pGDM women to prevent future complications related to prediabetes and undiagnosed type 2 diabetes.

One of the strengths in our study is the well-characterised cohort, consisting of young women with few comorbidities and the implementation of established OGTT based tools to assess insulin sensitivity and beta cell function. Our strength also lies in the methods of subphenotyping algorithm which can be performed at a single visit early postpartum and does not need sequential follow-up investigations. Another notable strength is the follow-up of 5-years postpartum.

There are some limitations in our study. One is the limited number of subjects in the present study and the calculation of cut-offs for HOMA-IR and AI relying on one cohort. We measured metabolic indices from OGTT and not with the gold-standard technique of a hyperinsulinemic clamp. However, we have confirmed a high correlation of both approaches for the PPSDiab cohort in a previous study [79]. The PPS-Diab study recruited primarily individuals of European descent, thus limiting the validity of our findings to young female Caucasian population. The subphenotyping algorithm must be validated in different cohorts and populations of different ethnicities. Thus, the present results cannot necessarily be generalised to the general population and community-based practice. A longer follow-up in women with a history of GDM is needed to assess whether this prediction model is still valid 10-20 years postpartum. Further studies should also analyse whether these post-GDM subtypes are also related to development of macro- and microvascular complications. Accurate subtyping of individuals may further benefit from integrating genetic and proteomic data [85, 96, 101] which were not available in the present cohort.

Conclusion

Understanding which clinical or serological biomarkers best represent disease progression in women with a history of gestational diabetes mellitus (GDM) is essential for postpartum management and for preventing post-GDM diabetes. Our study has demonstrated that clustering women by body mass index (BMI) and oral glucose tolerance test (OGTT)-based indices, such as HOMA-2-IR and AI, effectively distinguishes three distinct risk groups with varied prognostic metabolic trajectories. This novel subphenotyping provides a foundation for implementing individualized, risk-based preventive interventions, which can enhance the efficacy of treatments and improve long-term health outcomes in women with a history of GDM.

Importantly, this study adds to a growing body of literature highlighting the heterogeneity in metabolic outcomes among post-GDM women, underscoring the necessity of tailored clinical approaches. While all women with a history of GDM should receive postpartum follow-up, subphenotyping enables a more refined strategy. Women in high-risk clusters may require more frequent and intensive monitoring and intervention, while those in low-risk categories could benefit from less aggressive, but still diligent, surveillance.

Moreover, the potential for integrating additional biomarkers such as leptin—identified here as a predictor of postpartum pathological glucose metabolism (PGM)—suggests that future studies could refine these clusters further. Biomarkers like leptin, along with advanced genetic and proteomic data, hold promise for enhancing our understanding of metabolic risk factors and disease progression in this population.

Looking forward, further validation of our findings in diverse populations, including those of different ethnicities, is needed. While our study provides a valuable tool for early postpartum risk stratification, its applicability across various demographics requires more extensive research.

Additionally, longer follow-up periods, extending beyond the five years examined in this study, will be crucial for determining the long-term accuracy and utility of our prediction model. A better understanding of the links between GDM-related subtypes and the risk of developing diabetes-related complications, such as macrovascular and microvascular disease, will also inform future strategies for intervention.

In conclusion, our approach to subphenotyping based on simple clinical measures offers a reliable and accessible way to identify women at high risk for diabetes progression. This strategy opens new avenues for personalized medicine, optimizing preventive care, and potentially delaying or preventing the onset of type 2 diabetes in women after GDM. However, ongoing research and collaboration will be vital to further enhance our understanding of the complex metabolic pathways involved and to refine these clinical tools for broader application.

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Curriculum Vitae

- **12.07.1998** - geboren in Kobe (Japan)
- **06/2016** - Baccalauréat (Hochschulreife) am Lycée Jean-Renoir München
- **2016-2022** - Medizinstudium an der Ludwig-Maximilians-Universität München
- **09/2018** - Erster Abschnitt der Ärztlichen Prüfung (”Physikum”)
- **04/2021-07/2021** - Modul-6-Projektarbeit im Studienzentrum Diabetes der medizinischen Klinik IV des LMU Klinikums
  - Thema: ”*Hyperleptinämie verstärkt Hypertension in eine Kohorte von jungen Frauen, unabhängig von Glucose Metabolismus*”
- **10/2021** - Zweiter Abschnitt der Ärztlichen Prüfung
- **11/2021-03/2022** - Tertial des Praktisches Jahres in Paris (Frankreich) mit Austauschprogramm ERASMUS+
- **03/2022-06/2022** - Tertial des Praktisches Jahres in Tokyo (Japan) mit Austauschprogramm LMU-Exchange
- **06/2022-10/2022** - Tertial des Praktisches Jahres in Wasserburg a. Inn, Wahlfach: Psychiatrie und Psychotherapie
- **11/2022** - Dritter Abschnitt der Ärztlichen Prüfung
- **12/2022** - Approbation als Ärztin
- **02/2023-04/2024** - Promotionsarbeit in Vollzeit im Studienzentrum Diabetes der medizinischen Klinik IV des LMU Klinikums
  - Thema: ”*Subphenotypisierung von perimenopausaler Frauen nach Schwangerschaftsdiabetes*”
- **07/2023** - Teilnahme an ”GCP-Aufbaukurs”
- **Seit 09/2024** - Studienärztin in Teilzeit in der Studienzentrum Diabetes der medizinischen Klinik IV des LMU Klinikums
- **10/2024** - Teilnahme an ”GCP-Grundlagenkurs”
- **Seit 11/2024** Weiterbildung im Fach Viszeralchirurgie im HELIOS Klinikum Perlach

List of Publications

- Pappa E, Busygina K, Harada S, Hermann H, Then C, Lechner A, Ferrari U, Seissler J

  Association of GLP-1 secretion with parameters of glycemic control in women after gestational diabetes mellitus

  BMJ Open Diabetes Res Care. 2024 Jan 10;12(1):e003706. doi: 10.1136/bmjdrc-2023-003706. PMID: 38199777; PMCID: PMC10806896.

- Hesse N, Stohldreier Y, Schlaeger S, Theuerl S, Dietrich O, Hermann H, Kaiser I, Seissler J, Pappa E, Ferrari U, Gersing AS

  Association of breastfeeding duration with longitudinal changes in vertebral bone marrow, paraspinal muscle composition, and metabolic parameters in premenopausal women over five years

  [Manuscript submitted for publication]

- Hermann H, Seißler J, Ferrari U

  Subphenotyping of women after gestational diabetes mellitus identifies subjects at high and low risk for progression to prediabetes/type 2 diabetes mellitus

  [Manuscript in preparation]