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The German Gestational Diabetes Study (PREG), a prospective multicentre cohort study: rationale, methodology and design

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ABSTRACT

Introduction Even well-treated gestational diabetes mellitus (GDM) might still have impact on long-term health of the mother and her offspring, although this relationship has not yet been conclusively studied. Using in-depth phenotyping of the mother and her offspring, we aim to elucidate the relationship of maternal hyperglycaemia during pregnancy and adequate treatment, and its impact on the long-term health of both mother and child.

Methods The multicentre PREG study, a prospective cohort study, is designed to metabolically and phenotypically characterise women with a 75-g five-point oral glucose tolerance test (OGTT) during, and repeatedly after pregnancy. Outcome measures are maternal glycaemia during OGTTs, birth outcome and the health and growth development of the offspring. The children of the study participants are followed up until adulthood with developmental tests and metabolic and epigenetic phenotyping in the PREG Offspring study. A total of 800 women (600 with GDM, 200 controls) will be recruited.

Ethics and dissemination The study protocol has been approved by all local ethics committees. Results will be disseminated via conference presentations and peer-reviewed publications.

Trial registration number The PREG study and the PREG Offspring study are registered with Clinical Trials (ClinicalTrials.gov identifiers: NCT04270578, NCT04722900).

Strengths and limitations of this study

- The main strength of this multicentre PREG study is its in-depth phenotyping of mothers during pregnancy and repeatedly after delivery.
- Data acquisition and sample handling are performed in accordance with standard operating procedures at all study sites, thereby ensuring a high quality for each data point.
- A PREG biobank has been set up and samples are available to researchers of the German Center for Diabetes Research.
- Children of the study participants are repeatedly examined to cover the period of childhood and adolescence.
- The PREG study is not planned as a population-based cohort but is enriched for gestational diabetes mellitus cases.

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterised by a defined degree of hyperglycaemia that is first diagnosed during pregnancy. In 2008, the Hyperglycemia and Adverse Pregnancy Outcomes study[1] demonstrated the negative impact of even only moderately elevated blood glucose levels on pregnancy and birth outcome. In 2010, and prompted by these disquieting results, the International Association of the Diabetes and Pregnancy Study Group (IADPSG) amended its recommendation for plasma glucose thresholds for the diagnosis of GDM with a 2-hour 75-g oral glucose tolerance test (OGTT): fasting glucose 5.1 mmol/L, 1-hour glucose 10 mmol/L and 2-hour glucose 8.5 mmol/L.[2] Today, these GDM diagnostic criteria are in general use in many countries, and standardised screening procedures have been implemented. This approach enables the timely diagnosis of the majority of GDM cases. GDM treatment based on different thresholds has been shown to lead to a reduction in macrosomia and other unfavourable peripartal outcomes.[3–6]

However, even if maternal glucose levels can be therapeutically controlled and, as is usually the case, return to normal after delivery, GDM is not only a transient pregnancy-specific pathology. For the mother, GDM represents a looking glass into her own metabolic future.[7] Metabolic disturbances during pregnancy are predictive for a woman's future risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease. This has already been shown by a large number of studies and meta-analyses in which GDM was shown to increase the risk of developing not only T2DM[8 9] but also the metabolic syndrome,[10 11] cardiovascular disease[12] and some form of cancer.[13] A potential association with depression is currently being controversially discussed.[14 15]

For the offspring, who had already been exposed to elevated glucose and nutrient availability when GDM was diagnosed, maternal GDM is associated with an increased risk of adverse effects on (brain) development and metabolic health.[16] A number of studies show that GDM predisposes the offspring to becoming overweight and obese during childhood and adolescence.[17] Furthermore, disturbances of glucose metabolism in prepubertal children from GDM pregnancies have been reported.[18] Presumably via mechanisms termed fetal programming, high maternal glucose levels and glucose metabolism disorders cause epigenetic changes in the offspring that are detectable in, for example, placenta and blood.[19 20] The influence of GDM on the epigenome seems to be long-lasting, since differential methylation patterns persist even in teenagers whose mothers had increased glucose levels during pregnancy.[21] Adult offspring of mothers with GDM have a higher risk for glucose intolerance, insulin resistance[22] and T2DM.[23] Furthermore, register studies report that GDM has an adverse impact on cognitive development and intelligence.[24 25] Studies from our group demonstrated that the brain response of fetuses from mothers with GDM and insulin resistance is different from healthy controls, which is indicative of brain insulin resistance in these fetuses.[26] Furthermore, an association with attention-deficit/hyperactivity disorder in children exposed to diabetes in utero has been reported.[27] In addition to these potential central nervous effects, GDM appears to affect the fetal autonomic nervous system also.[28]

It is currently unclear as to whether standard GDM treatment will be successful in fully preventing the adverse long-term effects of GDM, such as obesity or disturbances of glucose metabolism in older children and adults. Some studies indicate that, while current GDM treatment approaches might not suffice to prevent increased childhood obesity,[29 30] they might still be beneficial for fasting glucose in female offspring aged 5–10 years.[31] However, this has been investigated in only a limited number of studies and further research is therefore required to optimise GDM screening and therapeutic strategies during pregnancy.

Transgenerational diabetes

Genetic and environmental factors such as sedentary lifestyle and poor nutrition are discussed as major drivers of the worldwide diabetes epidemic. Maternal hyperglycaemia, that is, GDM, might additionally drive the familial accumulation of T2DM via non-genetic inheritance mechanisms. However, these mechanisms are still not completely understood. An oversupply of nutrients to the fetus may not only induce overgrowth via fetal hyperinsulineamia but may also introduce long-term changes in regulation of satiety, activity and endocrine system. Women with a family history of diabetes have a higher risk of developing GDM themselves, thereby perpetuating this cycle of transgenerational diabetes.

Aims

While the detrimental effect of untreated GDM on the health of mother and child is undisputable, the long-term effects of a well-treated GDM on mother and child remain largely unknown. Even if the immediate threat of fetal overgrowth and macrosomia were to be dramatically reduced by nutritional intervention and medical treatment, fetal programming occurs from the very beginning of pregnancy, long before GDM is diagnosed. A treatment in the last trimester might not be sufficient to entirely counteract the adverse maternal metabolic influences that accumulated during the first two trimesters.

By in-depth phenotyping of the mother during pregnancy and repeated follow-up of both her and her offspring, the PREG study constitutes an outstanding opportunity to elucidate the relationship of the maternal metabolism during pregnancy and its impact on the long-term health of the mother and her child.

METHODS

Setting and study outline

The PREG study, a prospective multicentre cohort study, is being conducted at four sites of the German Center for Diabetes Research (DZD) (online supplemental table 1) and coordinated by the Tübingen site. At all four study sites, women are examined during pregnancy and up to four times in the decade following delivery. Birth outcomes as well as offspring anthropometric data are collected. Cord blood and placenta are collected and the offspring cohort is examined during subsequent visits (presently at the Tübingen site only).

The study outline is presented in table 1 and figure 1. Women are examined during pregnancy as well as 1, 2, 5 and 10 years after delivery. Offspring are followed up at the age of 2, 6, 10, 14 and 17 years. Harmonised standard operating procedures are established for OGTT, spiroergometry, MRI and magnetic resonance spectroscopy (MRS), sample generation, sample processing and sample storage across all study sites. The estimated primary completion date of the PREG study is May 2027 with the potential for further follow-up.

Table 1 Study outline

	Time point	OGTT	(Fasted) blood sample	Anthropometry	Continuous glucose measurement	Bioimpedance analysis	Liver ultrasound	Whole-body MRI/ liver fat MRS	Spiroergometry	Developmental and intelligence tests	Questionnaires and food diary	Accelerometry
During pregnancy	Gestational week 24–32	X		X		X	X				X	
	Delivery		X (cord blood and maternal HbA1c)	X (medical records)								
Follow-up mothers	1 year	X		X		X		X	X		X	
	2 years	X		X		X		X	X		X	
	5 years	X		X		X		X	X		X	
	10 years	X		X		X		X	X		X	
Follow-up children	2 years			X		X				X	X	
	6 years		X	X	X	X		X		X	X	X
	10 years		X	X	X	X		X		X	X	X
	14 years		X	X	X	X		X		X	X	X
	17 years		X	x	X	X		X		X	X	X

HbA1c, glycated haemoglobin; MRS, magnetic resonance spectroscopy; OGTT, oral glucose tolerance test.

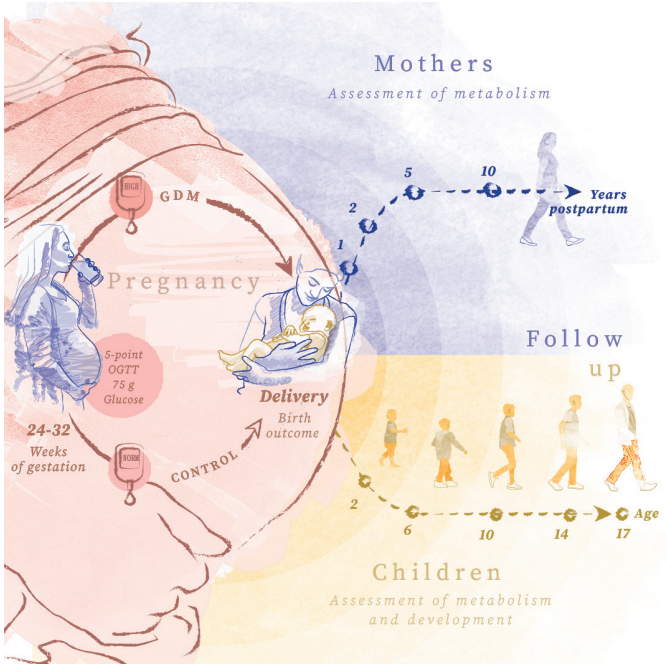

Figure 1 Study outline.

Calculation of sample size

On the basis of 2-hour glucose values from postpartum OGTT in our TUEFF cohort[32] on women with and without a history of GDM (group means 6.9±2.0 vs 6.4±2.0 mmol/L), we calculated the sample size required to detect these differences in glucose tolerance (effect size d=0.25, alpha=0.05, power (1-beta error probability)=0.8, allocation ratio 3:1). Taking potential dropouts into account, a total of 800 women (600 with GDM, 200 controls) will be recruited.

Recruitment

Eligible women are recruited via outpatient clinics for pregnant women, via the diabetes outpatient clinics, residential gynaecologist practices and advertising leaflets/broadcast emails. In Germany, GDM is currently usually diagnosed using a two-step approach. All pregnant women between gestational weeks 24 and 28 are recommended to take a 50-g glucose challenge test (GCT). This test can be performed at any time of day and does not require that the women fast. If the venous plasma glucose is ≥7.5 mmol/L at 1 hour after 50-g glucose ingestion, a diagnostic 2-hour-75-g-OGTT is performed on a separate day following an overnight fast. The 50-g GCT is not part of the PREG study. Residential gynaecologists refer patients with glucose concentrations above the threshold in the 50-g GCT to the diabetes outpatient clinic, where they are offered the opportunity to participate in the PREG study. With this recruitment strategy, women with GDM are enriched in the PREG cohort but although they constitute a high-risk group, they will not be representative of the general population.

Participants

Pregnant women are enrolled between gestational weeks 24+0 and 31+6. In addition, women with a confirmed diagnosis of GDM in a previous pregnancy are eligible for the study if detailed records of a previous diagnostic 2-hour OGTT with 75-g glucose are available. Exclusion criteria are age <18 years, pre-existing type 1 or type 2 diabetes mellitus, estimated glomerular filtration rate <60 mL/min/1.73 m² (MDRD formula[33]), C reactive protein (CRP) >1 mg/dL, transaminases >two times upper limit of the normal, pre-existing cardiac conditions, weight loss >10% within 6 months prior to study enrolment, psychiatric disorders, chronic alcohol or drug abuse, and blood glucose-increasing or -decreasing medication (eg, steroids, oral antidiabetic agents, insulin). For subjects enrolling after delivery, additional exclusion criteria are current breast feeding and new pregnancy.

Each of the facultative measurements—MRI/MRS and spiroergometry—has its own additional set of exclusion criteria; for MRI/MRS: any ferromagnetic metals in or on the body, claustrophobia, impaired thermal sensor reception, increased sensitivity to heating or loud noises and hearing loss; for spiroergometry: cardiac morbidities (acute coronary syndrome, cardiac arrhythmias, congestive heart failure, any form of acute carditis), history of pulmonary embolism, acute deep vein thrombosis, hyperthyroidism or hypokalaemia.

Exclusion criteria for offspring are severe malformations that preclude developmental tests.

Ethics and dissemination

Informed written consent is obtained from all study participants and from the legal guardian, respectively. Children aged 6 and upwards are asked to renew their written consent at each study visit. The study is conducted in accordance with the Declaration of Helsinki. The study protocol has been reviewed and approved by the Ethics Committee of the University Hospital Tübingen (protocol numbers 218/2012BO2 and 617/2020BO1), the Ethics Committee of the Technical University Dresden (protocol number EK263072013), the Ethics Committee of the Medical School of the Heinrich Heine University Düsseldorf (protocol number 4051) and the Ethics Committee of the Medical School of University of Leipzig (protocol number 038-15-09032015). Participants can withdraw their consent to the study at any time without stating a reason. In such a case, participants can decide whether their data and biosamples remain in the study for further analyses or if these are to be deleted. The results will be disseminated via conference presentations and peer-reviewed publications.

A PREG biobank is set-up and samples are available to researchers of the DZD on application and positive evaluation by the DZD Use and Access Committee.

Planned statistical analysis

We plan to analyse the effect of maternal metabolism during pregnancy on the long-term health consequences

for mother and child. This will be statistically tested with multivariate analysis of variance and mixed modelling. Associations will be tested by multivariate linear regression or non-parametric tests as appropriate. To address potential imbalance due to dropout, we plan to compare dropout cases with complete cases with regard to key factors. If differences are found, additional subgroup analyses with matched groups are planned.

Patients and public involvement statement

Patients were not directly involved in the initial design of the PREG and PREG Offspring studies. However, regular meetings between study participants and researchers take place at least once per year. This facilitates the dissemination of results and enables us to gain input from the participants. In addition, an annual newsletter providing information on the latest research results is published.

Examinations in women at baseline and follow-up

GDM diagnosis and treatment

GDM is diagnosed in accordance with the national guidelines[34] of the German Diabetes Association and with the WHO and IADPSG[2] criteria. Patients with GDM are treated in keeping with national guidelines[34] with nutritional counselling, self-monitoring of blood glucose, and, where necessary, with insulin. This treatment, while it is not part of the PREG study, is recorded in detail for each participant.

Oral glucose tolerance test

Following a fasting period of at least 10 hours, women undergo a 5-point 2-hour OGTT with 75-g glucose during pregnancy, as well as at all follow-up visits (table 1). Venous blood is taken from a venous catheter at 0, 30, 60, 90 and 120 min. Spot urine is collected prior to OGTT and directly after the last blood sample is withdrawn. All samples are immediately put on ice and quickly processed or transferred to long-term storage at −80°C, respectively.

Laboratory measurements

From fasted blood samples, routine laboratory parameters, including glycated haemoglobin, lipids, CRP, interleukin 6, cortisol, beta-human chorionic gonadotropin, prolactin, oestradiol, testosterone and sex hormone-binding protein are measured (online supplemental table 2). From each time point of the OGTT, glucose and non-esterified fatty acids are measured locally at each study site, while serum insulin and C-peptide are measured centrally at the Tübingen site by an immunoassay on ADVIA Centaur XP Immunoassay System (Siemens Healthcare Diagnostics, Erlangen, Germany). For batch analyses, plasma, serum and urine samples are stored at −80°C in the PREG biobank (ethics board number for biobank: 141/2019BO1).

DNA and RNA extraction

DNA is extracted at baseline from maternal whole blood with appropriate steps of cell lysis, protein precipitation and washing. RNA is extracted from the maternal PAXGene samples (BD, Heidelberg, Germany) at baseline and follow-up. DNA and RNA are stored at −80°C.

Anthropometry, body composition and blood pressure

Weight and height of participating women are measured using a calibrated scale and stadiometer at baseline and on every follow-up visit. Waist and hips are measured according to standard operating procedures with a tape measure. Body composition is assessed by bioimpedance with an Akern BIA101 (SMT medical, Würzburg, Germany) and calculated with Cyprus V.2.7 (RJL Systems, Clinton Township, Michigan, USA). Blood pressure is measured with an automated sphygmomanometer (BOSO Carat Professional; Bosch+Sohn, Jungingen, Germany).

Fetal magnetoencephalography (fMEG) and fetal magnetocardiography (fMCG)

In a subset of pregnant women, an additional measurement of fetal brain activity and heart rate is performed at the Tübingen site during the OGTT (ethics board number 339/2010BO1).[35–37] Measurements are conducted with the specially developed fMEG system SARA II (SQUID Array for Reproductive Assessment; VSM MedTech, Port Coquitlam, Canada). This device contains 156 integrated primary and 29 reference sensors (SQUID coils) to capture magnetic signals elicited by the fetal brain (fMEG) and heart (fMCG) and was developed to provide information on adverse in utero condition, for example, arrhythmias. The recording of biomagnetic signals is a unique tool to study the development of the human nervous system completely non-invasively in utero from as early as 28 weeks of gestation. At present, only two devices of the SARA type are available worldwide (University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA and fMEG Center of the University of Tübingen, Germany).

Sonography of liver fat in pregnant women

Liver fat accumulation is estimated from an ultrasonography of the liver carried out by an experienced sonographer. The ultrasound examination is performed with a curved-array transducer (34 Hz) and a CX50 POC ultrasound device (Philips, Hamburg, Germany). The degree of liver steatosis is quantified by a grading system (grades I–III, see online supplemental table 3).[38] In short, this classification is based on echogenicity, attenuation of sound and the visualisation/differentiation of vessels. The echogenicity of the liver is normalised by comparing renal and hepatic parenchyma.

Gestational weight gain and birth outcome data collection

As part of the standard care in Germany, gestational weight gain, birth outcome and complications are routinely documented in maternal logs by healthcare providers. Data from these logs are collected from all participants for study analyses. Cases of stillbirth, neonatal and infant death are recorded and the dataset remains in the sample.

Cord blood collection

Cord blood is collected from deliveries in the University Women's Hospital Tübingen. In addition to the assessment of glucose, blood count and blood gas analysis, cord blood serum and EDTA-plasma samples are stored at −80°C for batch analyses.

Questionnaires and food diary

During pregnancy, the Edinburgh Postnatal Depression Scale,[39] which has also been validated for pregnant women,[40] is used. The Beck Depression Inventory II[41] is used as a psychometric screening test for depression in non-pregnant women. The German version of the Patient Health Questionnaire (PHQ-D)[42] is used to assess psychiatric disorders. Chronic stress is assessed with the Trier Inventory for the Assessment of Chronic Stress.[43] Breastfeeding duration and intensity are assessed retrospectively with an in-house questionnaire (online supplemental material). A 7-day food diary is collected at each visit and assessed with OptiDiet PLUS software V.6.00.001 (GOE, Linden, Germany). At each appointment, the maternal physical activity is assessed using the habitual physical activity index.[44]

MRI and MRS

At all follow-up visits, non-pregnant adult participants undergo MR examinations on a 3 T whole body imager (Magnetom VIDA; Siemens Healthineers, Erlangen, Germany) for differentiation and quantification of whole-body adipose tissue (AT) and lean tissue (LT) compartments by MRI, and quantification of intrahepatic lipids (IHLs) by 1H-MRS in the early morning after overnight fasting as described in Machann *et al*.[45] From this, AT and LT of the lower extremities (feet to hips), AT of the trunk—subdivided into visceral adipose tissue (VAT) from hips to thoracic diaphragm and subcutaneous adipose tissue (SCAT, including internal AT between thoracic diaphragm and shoulders)—as well as AT of the upper extremities (from shoulders to hands, including the head)—are quantified. IHLs are quantified in the posterior part of segment 7 in the liver.

Spiroergometry

At follow-up, non-pregnant women participate in a continuous, incremental exercise test to volitional exhaustion using a cycle ergometer (Ergometrics 800 S; Ergoline, Bitz, Germany). Maximum oxygen consumption (VO_2max) is measured using a spiroergometer (MedGraphics System Breese; MedGraphics, St. Paul, Minnesota, USA). VO_2max is expressed as VO_2 per kilogram lean body mass (millilitre per minute per kilogram).

Data collection of subsequent pregnancies

Women with a subsequent pregnancy are offered a diagnostic 75-g OGTT. The data from both this examination and the birth outcome are recorded. Only the index pregnancy and the index child are followed up.

Follow-up of children

Offspring of PREG study participants are examined at the age of 2, 6, 10, 14 and 17 years (table 1) at the Tübingen study site.

Fasted blood sample and urine sampling

From children (aged 6 and older), a blood sample is taken in the morning following an overnight fast. A spot urine sample is collected from 2-year-old children at any time during the visit. Children aged 6 and above provide a urine sample before the blood sample is taken.

Anthropometry, body composition and blood pressure

Height, weight, abdominal girth and head circumference are measured at each study visit. Body composition is assessed by bioimpedance with an Akern BIA101 (SMT medical) and calculated with BodyGramPRO software (SMT medical). Blood pressure is measured with an automated sphygmomanometer with appropriate blood pressure cuff sizes for children (BOSO Carat Professional, Bosch+Sohn). In addition, the offspring's weight, length and head circumference are routinely and repeatedly documented in paediatric logs by the child's paediatrician. Data from these logs are collected from all participants for study analyses.

Heart rate variability

In children, a 10-min ECG is recorded with a BIOPAC MP36 (BIOPAC Systems, Goleta, California, USA) with a sampling rate of 1000 Hz. The children are positioned in a supine position and requested to lie still. In 2-year-old children, a parent reads a book aloud to encourage them to remain motionless. The time- and frequency-domain heart rate variability parameters are analysed using KUBIOS HRV Software V.2.2.[46]

Developmental tests

In 2-year-old children, the Bayley Scales of Infant Development, Third Edition (BSID-III)[47] in the German Edition are implemented to assess their developmental state. The BSID-III evaluates the development in infants aged between 1 and 42 months in the domains of cognition, language (receptive and active) and motor skills (fine and gross motor skills) on individual scales. However, although the BSID-III also provides both a social-emotional scale and an adaptive behaviour scale, neither of these was available in the German language at the beginning of the study and therefore could not be included.

To achieve a high level of standardisation, the developmental assessment of the children is always performed by the same researcher (JHa). A caregiver remains with the child throughout the procedure. Assessment with the BSID-III is always carried out in the morning and in the same specially prepared room. Disturbing factors due to environmental stimuli are largely excluded, and apart from the test material, the room is low in distractions to ensure that the child's attention remains focused on the assessment.

Intelligence assessment

The offspring's intelligence is assessed on the basis of the Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V).[48] This is one of the most widely implemented diagnostics for the evaluation of cognitive abilities in the age group of 6- to 16-year olds. The German version of the WISC-V consists of a total of 15 subtests that can be used to generate standardised scores in five primary indices (verbal comprehension, visual spatial, fluid reasoning, working memory, processing speed), and five secondary indices (quantitative reasoning, auditory working memory, non-verbal, general ability, cognitive proficiency) as well as a full-scale IQ score. The 15 subtests can be classified into two different categories, 10 primary and 5 secondary subtests. To build the full-scale IQ, the primary indices and three of the five secondary indices, the 10 primary subtests are combined in different constellations. To ensure a standardised implementation, the WISC-V is conducted in the absence of parents, but always by the same professional in the specially prepared room containing a minimum of distractions.

Physical activity

Daily physical activity is measured with a wrist accelerometer for children aged 6 years for up to a period of 14 days following each study visit (Fitbit Ace; Fitbit, San Francisco, California, USA).

Continuous glucose monitoring

In children aged 6 years and older, 24-h glucose profiles and postprandial glycaemic excursions (area under the curve) are measured by flash glucose monitoring following each visit (Freestyle Libre Pro iQ; Abbot Diabetes Care, Witney, UK). A single-use sensor is placed on the back of the upper arm of the child and remains in place for 14 days. Children and parents are unaware of the glucose levels, since the sensor is read-out only after the recording period by study personnel.

Questionnaires and food diary

The children's physical activity is assessed with the MoMo Questionnaire,[49] and pubertal status is self-assessed with a German pubertal questionnaire for children.[50] A 7-day food diary is collected at each appointment and assessed with OptiDiet PLUS software V.6.00.001 (GOE).

MRI and MRS

Children aged 6 years and above are measured in the early morning following an overnight fast on the same MR unit. Using a chemical-shift-selective imaging technique (VIBE-Dixon), gapless fat- and water-selective images are acquired from shoulder to hip within two to three breath-hold examinations for the quantification of VAT and SCAT in the trunk. Additionally, IHL are quantified as described above and a multiecho Dixon sequence is applied for the quantification of proton density fat fraction in abdominal organs (liver, pancreas) in a single-breath-hold examination. Measurement time in total, including preparation, amounts to approximately 40 min for adult participants and 15 min for children.

DISCUSSION AND OUTLOOK

The prospective design of the PREG and PREG Offspring studies, with detailed phenotyping during and after pregnancy, provides a unique platform to address crucial research questions in an aim to gain a better comprehension of the trajectories of subsequent maternal metabolism, fetal and offspring development as well as the long-term health of mother and child. Several questions will be addressed. One key question is whether initiating the standard GDM treatment after diagnosis in the third trimester of pregnancy is sufficient to prevent adverse effects on the offspring's long-term health and development, thereby breaking the cycle of transgenerational diabetes.

The PREG study is also designed to examine the impact of maternal glucose excursions and other metabolic and anthropometric factors on the child's brain development and certain aspects of the proper formation of the autonomous nervous system. Close-meshed tracking of the development of cognition and intelligence, as well as of the autonomous nervous system throughout childhood and analysing this information together with fetal data obtained from fMEG/fMCG may provide unique insight into the effect of maternal metabolism on the neuronal development of human offspring.[51]

Worldwide, there are only a very limited number of mother-child cohorts with focus on GDM. These range from large register studies[52 53] over prospective studies[30 54–56] to case-control studies[52] and follow-up studies of randomised clinical trials for the prevention of GDM with lifestyle intervention early in pregnancy.[57] However, this PREG study covers crucial aspects that have not yet been comprehensively addressed in other larger trials. While register studies provide large datasets for epidemiological research, they lack the detailed characterisation of subjects necessary for uncovering details of potential pathomechanisms of GDM. Prospective birth cohort studies do not usually recruit mother-child dyads before birth and retrieve data on GDM diagnosis/OGTT from medical records, where often only potentially imprecise glucose measurements from baseline, 1-hour and 2-hour postglucose load are available. The PREG study is unique as it not only performs an OGTT with five strictly quality-controlled glucose measurements, but also measures insulin, C-peptide, proinsulin and free fatty acids at each time point. Furthermore, biomaterial is properly stored for the measurement of upcoming novel parameters.

These detailed OGTT data will also be used to search for potential novel subtypes of GDM. Novel clusters of pre-diabetic metabolism[58] and diabetes[59] were recently defined in an aim to gain a better understanding of the underlying disease pathomechanisms and the prediction of disease progression and complications. These studies

demonstrated that combining glucose levels with additional biomarkers can lead to the detection of clinically relevant metabolic subphenotypes.[58] The concept of subtyping of overt diabetes was recently validated and its role in the early development of diabetes-related complications was defined.[60] Although subclassification of GDM has already been proposed in the past,[61–63] these studies relied only on glucose assessments, combined in part with insulin sensitivity. While the use of such subclassification might enable us to identify a potential future T2DM risk more precisely, the clinical relevance of such approaches has yet to be proven in appropriate prospective studies. The comprehensive dataset collected from each pregnant woman in the PREG study will facilitate an investigation of possible subtypes of GDM with much higher discriminating power, not only for the future diabetes risk of the mother but also for birth outcome and the offspring's development.

The unique strengths of this PREG study are its in-depth phenotyping of mothers during pregnancy and repeatedly after delivery, comprehensive laboratory measurements and state-of-the-art examination procedures in combination with biobanking. Data acquisition and sample handling are performed in accordance with standard operating procedures at all study sites, thus ensuring a high quality for each data point. Biosamples are collected during and after pregnancy, facilitating the measurement of potential new biomarkers or pathogenetic factors that might be identified in the future. The PREG Offspring study protocol provides a platform to comprehensively cover the period of childhood and adolescence. By design, the PREG study is not a population-based cohort, but is enriched for GDM cases.

In conclusion, the comprehensive phenotyping of maternal metabolism in this study will enable us to study the effect of hyperglycaemia and other maternal factors on subsequent diabetes risk of the mother and on body composition, metabolism and development of the offspring with respect to standard GDM treatment. These findings could help ameliorate current treatment recommendations and thereby help to fight the ever-increasing diabetes epidemic.

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