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ORIGINAL DATA

Postpartum Outcomes in Women with Gestational Diabetes and their Offspring: POGO Study Design and First-Year Results

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■ Abstract

BACKGROUND: Gestational diabetes mellitus (GDM) is a risk factor for mothers to develop type 2 diabetes (T2D) postpartum, and for their children to develop obesity. The aim of the ongoing POGO study is to identify long-lasting changes in the maternal and fetal metabolism and microbiome, after GDM, which contribute to subsequent development of T2D and obesity. **METHODS**: Women screened for GDM are asked to attend a postpartum study visit together with their offspring. At the visit, demographic, nutritional, and anthropometric data are recorded. Additionally, data about physical activity, metabolism, and genetic susceptibility are collected using accelerometers, breath gas analyses, 75g oral glucose tolerance tests (OGTT), and biosamples such as blood and stool. **RESULTS**: To date, 121 women (median follow-up time postpartum: 5.5 years) have been enrolled together with 133 index children. GDM has been diagnosed using OGTT in 105 women (and 117 children). It showed that 47 mothers had abnormal glucose tolerance, including 19 cases of impaired glucose tolerance, 19 of impaired fasting glucose, eight with T2D, and one with type 1 diabetes (T1D). The prevalence of obesity in the offspring of GDM mothers was 5.1%. Of 61 children tested by OGTT, three were diagnosed with impaired glucose tolerance, another three with impaired fasting glucose, and none with T1D or T2D. **CONCLUSIONS**: The POGO study will contribute to the understanding of the pathogenesis of T2D and obesity after GDM, and will thus help to develop appropriate prevention and intervention strategies. This article presents the first results of the ongoing study, which are looking promising.

Keywords: gestational diabetes · postpartum type 2 diabetes · overweight · obesity · cohort-study · offspring · metabolomics · microbiome

Introduction

Gestational diabetes mellitus (GDM) is a complication occurring in 2-6% of all pregnancies in industrialized countries [1]. It is strongly associated with maternal obesity [2]. Women with GDM are at increased risk of developing type 2 diabetes (T2D) postpartum [3]. *In utero* exposure to GDM is a risk factor for the development of obesity during childhood and adolescence [4] and has been associated with an increased risk of T2D later in life [5]. Factors responsible for these risks are not fully understood. As the prevalence of pregnancies complicated by GDM and maternal obesity is increasing [2, 6], the number of children at risk of obesity due to *in utero* exposure to GDM is also expected to increase. Furthermore, female offspring of GDM mothers have an elevated risk of developing GDM themselves, thus contributing to increased GDM prevalence. To halt this epidemic and consequently reduce the burden for the individual, it is essential to identify risk factors and

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underlying mechanisms that contribute to the pathogenesis of postpartum T2D and offspring's obesity.

Previous studies have shown that maternal obesity and insulin treatment increases the risk for postpartum development of T2D in GDM mothers [3], while breastfeeding seemed to have protective effects [7]. However, these studies were not able to provide insight into the underlying mechanisms of these associations. The contribution of maternal obesity in this context may point to the involvement of genetic and/or environmental factors, but data supporting this assumption are scarce. Even less is known about potential modifying factors or pathways involved in the association of GDM and offspring's obesity.

The objective of the so called POGO study (= Postpartum Outcomes in mothers with Gestational diabetes and their Offspring) is to identify potential changes in the metabolism and microbiome of mothers, after exposure to GDM, through studies of metabolomics, volatile organic compounds (VOCs), and the intestinal microbiota, and whether these changes are predictive for T2D. Likewise, we want to find out how *in utero* exposure to GDM contributes to fetal and postnatal programming of the risk of obesity by potential alterations of metabolism and microbiome in offspring. Further research will include investigating potential pathways modified by genetic disposition, environmental factors (such as physical activity and nutrition) or GDM treatment strategy (insulin or diet). Furthermore, a biobank of DNA, RNA, peripheral blood mononuclear cells (PBMCs), serum, plasma, and stool will be established for future analyses including potential gene-environment interactions, epigenetics, and transcriptomics.

It is expected that the POGO study will increase our knowledge of the pathogenesis of T2D and obesity in mother and offspring and thus help to develop appropriate prevention and intervention strategies. In this paper, we present first results that describe the future potential of the POGO study.

Abbreviations:
BG - blood glucose
BMI - body mass index
DNA - deoxyribonucleic acid
EDTA - ethylenediaminetetraacetic acid
GDM - gestational diabetes mellitus
HbA1c - glycosylated hemoglobin
HOMA - homeostasis model assessment
HOMA-IS - homeostasis model assessment insulin sensitivity
IFG - impaired fasting glucose
IGT - impaired glucose tolerance
NGT - normal glucose tolerance
OGTT - oral glucose tolerance test
PBMC - peripheral blood mononuclear cell
POGO - Postpartum Outcomes in mothers with Gestational diabetes and their Offspring
PTR-MS - proton transfer reaction mass spectrometry
RNA - ribonucleic acid
T1D - type 1 diabetes
T2D - type 2 diabetes
VOC - volatile organic compound

Materials and methods

Study design and organization

The recruitment in the POGO study is ongoing. The women recruited to date were contacted to participate in the POGO study if they were screened for GDM in the Klinikum Schwabing, a former outpatient clinic in Munich, during at least one pregnancy between 1998 and 2009. Data available from the screening included assessment of weight, height, HbA1c, oral glucose tolerance test (OGTT), blood pressure, and family history of diabetes. Before 2012, screening of GDM was not a regular part of the pregnancy check-ups in Germany. Therefore, women were usually screened if they were at increased risk for GDM (e.g. family history of diabetes, GDM in a previous pregnancy, previous birth of a large-for-gestational-age infant, habitual abortion, fetal macrosomia) [8], or because a glucosuria and/or hyperglycemia was detected.

Of the 1829 pregnant women screened, 1226 were complicated by GDM, defined by exceeding at least 2 out of the following 3 reference values (according to the guidelines of the German Diabetes Association from 2001 [8]):

1. Fasting plasma glucose levels >95 mg/dl (5.3 mmol/l)
2. 1-hour values >180 mg/dl (10 mmol/l)
3. 2-hour values > 155 mg/dl (8.6 mmol/l)

Women with GDM received dietary counseling and treatment of GDM (diet or insulin) with repeated follow-up visits until delivery according to the Guidelines of the German Diabetes Association. Mothers and their offspring were invited for a single clinical visit that is performed at the clinical study center of the institute of diabetes research. Older or younger siblings born before or after the index pregnancy (i.e. without GDM screening data

available in the Klinikum Schwabing) were also invited to participate in POGO. Recruitment of mothers and their offspring was initiated in 2011 and is expected to continue until 2017 at a rate of 100 mother-offspring pairs per year aiming to enroll at least 500 mother-offspring pairs. As screening data were collected from 1998 to 2009 as mentioned above, child's age at the study visit can vary from 1 to 19 years. The study protocol was approved by the Ethical Committee of the Technical University, Munich (No. 2937). All participants gave written informed consent; for the children informed consent was obtained from both parents.

In 2012, three other German centers (University of Tübingen, University of Düsseldorf and Ludwig-Maximilians University of Munich) initiated the recruitment of mother-child pairs in the same way. Combining these data with those from the POGO study will increase statistical power.

Study objective

The POGO study investigates potential mechanisms and pathways leading to the development of T2D in women with GDM, and to the development of obesity in offspring exposed to GDM during fetal life. We hypothesize that GDM induces long-lasting changes in the maternal and fetal metabolism and microbiome, which increase the risk for subsequent development of T2D and obesity, respectively, and may be modified by genetic and environmental factors. The main topics and research questions of the POGO Study are as follows:

For the mother:

1. How do women with GDM differ from healthy mothers with respect to their metabolomics profiles and microbiome?
2. Are there specific differences in the metabolism and/or microbiome between GDM mothers who subsequently develop abnormal glucose tolerance or T2D and those who did not?
3. How do maternal obesity, insulin treatment during pregnancy and breastfeeding affect maternal metabolism (e.g. metabolomics profiles) and thereby influence the T2D risk?
4. Do genetic variants and environmental factors (e.g. nutrition, life-style) have additional effects on the maternal metabolomics profile and further modify the mother's T2D risk?
5. How do glucose levels during the diagnostic OGTT in pregnancy correlate with the respective maternal and offspring outcome postpartum?
6. What is the prevalence of T2D and impaired glucose tolerance in women with preceding GDM compared to women without GDM?

For the offspring:

1. Does fetal exposure to GDM program metabolic pathways in childhood?
2. Does fetal exposure to GDM program the childhood gut microbiome and inflammatory pathways (e.g. cytokines) with respect to an increased risk for obesity?
3. Is the risk of obesity in children exposed to GDM *in utero* further modified by other factors, e. g. T2D susceptibility genes or environmental factors, e.g. nutrition and physical activity?
4. What is the prevalence of overweight and obesity in offspring who have been exposed to GDM *in utero*?

Data items

The following data were collected from each mother-child pair at the study visit or afterwards. While data collection for the participants included in the study to date has been carried out and described in past tense, recruitment and data collection are still ongoing.

Demographic and socioeconomic status

Information regarding demographics and socioeconomic status was obtained from questionnaires distributed at study entry. The questionnaires requested information on the parents (main language spoken, nationality, education, smoker/nonsmoker, weight, height, and family income), their community (rural or urban), family history of diabetes, and the child.

Prenatal and perinatal factors

Prenatal data were retrieved from the mother's pregnancy booklet, which is issued to every pregnant woman in Germany for complete documentation of antenatal care visits, and from the patient file. These included information about maternal body mass index (BMI) in early/late pregnancy, gestational weight gain, maternal OGTT values,

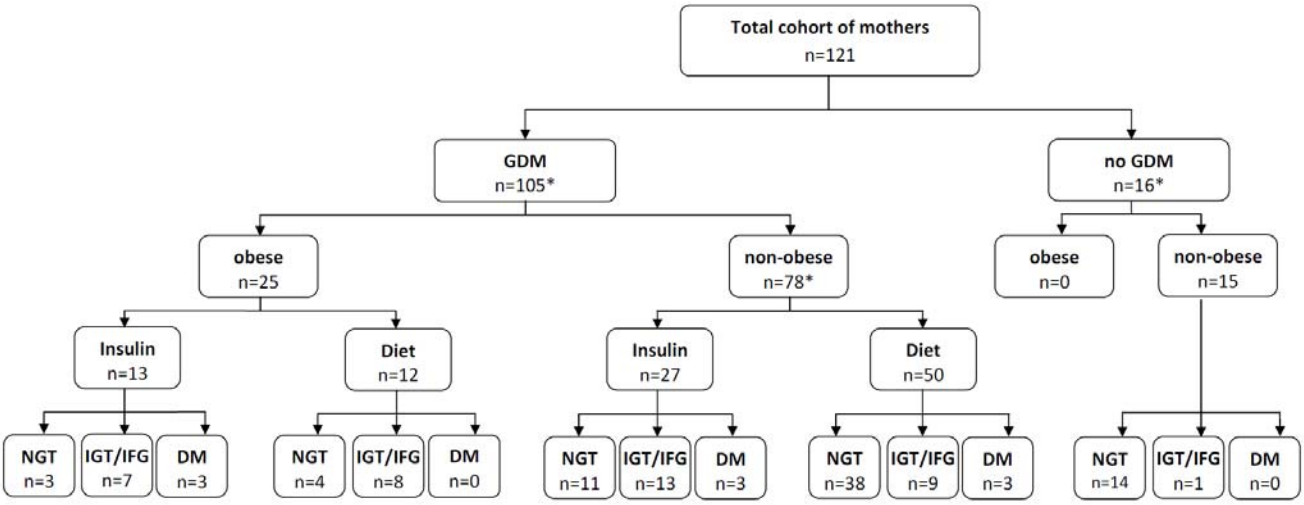

Figure 1. Flow chart of mothers enrolled in the POGO study. GDM was defined as observed in at least one index pregnancy. Insulin/diet refers to treatment of the last index pregnancy with GDM, while NGT (normal glucose tolerance), IGT/IFG (impaired glucose tolerance/fasting glucose) and diabetes (type 1 or type 2) refers to diagnosis at study visit postpartum. An asterisk denotes missing values in the next-lower level.

GDM therapy (diet/insulin treatment, insulin dose), mode of delivery, and gravida/para status. In index pregnancies, GDM was defined based on OGTT values obtained at screening as described above. In non-index pregnancies, GDM history was extracted from the respective pregnancy booklets.

Perinatal data such as gestational age at delivery, birth weight, and weight/height development during childhood were retrieved from records of the regular examinations of the preventive health program offered to all children in Germany. These records were collected in a booklet (the so-called 'U-Heft'), which mothers were advised to bring with them to the POGO visit. If available, and if the mothers agreed, these data were also collected if the respective child did not attend the study visit.

Anthropometric data

Weight and height of mothers and children were measured under standardized conditions by trained study personnel. Infants were weighed lying on their back without clothes and diaper. In infants ≤2 years of age, length was measured while lying on their back, from the bare heels to the top of the head avoiding toe pointing. In children old enough to stand, weight was measured in light clothing on a scale; standing height was assessed barefoot with a wall-mounted stadiometer. BMI was calculated from these measures as weight/height² (kg/m^2).

Waist circumference (cm) was taken with a tape measure as the point midway between the costal margin and iliac crest in the mid-axillary line, with the subject standing and breathing normally. Hip circumference (cm) was measured at the widest point around the greater trochanter. The waist-to-hip ratio was calculated as the waist measurement divided by the hip measurement. Skinfold thickness was measured by a Harpenden skinfold caliper using the 2 site system (triceps and subscapular), and the measurements were used to estimate fat mass proportions of mother and child.

Blood sample collection

After an overnight fast, venous blood was collected by a paediatrician from the mother and her child. Fasting blood samples were processed into serum, plasma (aprotinin and EDTA), PBMC, and DNA. An OGTT was performed in the mothers and children aged ≥2 years, unless T2D has been previously diagnosed. Blood samples for the measurement of insulin, C-peptide, and glucose were obtained at 0, 30, 60, 90, and 120 minutes after the OGTT glucose-drink has been consumed. The study subjects were classified as normoglycemic or

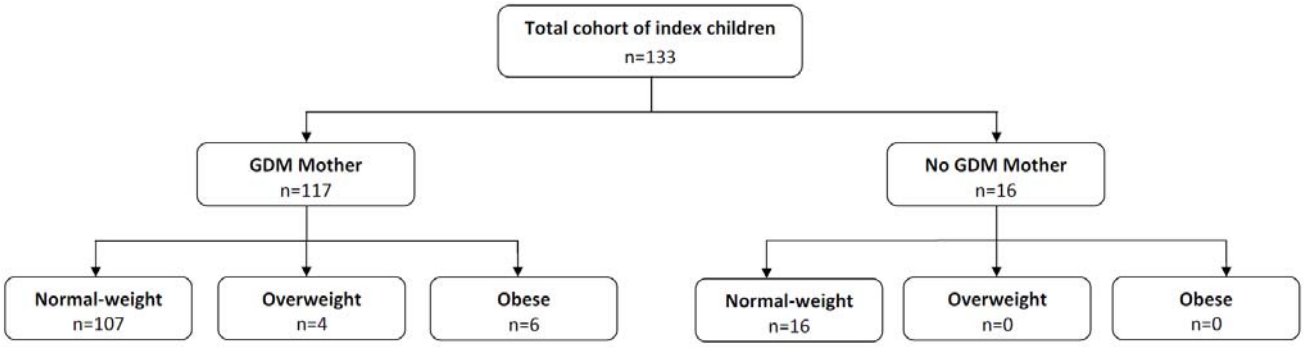

Figure 2. Flow chart of index children enrolled in the POGO study. Normal-weight, overweight (excluding obesity), and obesity refer to the values at study visit or last height and weight measurement before study visit.

abnormal, i. e. having impaired glucose tolerance, impaired fasting glucose, or (additionally based on previous medical records, e. g. autoantibody measurements, if available) type 1 diabetes (T1D) or T2D. The OGTT values were further used to determine the HOMA index as a measure of insulin sensitivity [9].

Specimens were stored for both immediate and future testing requirements, e.g. insulin sensitivity, β-cell function, genotyping, and determination of islet autoantibodies. Additional aliquots were stored at the biobank of the Institute of Diabetes Research, Helmholtz Zentrum, Munich.

Outcome definition

In mothers, T2D was defined according to the new guidelines of the German Diabetes Association from 2011 [10], including the use of the HbA1c as a novel decisive factor/an important criterion for T2D diagnosis. A patient was classified as having T2D if at least one of the following reference values was exceeded:

1. HbA1c ≥6.5% (≥48 mmol/mol)
2. Random plasma glucose value ≥200 mg/dl (≥11.1mmol/l)
3. Fasting plasma glucose value ≥126 mg/dl (≥7.0 mmol/l)
4. 2-hour OGTT value in venous plasma is ≥200mg/dl (≥11.1 mmol/l)

Impaired fasting glucose was defined as fasting plasma glucose values between 100-125 mg/dl (5.6-6.9 mmol/l). Impaired glucose tolerance was defined as having a 2-hour OGTT plasma glucose value between 140-199 mg/dl (7.8-11.0 mmol/l) plus a fasting plasma glucose value <126 mg/dl (<7.0 mmol/l).

In children who exceeded the reference values, the test result had to be confirmed with a second OGTT on another day (fasting plasma glucose: >126 mg/dl (>7.0 mmol/l), 2-hour OGTT value >200 mg/dl (>11.1 mmol/l)) [11].

Overweight and obesity were defined as BMI ≥25 kg/m² and ≥30 kg/m² in mothers, and as BMI at or above the 90th and 97th age- and sex-specific reference percentile, according to German reference data [12] in children, respectively.

Gender-specific aspects

Pubertal growth relative to age differed between boys and girls, meaning that ostensible gender differences might occur for many of the data items examined. Therefore, Tanner stages were recorded in children ≥8 years old by pediatricians, so that it will be possible to correct for pubertal status.

Physical activity

Physical activity of both mothers and children was assessed by questionnaires and accelerometers (Acti Graph, GT3X Activity Monitor, USA).

For children, a physical activity questionnaire was used, which was developed in the German Health Interview and Examination Survey of Children and Adolescents (KiGGS) in the module "Motorik" [13]. The mothers received the "Freiburger Fragebogen zur körperlichen Aktivität" [14].

Table 1. Characteristics of the six children with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) at the study visit, when blood glucose (BG, mg/dl) was measured at 0, 30, 60, 90, and 120 minutes after OGTT

Diagnosis	Gender	Age (y)	BMI (kg/m²)	BMI percentile	HOMA-IS	BG0	BG30	BG60	BG90	BG120
IGT	Female	3.93	15.3	47	0.61	80	140	112	91	153
IGT	Male	5.89	14.5	26	0.17	85	113	119	103	162
IGT	Female	6.29	14.5	28	-	85	165	100	125	174
IFG	Female	6.46	14.3	23	2.17	119	185	192	161	129
IFG	Female	9.06	20.6	92	2.99	101	121	129	111	111
IFG	Male	11.35	15.0	8	2.09	103	125	105	91	110

Legend: Body mass index (BMI) percentiles were defined according to German reference data. In one subject, no HOMA insulin sensitivity (IS) index could be calculated due to missing insulin measurements.

Furthermore, the mothers and their children were instructed to wear an accelerometer for 7 days from the study visit, and to document times of physical activity or inactivity (e.g. swimming, sleeping) in a structured diary.

Dietary evaluation

The mother's diet was assessed at the study visit by a food frequency questionnaire (by Toeller©, 2004), reflecting the nutritional habits of the past 4 weeks [15].

Furthermore, mothers and their children were asked to keep a 3-day record of the child's dietary intake within the 10 days after the visit in the study center (including two week-days and one week-end day). For assistance, the families received a picture booklet including food portion sizes usually consumed by children in that age range. The diet records were reviewed by trained study personnel for plausibility and entered into a food database (PRODI® 5 basis, Wissenschaftliche Verlagsgesellschaft, Germany) to calculate energy and nutrient intake and assess food categories consumed. Moreover, the primary caretakers and the child were questioned on whether they ingested pro-/prebiotic supplements during the last 6 months preceding the clinical visit, and also whether the child was fully breastfed during the first 3 months of life.

Stool sample for microbiome analysis

The primary caretaker and the child were asked whether they were sick or received medication during the 6 months preceding the study visit, with a focus on the use of antibiotics. If no antibiotics were taken within the last 6 months preceding the study visit, the mothers were asked to collect fresh stool samples in tubes, from both themselves and their participating children, in the weeks after the clinical visit, and send them back by courier to the Institute of Diabetes Research, Helmholtz Zentrum München. Stool samples were processed and stored at -80°C until analysis of stool bacterial.

Metabolomics and breath gas analyses

To investigate changes in the metabolic profile after glucose challenge, blood samples (plasma aprotinin) and breath gas samples of either the mother or her child were collected during the OGTT. Sample donors breathed at 6-10-minute intervals in a buffered end-tidal sampler (Ionicon GmbH) connected to the proton transfer reaction mass spectrometry (PTR-MS) device with a heated flexible tube, as described recently [16].

A targeted and non-targeted metabolomics approach was used where quantification of metabolites was performed using high throughput platforms like the Biocrates Absolute IDQTM kit 2 technology for amino acid and lipid metabolites [17]. The non-targeted Metabolon-based technology was used for other metabolites including carbohydrates, nucleotides, peptides, steroids, and xenobiotics.

Data management

The results of all clinical and laboratory evaluations have been entered in case report forms (CRFs) which have been stored at the Institute of Diabetes Research, Helmholtz Zentrum München. Data were entered into an established SQL database.

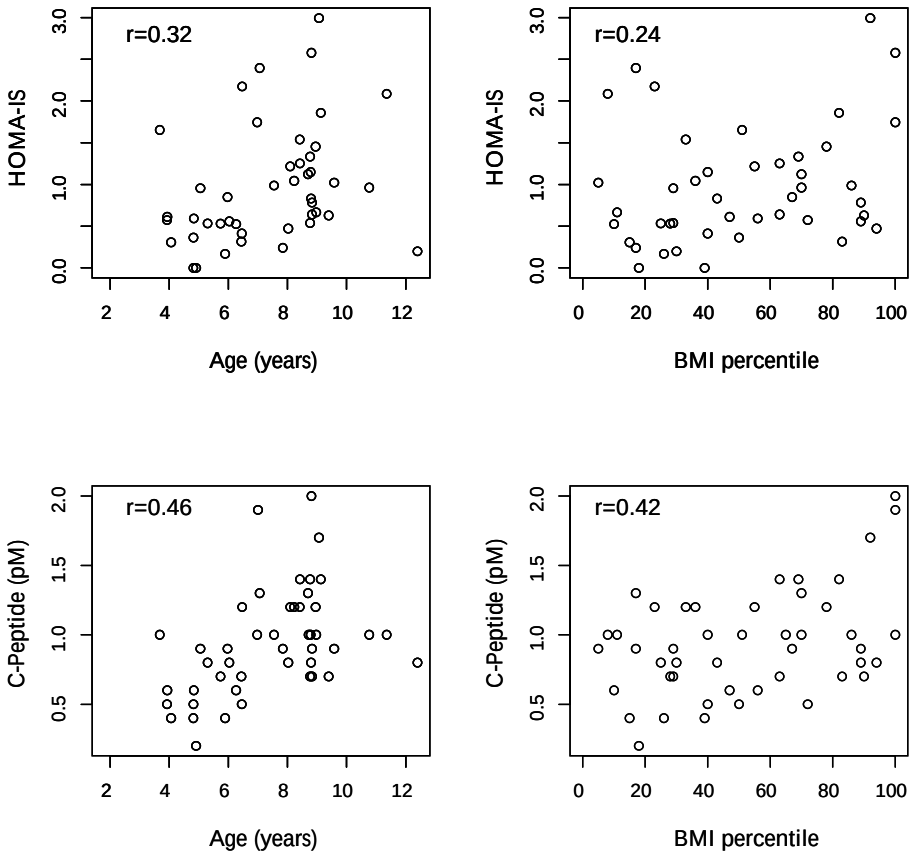

Figure 3. Scatterplots of fasting HOMA insulin sensitivity (IS) index and C-peptide by age and BMI percentile, respectively, with Pearson correlations r.

Results

Of 121 mothers recruited up to 1 July 2013, 105 (86.8%) had been exposed to GDM in at least one index pregnancy (**Figure 1**), as diagnosed through OGTT between the 24th and 28th week of gestation. Of the 105 GDM mothers, 62 (59.0%) had received dietary treatment, and 40 (38.1%) had been treated with insulin (three missing values) during their last index pregnancy with GDM.

The median follow-up time after the last index pregnancy was 5.5 years, with a range of 1.8 to 11.4 years. At follow-up, 47 mothers (38.8%) had abnormal glucose tolerance, comprising 19 subjects (15.7%) with impaired glucose tolerance, 19 (15.7%) with impaired fasting glucose, eight (6.6%) with T2D and one (0.8%) with T1D.

In total, 133 index children (median age: 6.2 years) and 49 non-index children (median age: 6.9 years) have been enrolled in the POGO study. For 13 mothers, more than one index child was enrolled, while for six mothers only data of non-index children were available. Exposure to GDM was found in 117 (88.0%) of the index children (**Figure 2**). Six (5.1%) of the GDM-exposed children were obese at the study visit or at the last available measurement before it, while there were no obese children in the group with no exposure to GDM. Of 61 index children tested by OGTT, three (4.9%) had impaired glucose tolerance and another three (4.9%) had impaired fasting glucose at the study visit, all six having been exposed to GDM *in utero*. Detailed characteristics of these six children are shown in **Table 1**. No child was diagnosed as T1D or T2D.

Insulin sensitivity and fasting C-peptide levels could be calculated in 44 and 46 index children and were positively correlated with age and BMI percentile, respectively (**Figure 3**).

Discussion

The POGO study addresses mechanisms behind the increased incidence of pre-diabetes and T2D in women with a history of GDM and further focuses on identifying the causes that might contribute to the subsequent development of obesity and insulin resistance in the offspring exposed to GDM *in utero*. A microbiome and metabolomics approach is used to investigate the relationship between the composition of the intestinal microbiota and/or (concomitant) changes in the metabolite profile with GDM exposure and subsequent development of obesity, insulin resistance and T2D in this cohort.

As presented in this paper, preliminary results appear promising. Unique to the POGO cohort its focus is on both maternal and child associated changes in lifestyle and metabolism, β-cell function and insulin sensitivity, and their relationship with the development of T2D and obesity. Our study also includes extensive documentation of lifestyle habits (e.g. nutrition, physical activity) and environmental factors (e.g. breastfeeding, siblings, smoking exposure). This will enable us to examine a variety of relevant confounders within this association, and thus to investigate the independent contribution of GDM to T2D development and the offspring's obesity risk.

Consequently, there is a focus on the perinatal and early postnatal phases, which may be highly relevant for offspring's disease susceptibility [18]. If available, timing of fetal exposure to elevated maternal glucose levels will be documented, together with maternal therapy during pregnancy, early infant growth and breastfeeding status. These factors are suggested as highly relevant for maternal metabolic outcome [3, 7] and child's obesity risk [4, 19]. Further we plan to investigate how the glucose levels during the diagnostic OGTT in pregnancy correlate with the respective maternal and offspring outcome postpartum.

Our data may even enable us to compare outcomes between siblings with discordant exposure to GDM, thus providing an opportunity to control for shared genetic and lifestyle factors.

As GDM was confirmed in 67% (1229/1826) of all women screened, the final sample is expected to contain about 335 mother-offspring pairs with GDM exposure and 165 controls, if the goal of 500 recruited pairs will be reached. Assuming a prevalence of 1.0% of mothers who develop T2D after a pregnancy without GDM, an increased risk of 7.43 in GDM mothers [20] and a significance level of 5%, this sample size would allow the detection of an association between GDM and later T2D with a statistical power of 89%. Similarly, based on a prevalence of childhood overweight (including obesity) of 15% [21] and an increased risk of 1.81 in offspring of GDM mothers [22], the statistical power to detect an association of this size would be 67%. In the data collected so far, the group of mothers without GDM comprised only 13%, because the initial recruitment focus was on GDM mothers. However, the clear aim is to contact all women who had been originally screened, so that the proportions of GDM women and controls will be similar to those in the screening data. Although addition of the data from the three other participating centers will certainly increase the statistical power, it should be acknowledged that these questions have already been addressed in epidemiological studies with much larger sample sizes [20, 22, 23]. However, we do not consider this as a major drawback of the POGO study, as its main focus lies on comparing mothers and children with and without adverse health outcomes, respectively, after exposure to GDM, and not to compare individuals who were or were not exposed to GDM.

It is expected that our study will lead to a better understanding of the impact of e.g. genetic, physiological, intrauterine, and postnatal factors on the development of childhood obesity and T2D in the offspring of women with GDM. We believe that our results will strengthen the notion that T2D etiology and offspring obesity risk after GDM pregnancy are multifactorial. This should set new focal points for investigating the causes of the disease and may further stimulate research to specify whom to target for T2D and childhood obesity prevention efforts.

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