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Epidemiology of diabetic disorders and its long-term impact on adiposity in the offspring

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Content

List of Abbreviations

ALT	Alanine aminotransferase
BMI	Body mass index
CI	Confidence interval
GDM	Gestational diabetes mellitus
GGT	γ-glutamyl transferase
hs-CRP	High-sensitive C-reactive protein
MetS	Metabolic syndrome
OGDM	Offspring of mothers with gestational diabetes mellitus
OnonDM	Offspring of mothers without diabetes
OT1DM	Offspring of mothers with type 1 diabetes mellitus
OT2DM	Offspring of mothers with type 2 diabetes mellitus
SDS	Standard deviation scores
SHIP	Study of Health in Pomerania
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TEDDY	The Environmental Determinants of Diabetes in the Young

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1. Introduction

Globally, the disease burden of diabetes mellitus in the recent decades is alarming and is considered a major public health problem, as it is the seventh major cause of death[1]. The estimated global prevalence of diabetes was 425 million in 2017, and is expected to rise to 629 million by 2045[2]. Diabetes mellitus can be principally classified into 4 categories: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM) and diabetes with specific causes, e.g., monogenic diabetes syndromes and drug- or chemical- induced diabetes[3]. Type 1 and type 2 together account for the major burden of diabetes with T2DM contributing for more than 85% of the total diabetes cases[2]. The etiology of T2DM is multifactorial and not completely understood yet. Genetic predisposition to T2DM, overweight and obesity, physical inactivity, smoking and increased consumption of unhealthy diets are some major known risk factors of T2DM[4]. Apart from these factors, iron status has been suggested to play a role in the pathogenesis of T2DM and other metabolic disorders[5]. This hypothesis evolved from the fact that the prevalence of T2DM was higher in individuals with hereditary hemochromatosis – a genetic disorder characterized by massive iron overload[6]. Thus, of the many less established risk factors, the relationship between iron markers and T2DM and metabolic syndrome (MetS) were investigated as part of my doctoral thesis.

In addition to gaining insight into the risk factors for diabetes mellitus, there is dire need to gain a wider understanding into the consequences of diabetes mellitus. It has been suggested that a maternal diabetic environment can impact the intrauterine development of the fetus through excessive maternal glucose crossing the placenta and leading to excess fetal glucose and insulin, thereby causing overgrowth of the fetus[7]. These exposures to maternal hyperglycemia during fetal life have been reported to extend beyond the neonatal period and influence metabolic complications in later life. While the immediate effects of maternal hyperglycemia on fetal growth are well known, the long-term consequences on the offspring are less clear. Hence, the present dissertation also focuses on 1) the influence of maternal diabetes on offspring adiposity and metabolic health during childhood and adolescence and 2) the potential pathways through which maternal diabetes may affect the offspring's metabolic health.

1.1 Iron markers and type 2 diabetes mellitus/ metabolic syndrome

Although iron is an essential mineral responsible for physiological processes such as DNA synthesis and oxygen transport, excessive iron stores have been considered a health hazard as it leads to the formation of hydroxyl radicals[8,9]. As a result, iron is said to affect glucose metabolism and several observational studies have observed positive associations between ferritin concentrations – a widely used marker of body iron stores – and increased risk of impaired glucose metabolism, MetS and T2DM[10-13]. However, serum ferritin is an acute phase protein which may also reflect systemic inflammation or liver dysfunction rather than high iron stores. Thus, it is not clear whether the association of ferritin concentrations with T2DM or MetS is independent of inflammation or hepatic dysfunction. Besides, investigation of additional iron markers may help to further understand the role of iron in the development of metabolic disorders. Studies investigating other iron markers such as transferrin – an iron transport protein – and T2DM and Mets are scarce and inconsistent[12,14,15].

Hence, based on the population-based SHIP cohort, we aimed to evaluate 1) the association of ferritin and transferrin concentrations at baseline with prevalent and incident T2DM and MetS during a follow-up of nearly 11 years, and 2) whether these associations are independent of inflammatory markers and hepatic enzymes. If a causal relationship between iron metabolism and metabolic disorders is established, decreasing iron stores may play an integral role in the prevention and management of diabetes and other metabolic disorders.

1.2 Maternal diabetes and offspring adiposity/ metabolic health

Childhood obesity and overweight is a growing problem worldwide. Both subsequently increase the risk for later obesity, MetS, diabetes, and cardiovascular disease in adulthood[16,17], which calls for a need to identify the determinants of obesity in early life or even before birth. Emerging evidence suggests that childhood obesity and metabolic complications in later life may have their origins in utero via exposure to maternal diabetes[18-20]. It is well known that intrauterine hyperglycemia is strongly associated with infant macrosomia[21,22]. However, evidence to support long-term consequences of maternal diabetes during early childhood, late childhood and adolescence are not consistent[23-28]. Moreover, the majority of the previous studies have shown higher risk of obesity, insulin resistance, or T2DM in the offspring exposed to GDM and T2DM[18,19,29], but there is only scant evidence to support a similar effect in the offspring exposed to maternal T1DM[15,18]. However, it is important to investigate the effects of all diabetes forms as they may have differential effects on the offspring or act

through different pathways owing to the differences in the timing of hyperglycemia or associated risk factors like maternal obesity.

Hence, our study aimed to investigate 1) whether exposure to maternal diabetes during pregnancy is associated with offspring adiposity and metabolic risk during childhood and adolescence and 2) whether this association varies by offspring age or maternal diabetes status, based on the data from three prospective cohorts which followed offspring from birth until about 18 years of age. This will aid in 1) recognizing the children whose mothers had diabetes during pregnancy as a particular risk group with respect to excess weight gain and metabolic risk in later life and 2) providing early intervention at the right time which may have measurable impact on the health of the population.

1.2.1 Potential pathways linking maternal diabetes and offspring adiposity

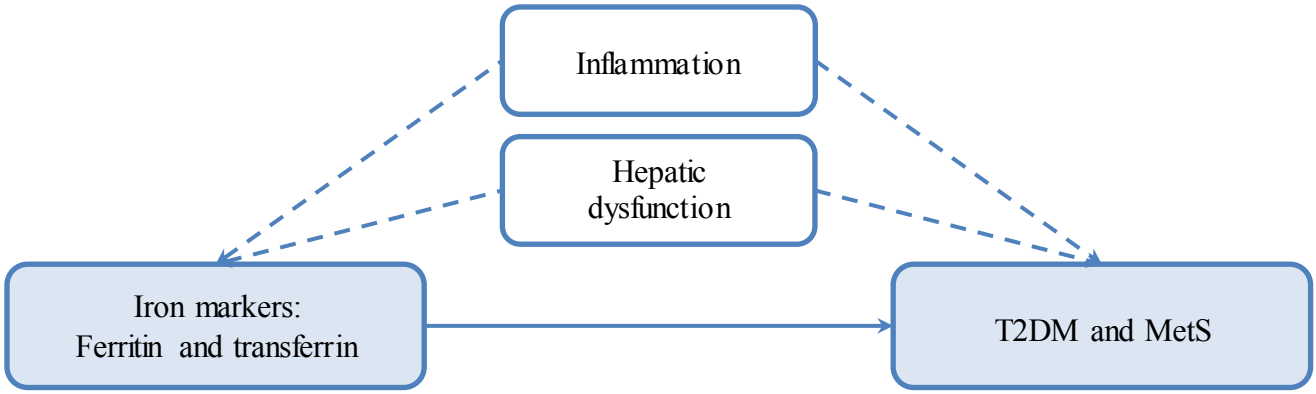
While there are several studies showing an association between maternal diabetes and excess adiposity and poor metabolic health in the offspring, potential pathways were not investigated in detail. Evidence suggests that maternal obesity may also increase the risk of overweight and obesity in the offspring[30]. Previous studies associating GDM with offspring overweight and obesity have reported that maternal obesity largely confounds this association[24,31-33], which is in contrast to one study demonstrating independent associations of GDM with childhood overweight[34]. Therefore, it remains unclear whether GDM as well as the other diabetes forms, T1DM and T2DM are associated with offspring adiposity independent of maternal pre-pregnancy BMI.

High birth weight is a known risk factor of maternal hyperglycemia in pregnancy, regardless of the type of diabetes[35,36]. Further, high birth weight is considered as an early marker of overweight/obesity at later ages[37]. However, little remains known about the influence of birth weight on the pathway from maternal diabetes to offspring overweight/obesity.

Furthermore, epidemiological studies have suggested that metabolic programming caused by the obese and/or diabetic intrauterine environment is one of the critical factors contributing to the etiology of obesity and poor metabolic health in the offspring[38]. While some studies have reported a link between metabolic concentrations and childhood obesity[39-41], the effects of maternal T1DM on the offspring metabolic profile have not yet been examined.

Hence, our aim was to investigate whether the association between maternal diabetes and offspring adiposity is independent of maternal pre-pregnancy BMI and whether birthweight and/or changes in the offspring's metabolome are in the potential causal pathway.

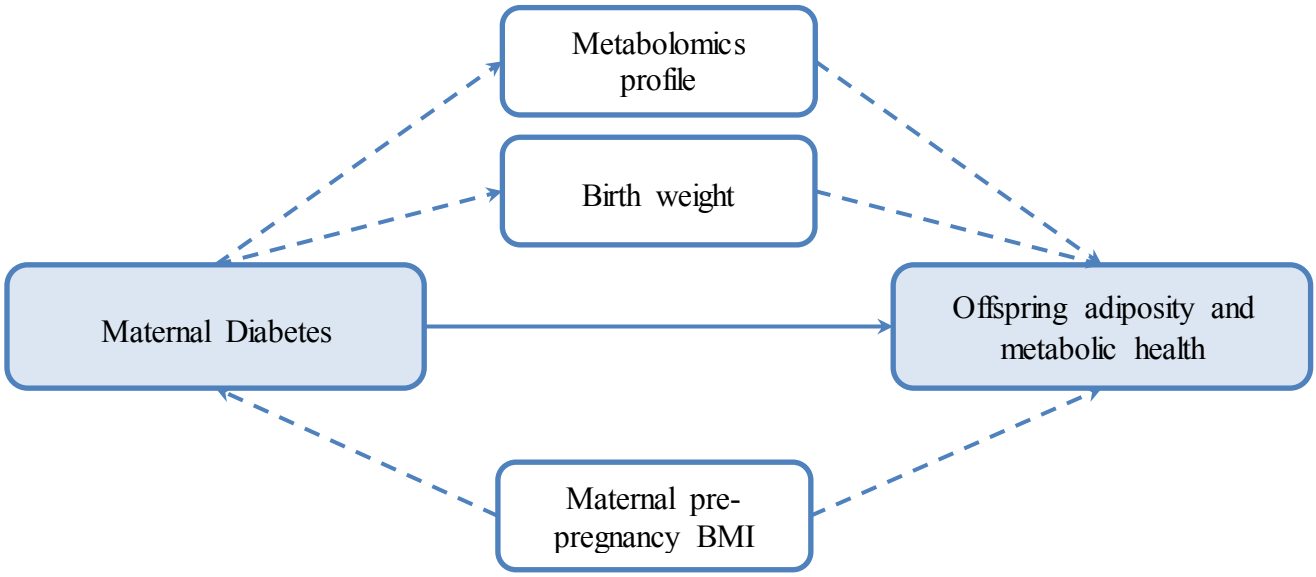
1.3 Aims of the project:



Aims of the project 1: a) to investigate the associations between iron markers and prevalent and incident T2DM/ MetS and b) to investigate whether these associations are independent of inflammation and hepatic dysfunction.

The findings are reported in this scientific paper:

1. Pitchika A, Schipf S, Nauck M, Dörr M, Lerch MM, Felix SB, Markus MRP, Völzke H, Ittermann T. Associations of iron markers with type 2 diabetes mellitus and metabolic syndrome: Results from the prospective SHIP study. Diabetes Res Clin Pract. 2020;163:108149. Epub 2020 Apr 15.



Aims of the project 2: a) to investigate the associations between maternal diabetes (GDM, T1DM, T2DM) and offspring adiposity and metabolic health, b) to investigate whether these associations are independent of maternal pre-pregnancy BMI and c) to investigate whether birthweight and/or changes in the offspring's metabolome are in the potential causal pathway.

The findings are reported in these two scientific papers:

1. Pitchika A, Vehik K, Hummel S, Norris JM, Uusitalo UM, Yang J, Virtanen SM, Koletzko S, Andrén Aronsson C, Ziegler AG, Beyerlein A; TEDDY study group. Associations of Maternal Diabetes During Pregnancy with Overweight in Offspring: Results from the Prospective TEDDY Study. Obesity (Silver Spring). 2018 Sep;26(9):1457-1466.

2. Pitchika A, Jolink M, Winkler C, Hummel S, Hummel N, Krumsiek J, Kastenmüller G, Raab J, Kordonouri O, Ziegler AG, Beyerlein A. Associations of maternal type 1 diabetes with childhood adiposity and metabolic health in the offspring: a prospective cohort study. Diabetologia. 2018 Nov;61(11):2319-2332

2 Materials and methods

2.1 Study population

The study populations included in the current dissertation are based on four prospective cohort studies: Study of Health in Pomerania (SHIP), TEENDIAB, BABYDIAB/BABYDIET and The Environmental Determinants of Diabetes in the Young (TEDDY).

2.1.1 Project 1: Iron markers and type 2 diabetes mellitus/ metabolic syndrome

This study project was based on data from the population-based SHIP cohort, which was conducted in West Pomerania, Germany. The study sample was drawn from the target population after stratification by age, sex and region. Details on the study design, protocol and sampling methods have been reported elsewhere[42,43]. A total of 6,265 subjects aged 20-79 years were invited, of which, 4,308 participated in the first examination of the SHIP study (response rate 68.8%). The follow-up examinations of the cohort were conducted during 2002-2006 (SHIP 1) and 2008-2012 (SHIP 2) among 3,300 and 2,333 participants after a mean follow-up time of 5 and 11 years, respectively. All participants provided written informed consent and the study was approved by the medical ethics committee of the University of Greifswald. Our final study sample comprised a maximum of 3,232 individuals, but the sample size varied according to specific outcome, analysis and exclusion criteria (Figure 1).

2.1.2 Project 2: Maternal diabetes and offspring adiposity/ metabolic health

This study project was based on the data from three prospective cohort studies: The Environmental Determinants of Diabetes in the Young (TEDDY), TEENDIAB, and BABYDIAB/BABYDIET.

The TEDDY study is an ongoing international, multicenter, prospective cohort study conducted in six clinical research centers located in the United States, Finland, Germany and Sweden. Between 2004 and 2010, this study enrolled 8,676 newborns with T1DM associated human leukocyte antigen genotypes to identify the environmental factors triggering islet autoimmunity and T1DM. This large longitudinal cohort also offers the opportunity to investigate the factors influencing childhood overweight and obesity. Children were followed every 3 months from birth until 4 years and every 6 months thereafter. Our final study sample comprised 5,324 children after excluding 3,352 children with missing data on height and

weight measurements after age 5 (n = 3,181) or maternal diabetes status during pregnancy (n = 171).

TEENDIAB study is a prospective cohort study conducted in the cities of Hannover and Munich, Germany. During 2009-2015, this study enrolled 610 children aged 6-16 years who had at least one parent or sibling with T1DM. Children were followed, on average, every 6 months from 6 to 18 years of age until 2016. Our final study sample comprised all 610 children.

BABYDIAB/BABYDIET studies are two ongoing prospective studies of German birth cohorts. Between 1989 and 2006, these two studies enrolled 2,441 newborns with a first-degree relative with T1DM. A total of 1,650 offspring of individuals with T1DM were recruited for the BABYDIAB study between 1989 and 2000. An additional sample of 791 offspring or siblings of individuals with T1DM were screened in the context of the BABYDIET study. Data from these two cohorts were combined for longitudinal analyses of maternal T1DM and anthropometric outcomes in the offspring. Our final study sample comprised 2,169 children after excluding 272 children who had no height and weight measurements (n=14), were lost to follow-up after 0.3 years (n=44), or who also participated in the TEENDIAB study (n=14).

Detailed information on these studies regarding recruitment criteria, follow-up characteristics, exposures and outcomes are displayed in Table 1. Further details on study design, eligibility and data collection have been described elsewhere[44-49]. All parents gave written informed consent for participation. All studies were approved by the local ethics committees.

2.2 Measurements

In this section, the measurements of the main variables used in the projects are described. Further detailed description of the measurements can be found in the respective papers[50-52].

2.2.1 Project 1: Iron markers and type 2 diabetes mellitus/ metabolic syndrome

Definition of type 2 diabetes mellitus and metabolic syndrome

Newly diagnosed T2DM at baseline and follow-up was defined based on HbA1c values ≥ 6.5%, whereas known T2DM was defined based on self-reported physician diagnosed diabetes or use of hypoglycemic medication (ATC code A10). MetS was defined as the presence of at least three out of the following 5 criteria: 1) waist circumference ≥ 94 cm in

men and ≥ 80 cm in women; 2) high-density lipoprotein (HDL) cholesterol < 1.03 mmol/l in men and < 1.29 mmol/l in women; 3) blood pressure ≥ 130/85 mmHg or antihypertensive treatment (ATC code C02); 4) random serum glucose ≥ 8 mmol/l or antidiabetic medication (ATC code A10); and 5) non-fasting triglycerides ≥ 2.3 mmol/l or lipid lowering medication (ATC code C10AB or C10AD). It was defined according to NECP/ ATP III modified with AHA/NHLBI and IDF criteria based on non-fasting blood values[53].

Measurement of iron markers and other covariates

Data on sociodemographic and lifestyle factors, medical histories and medication use were assessed by standardized computer-assisted personal interviews. During the physical examination, standardized measurements of height, weight, waist circumference, hip circumference and blood pressure were performed. Non-fasting blood samples were used to measure iron markers and other laboratory measurements. Serum ferritin concentrations were determined by an immunoturbidimetric assay (Cobas Mira Plus, F. Hoffmann-La Roche Ltd, Basel, Switzerland) and transferrin by chemiluminescent assay (Siemens Vista, TRF Flex_ reagent cartridge, Siemens Healthcare Diagnostics Inc., Newark, DE, USA). Serum ALT and GGT were determined photometrically using Hitachi 717 device. High-sensitive C-reactive protein (hs-CRP) was determined immunologically on a Behring Nephelometer II.

2.2.2 Project 2: Maternal diabetes and offspring adiposity/ metabolic health

Maternal characteristics

In TEENDIAB, BABYDIAB/BABYDIET, and TEDDY studies, information on maternal characteristics was obtained via self-administered questionnaires. Data on the presence of maternal diabetes during pregnancy and maternal smoking was available for all three studies, whereas data on parental education and family income was available only for the TEENDIAB study and data on maternal age, pre-pregnancy BMI, breastfeeding duration, gestational weight gain, gestational age at delivery and alcohol intake during pregnancy was available only for the TEDDY study.

Offspring measurements

In all three studies, data on birth weight was taken from pediatric health records or via self-administered questionnaires or structured interviews conducted during one of the follow-up visits. During each study visit, children's body weight was measured in kilograms using regularly calibrated electronic scales. Height was measured as length before age 2 and as

standing height to the nearest 0.1 cm after age 2, using a wall mounted stadiometer. Further height and weight measurements for the BABYDIAB/BABYDIET study were obtained from health records from the well-baby preventive health programme visits, which were regularly conducted at birth and at the age of 3-10 days, 4-6 weeks, and 3-4, 6-7, 10-12, 21-24, 46-48 and 60-64 months. Further in the TEENDIAB study, waist circumference was measured using a measuring tape between the pelvic crest and the lower ribs. Venous blood samples were collected to assess fasting blood glucose, insulin and C-peptide after an overnight fast of at least 10h.

Metabolomic profiling – TEENDIAB study

Non-targeted metabolomic profiling was performed on fasting serum samples taken from 500 children at the first visit using ultra high-performance liquid chromatography and mass spectrometry on the Metabolon platform (Metabolon, Durham, NC, USA). A total of 575 metabolites were quantified, of which 239 were unknown. Metabolites and samples which had more than 30% missing values were excluded, leaving a total of 441 metabolites, including 294 known and 147 unknown ones, and 485 samples. Metabolite concentrations in terms of raw ion counts were normalised to account for run-day differences and log-transformed to bring them closer to a normal distribution. Missing data were imputed using random forest imputation.

2.3 Statistical analyses

Data analyses were carried out using STATA 14.2 (Stata Corporation, College Station, TX, USA), SAS 9.4 (SAS Institute, Cary, NC, USA) and R 3.4.1 (http://cran.r-project.org).

2.3.1 Project 1: Iron markers and type 2 diabetes mellitus/ metabolic syndrome

Baseline characteristics of the study sample were expressed as median and interquartile range for continuous data and as absolute numbers and percentages for categorical data. Differences between the subjects with and without T2DM at baseline were tested by Mann-Whitney U test for continuous data and χ^2 test for categorical data. Logistic and linear regressions were performed to test associations between each baseline iron markers (ferritin and transferrin) and prevalent T2DM and MetS. Further, cox-proportional hazards regression model was performed to test the association of baseline iron markers with incident T2DM and MetS, separately. Associations were analyzed based on stepwise adjustment for all outcomes. The

first model was adjusted for age, sex, education, smoking, alcohol intake, physical inactivity, BMI, waist/hip ratio, hypertension, triglycerides, total/ HDL cholesterol ratio, serum creatinine, urinary albumin/creatinine along with the inflammatory markers hs-CRP and leukocytes, and the second additionally for the hepatic enzymes ALT and GGT to explore pathways. For the outcome MetS, the covariates BMI, waist/hip ratio, triglycerides and total/ HDL cholesterol ratio, which are components of MetS, were not used for adjustment. Several sensitivity analyses were performed to confirm the observed findings, e.g., stratified analyses in men and women, effect modification of association by sex, hs-CRP and ALT etc.

2.3.2 Project 2: Maternal diabetes and offspring adiposity/ metabolic health

In all studies, we compared offspring of mothers with diabetes to offspring of mothers without diabetes. The analyses were done separately for TEDDY, TEENDIAB and BABYDIAB/BABYDIET studies because the studies differed in the number of exposure groups, outcomes assessed, availability of covariates and the timing of the respective measurements.

Study 1: TEDDY study

Children were classified into four different groups according to maternal diabetic status during pregnancy. (1) offspring of mothers with GDM (OGDM), (2) offspring of mothers with T1DM (OT1DM), (3) offspring of mothers with T2DM (OT2DM), and (4) offspring of mothers without diabetes (OnonDM). Prior to analysis, height, weight, and BMI were transformed to standard deviation scores (SDSs) using World Health Organization (WHO) reference values[54,55]. BMI SDS values were also used to define overweight (including obesity; BMI SDS > 1) and obesity (BMI SDS > 2) according to WHO recommendations. Birth weight was transformed to a *z* score adjusting for country, sex, gestational age, maternal height, and birth type (singleton or multiplet), similar to previous analyses of the TEDDY data[56,57].

Linear and logistic regression models were performed to assess the cross-sectional associations between maternal diabetes and offspring anthropometric outcomes (BMI, height, weight, overweight, and obesity) measured at 5.5 years of age. Mixed-effects regression models were performed for longitudinal analyses of outcomes measured between 0.25 and 6 years of age. Associations were analyzed based on stepwise adjustment. In the first model, we adjusted for age (only longitudinal analysis), sex, and country for all outcomes; in the second model, we additionally adjusted for maternal pre-pregnancy BMI. Next, we included maternal

age, gestational weight gain, smoking and alcohol intake during pregnancy, maternal education, and duration of any breastfeeding in the third model and, additionally, birth weight *z* scores in the fourth model to explore potential pathways. Furthermore, we explored interaction terms between maternal diabetes and child's age (in years) in the fully adjusted longitudinal model to explore whether the association changed with an increase in age.

Study 2: TEENDIAB and BABYDIAB/BABYDIET studies

Children were classified into two groups based on maternal type 1 diabetic status: (1) Offspring of mothers with T1DM (OT1DM) and (2) Offspring of mothers without diabetes (OnonDM). Prior to analysis, height, weight, BMI and waist circumference were transformed into age- and sex-specific SD scores (SDSs) according to German reference values[58,59]. Overweight was defined as a BMI at or above an SDS of 1.31, corresponding with the 90th percentile. Abdominal obesity was defined as a waist circumference at or above the 90th percentile or the adult threshold set by the International Diabetes Federation[60]. Birthweight was transformed into age- and sex-specific percentiles based on German reference values[61], and categorized as small for gestational age (birthweight < 10th percentile), appropriate for gestational age (10th–90th percentile) or large for gestational age (> 90th percentile). Linear and logistic mixed-effect models accounting for repeated observations were performed to test the association of maternal T1DM with offspring anthropometric and metabolic outcomes, adjusting for age, sex, BMI, Tanner's staging, maternal smoking and socio-economic status and additionally, birth weight in the second model to explore potential pathways.

Within the TEENDIAB study, we further explored the extent to which the offspring's metabolomic profile may play a mediating role in the association between maternal T1DM and being overweight. First, we examined associations between the principal components as well as each metabolite concentration with offspring overweight status using logistic regression models. Second, we investigated whether maternal T1DM was associated with principal components or metabolites that were significant for overweight status, adjusted for age and sex. Third, associations between maternal T1DM and overweight status in the offspring were assessed after adjusting for metabolites or principal components which were significantly associated with being overweight. In addition, metabolite concentrations were categorized into 68 sub- and eight superpathways and were associated with offspring overweight status and maternal T1DM. The Benjamini–Hochberg procedure was used to control the false-discovery rate in order to account for multiple comparisons.

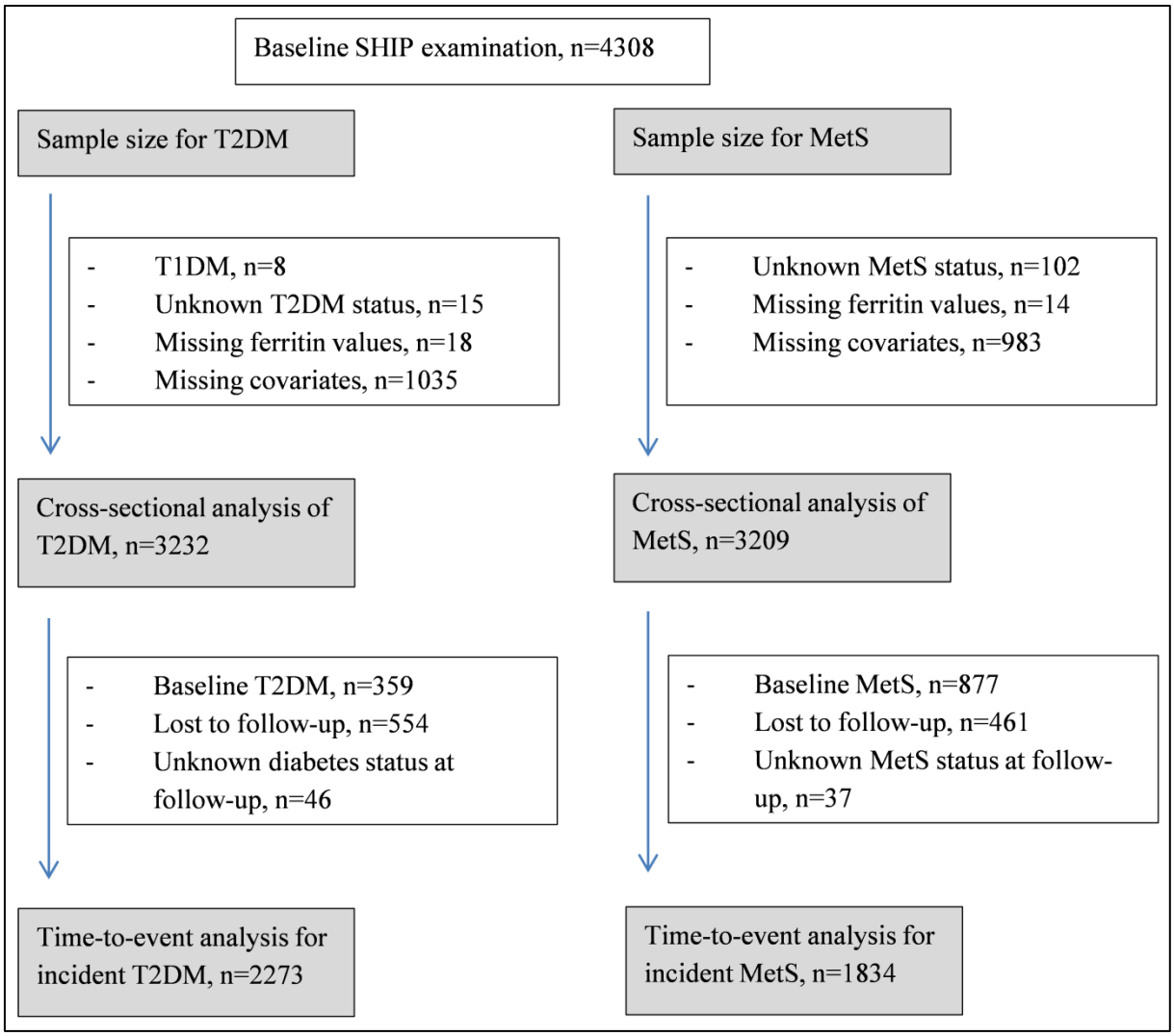


Figure 1: Flowchart of study participants used in the analyses of iron markers and type 2 diabetes mellitus/ metabolic syndrome (project 1). MetS, metabolic syndrome; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus

Table 1: Characteristics of the studies used in the analyses of maternal diabetes and offspring adiposity/ metabolic health (Project 2)

Study population	**TEDDY**	**TEENDIAB**	**BABYDIAB/ BABYDIET**
Study criteria	Children with T1DM associated HLA genotypes	Children > 6 years of age and with first degree relative with T1DM	Children with first degree relative with T1DM
Recruitment period	2004-2010	2009-2015	1989-2006
Follow-up duration	Every 3 months until 4 years and every 6 months thereafter	Every 6 months/ until 2016	Every 3 years in BABYDIAB and yearly in BABYDIET
Median follow-up	5.25 years/ 21 visits	3 years/ 6 visits	10.7 years/ 6 visits
Age range	0.25-6 years	6-18 years	0.3-18 years
Enrolled sample	n=8,676	n=610	n=2,441
Final study sample	n=5,324	n= 610	n=2,169
Intrauterine exposure for offspring	Maternal GDM, n= 326 Maternal T1DM, n=225 Maternal T2DM, n=14	Maternal T1DM, n=257	Maternal T1DM, n=1,287
Outcomes assessed in the offspring	Anthropometric: height, weight, BMI, overweight, obesity	Anthropometric: height weight, BMI, overweight, waist circumference, abdominal obesity, triceps and subscapular skinfold thickness Metabolic: blood pressure, lipids, fasting glucose, insulin and C-peptide, and HOMA-IR	Anthropometric: height, weight, BMI, overweight
Pathways analyzed	Birthweight and maternal pre-pregnancy BMI	Birthweight and changes in the metabolomics profile	Birthweight

3 Results

3.1 Project 1: Iron markers and type 2 diabetes mellitus/ metabolic syndrome

The prevalence of T2DM and MetS was 11% and 27% at baseline, respectively. In general, subjects with T2DM were older, more often hypertensive, and had significantly increased concentrations of ferritin, hepatic enzymes, inflammatory markers and metabolic parameters compared to individuals without T2DM (Table 2). During follow-up, 228 (10%) individuals and 479 (26.12%) individuals developed T2DM and MetS, respectively. The median follow-up time was 10.6 years (range; 4.4-14.6 years).

In the cross-sectional analyses, a higher value in baseline serum ferritin concentration was associated with a greater prevalence of T2DM and MetS in the total population (T2DM OR: 1.16 [95% CI: 1.07, 1.26]; MetS OR: 1.27 [95% CI: 1.16, 1.38] per 100 µg/l) and men (T2DM OR: 1.18 [95% CI: 1.08, 1.30]; MetS OR: 1.26 [95% CI: 1.15, 1.38] per 100 µg/l) independently of known diabetes risk factors, renal function, inflammatory markers and hepatic enzymes (Table 3). Further, in interaction analyses, serum ferritin concentrations were positively associated with prevalence of T2DM in participants with hs-CRP (OR: 1.24 [95% CI: 1.12, 1.37]) and ALT (OR: 1.20 [95% CI: 1.10, 1.30]) concentrations above the median, but we found no significant associations in participants with hs-CRP or ALT concentrations below the median.

In the longitudinal analyses, baseline ferritin concentrations were associated with a higher risk of incident T2DM in women (HR: 1.38 [95% CI: 1.10, 1.71]), but not in men or in the total population (Table 3). Baseline ferritin concentrations were also associated with a higher risk of incident MetS (HR: 1.09 [95% CI: 1.01, 1.17]) in the total population. However, these longitudinal associations attenuated considerably after adjustment for hepatic enzymes but not after adjustment for inflammatory markers. Further, in interaction analyses, there was a significant interaction between ferritin and sex for incident T2DM (p-value for interaction = 0.005) suggesting that increasing ferritin concentrations have a greater risk for incident T2DM in women (HR: 1.35 [95% CI: 1.10, 1.65]) than in men (HR: 0.97 [95% CI: 0.86, 1.09]). We found no significant associations of transferrin with prevalent or incident T2DM and MetS in the total population, men or women after adjustment for potential confounders and also no significant effect modifications by sex, hs-CRP or ALT.

Table 2: Baseline characteristics of SHIP population stratified by type 2 diabetes mellitus at baseline

Characteristics	**Overall cohort (n= 3,232)**	**No diabetes (n= 2,873)**	**Type 2 Diabetes (n= 359)**	**p-value**[a]
Age (years)	50 (36-63)	47 (34-61)	66 (57-72)	<0.0001
Sex, males	1652 (51.11%)	1448 (50.40%)	204 (56.82%)	0.02
Education				
< 10 years	1272 (39.36%)	1024 (35.64%)	248 (69.08%)	<0.0001
10 years	1408 (43.56%)	1334 (46.43%)	74 (20.61%)	
> 10 years	552 (17.08%)	515 (17.93%)	37 (10.31%)	
Smoking				
Never smoker	1147 (35.49%)	1015 (35.33%)	132 (36.77%)	<0.0001
Ex-smoker	1100 (34.03%)	933 (32.47%)	167 (46.52%)	
Current smoker	985 (30.48%)	925 (32.20%)	60 (16.71%)	
Alchohol intake				
0 g/day	540 (16.71%)	424 (14.76%)	116 (32.31%)	<0.0001
0.01-39.99 g/day in men & 0.01-19.99 g/day in women	2409 (74.54%)	2196 (76.44%)	213 (59.93%)	
> 40 g/day in men & > 20 g/day in women	283 (8.76%)	253 (8.81%)	30 (8.36%)	
Physically inactive	1869 (57.83%)	1588 (55.27%)	281 (78.27%)	<0.0001
Alanine aminotransferase (µkat/l)	0.39 (0.28-0.56)	0.38 (0.28-0.54)	0.46 (0.33-0.67)	<0.0001
Serum ferritin (µg/l)	74.6 (38-134.3)	70.9 (35.4-127.2)	110.4 (64-196.6)	<0.0001
Serum transferrin (g/l)[*]	2.5 (2.2-2.7)	2.5 (2.2-2.7)	2.4 (2.2-2.7)	0.15
γ-glutamyl transferase (µkat/l)	0.34 (0.23-0.57)	0.33 (0.23-0.55)	0.43 (0.31-0.75)	<0.0001
hs-CRP (mg/l)	1.36 (0.65-3.14)	1.28 (0.62-2.95)	2.09 (1.04-5.11)	<0.0001
Leucocytes (10^9/l)	6.4 (5.4-7.8)	6.4 (5.3-7.7)	6.7 (5.6-8.1)	0.0004

Data are median (interquartile range) or number (%). hs-CRP, high-sensitive C-reactive protein.

[a] Chi-square tests for categorical variables and Mann-Whitney U-test for continuous variables

[*] Based on 2162 individuals due to missing values in transferrin measurements.

Table 3: Cross-sectional and longitudinal analysis of serum ferritin at baseline with type 2 diabetes mellitus and metabolic syndrome

Outcome		Total population	Men	Women
		Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
Cross-sectional analysis				
Odds ratios				
T2DM at baseline	Model 1	1.19 (1.10, 1.28)*	1.19 (1.10, 1.30)*	1.17 (0.93, 1.48)
	Model 2	1.16 (1.07, 1.26)*	1.18 (1.08, 1.30)*	1.08 (0.84, 1.36)
MetS at baseline	Model 1	1.39 (1.28, 1.51)*	1.35 (1.24, 1.47)*	1.38 (1.10, 1.73)*
	Model 2	1.27 (1.16, 1.38)*	1.26 (1.15, 1.38)*	1.18 (0.94, 1.48)
Longitudinal analysis				
Hazard ratios				
Incident T2DM	Model 1	1.06 (0.96, 1.17)	1.04 (0.92, 1.17)	1.37 (1.10, 1.72)*
	Model 2	1.02 (0.92, 1.13)	1.02 (0.90, 1.15)	1.25 (0.98, 1.60)
Incident MetS	Model 1	1.09 (1.01, 1.17)*	1.09 (0.99, 1.19)	1.04 (0.94, 1.15)
	Model 2	1.02 (0.94, 1.11)	1.16 (0.93, 1.45)	1.05 (0.84, 1.33)

MetS, metabolic syndrome; T2DM, type2 diabetes mellitus. * indicates $p < 0.05$.

Model 1: Adjusted for age, sex (only in total population models), education, smoking, alcohol consumption, physical activity, BMI, waist /hip ratio, hypertension, triglycerides, total/HDL cholesterol ratio, serum creatinine, urinary albumin/creatinine, hs-CRP, leucocytes; Model 2: Model 1 + alanine aminotransferase, γ-glutamyl transferase. For MetS, the covariates triglycerides, total/HDL cholesterol, BMI and waist/hip ratio which are components of MetS, were not used for adjustment

3.2 Project 2: Maternal diabetes and offspring adiposity/ metabolic health

Study 1: TEDDY study

The TEDDY study consisted of 326 and 225 children who were OGDM and OT1DM, respectively and only 14 were OT2DM (Table 4). At 5.5 years of age, 1,154 and 303 children were classified as being overweight and obese, respectively. Birthweight z score was significantly lower in OnonDM than that in OGDM or OT1DM ($p < 0.005$; Table 4).

In the cross-sectional analyses at 5.5 years of age, OGDM and OT1DM had a significantly higher BMI SDS (OGDM: +0.19 (95% CI: 0.07, 0.29); OT1DM: +0.22 (95% CI: 0.08, 0.35)) and increased risk for overweight (OGDM OR: 1.48 (95% CI: 1.14, 1.92); OT1DM OR: 1.60 (95% CI: 1.16, 2.20) and obesity (OGDM OR: 1.98 (95% CI: 1.34, 2.93); OT1DM OR: 1.84 (95% CI: 1.09, 3.10)) than OnonDM after adjustment for sex and country (Table 5). When further adjusted for maternal pre-pregnancy BMI, the corresponding associations for OGDM were attenuated and became nonsignificant (for example, OR for overweight: 1.05 (95% CI: 0.80, 1.38)), while the estimates for OT1DM attenuated considerably only after adjustment for birthweight z scores (OR for overweight: 1.15 (95% CI: 0.81, 1.62)). OGDM, OT1DM or OT2DM showed no significant differences in height SDS and weight SDS when compared to OnonDM (Table 5).

In the longitudinal analyses comprising the ages 0.25-6 years, OGDM was not significantly associated with any outcome, when adjusted for potential confounders and maternal pre-pregnancy BMI. Similarly, OT1DM was not associated with any outcome except height SDS, which was significant even after inclusion of birthweight z scores. However, an interaction term between child's age and maternal diabetes in the full adjusted longitudinal model showed that OGDM, OT1DM and OT2DM had comparatively greater increases in BMI SDS, and greater risk for overweight and obesity per year increase in age when compared with OnonDM (Figure 2). This indicates that the potential impact of maternal diabetes on childhood adiposity grows stronger with increasing age.

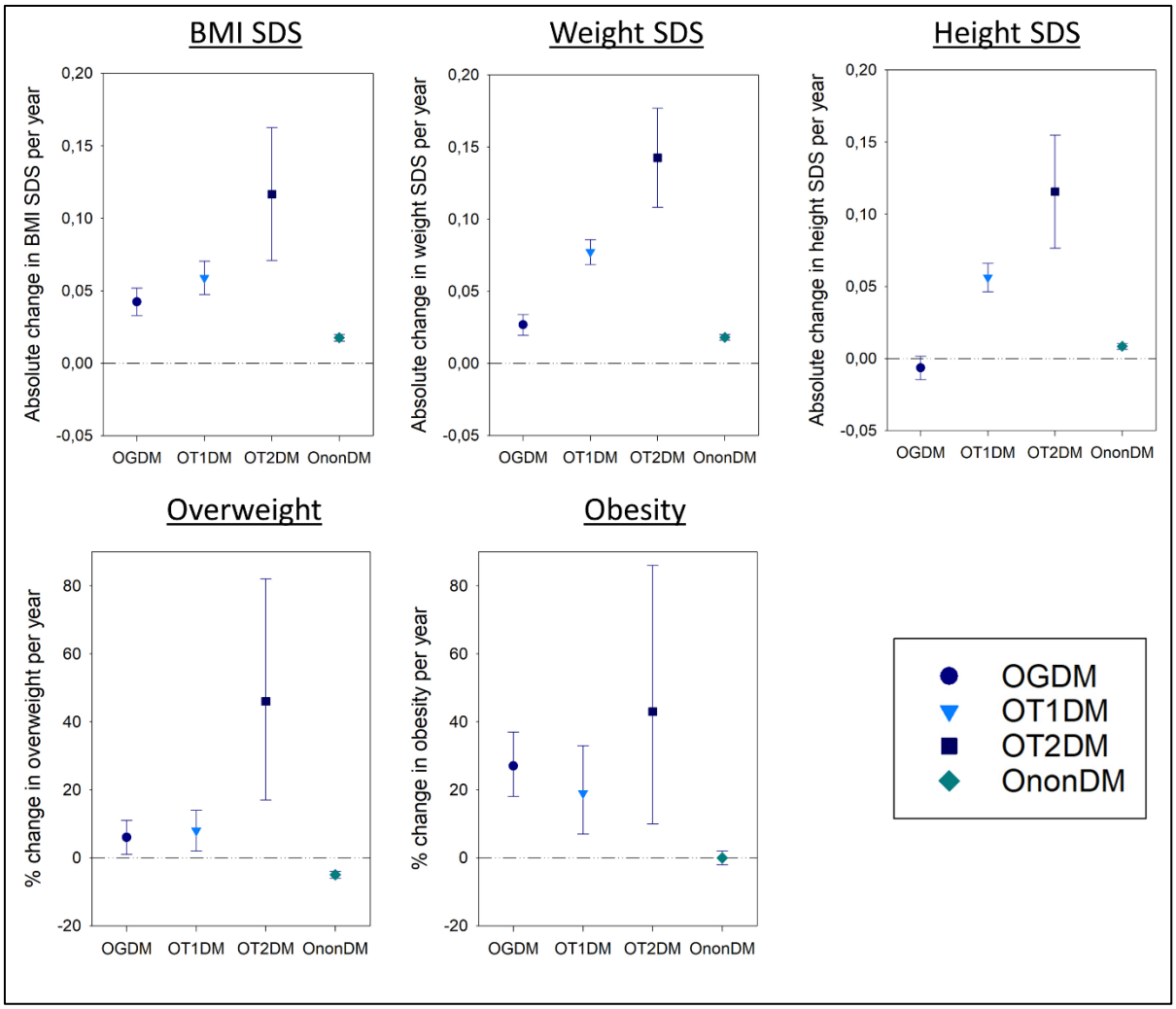


Figure 2: Modifications of association between child's age (per year) and anthropometric outcomes by maternal diabetes status presented as estimates (symbols) with 95% confidence intervals (lines). OGDM: Offspring of gestational diabetic mothers; OnonDM: Offspring of non-diabetic mothers; OT1DM: Offspring of type 1 diabetic mothers; OT2DM: Offspring of type 2 diabetic mothers; SDS: Standard deviation scores

Study 2: TEENDIAB and BABYDIAB/BABYDIET studies

The TEENDIAB and BABYDIAB/BABYDIET studies comprised of 257 and 1,287 OT1DM, respectively. Children born large for gestational age were significantly higher in OT1DM than those in OnonDM in both studies (Table 4). In the longitudinal analyses, OT1DM had a significantly higher BMI SDS (TEENDIAB: 0.36 [95% CI: 0.19, 0.53]; BABYDIAB/BABYDIET: 0.14 [95% CI: 0.07, 0.21] and increased risk for being overweight (TEENDIAB OR: 2.28 [95% CI: 1.29, 4.01]; BABYDIAB/BABYDIET OR: 1.45 [95% CI: 1.20, 1.74]) compared with OnonDM after adjustment for potential confounders in both TEENDIAB and BABYDIAB/BABYDIET studies (Table 6). However, when adjusted for birthweight, the observed associations were diminished in TEENDIAB and remained no longer significant in BABYDIAB/BABYDIET.

In TEENDIAB, OT1DM had significantly higher waist circumference SDS, increased abdominal obesity risk, higher levels of fasting glucose and insulin, and HOMA-IR, when compared with OnonDM independent of potential confounders and birthweight (Table 6). Significant associations with height SDS and fasting C-peptide were observed only after adjustment for birthweight.

Analyses of metabolomic profiles from the TEENDIAB study

The blood samples used for metabolomics analyses were drawn at a median age of 10 years (range 6-16 years). Of the 485 children included in the metabolomics analyses, 48 were overweight and 197 had mothers with T1DM. 441 metabolites were analyzed, of which 28 metabolites including 19 of known identity were significantly associated with offspring overweight after multiple testing correction (Table 7). The single metabolites upregulated in overweight individuals were mainly amino acids (e.g., branched chain amino acids) and lipids (e.g., androgenic steroids and carnitine). Similarly, three principal components representing the variability of androgenic steroids, branched chain amino acids and related metabolites showed significant associations with overweight. No significant associations between maternal T1DM and offspring metabolites or principal components were seen. Pathway analyses showed similar patterns of androgenic steroids and branched chain amino acids to be associated with overweight but not with maternal T1DM. Further, the associations between maternal T1DM and offspring overweight remained almost similar and significant even when adjusted for any potentially relevant single metabolites or principal components, suggesting that none of the metabolites are in the causal pathway.

Table 4: Characteristics of the study population stratified according to maternal diabetes

Variable		TEDDY n=5,324				TEENDIAB n=610		BABYDIAB/BABYDIET n=2,169	
		OnonDM n=4,759	OT1DM n=225	OGDM n=326	OT2DM n=14	OnonDM n=353	OT1DM n=257	OnonDM n=882	OT1DM n=1,287
Sex	Males	2,457 (51.6)	109 (48.4)	174 (53.4)	6 (42.9)	187 (53.0)	126 (49.0)	445 (50.5)	661 (51.4)
Country	US	1807 (38.0)	78 (34.7)	117 (35.9)	11 (78.6)	-	-	-	-
	Finland	1,052 (22.1)	47 (20.9)	138 (42.3)	0	-	-	-	-
	Germany	192 (4.0)	65 (28.9)	17 (5.2)	0	-	-	-	-
	Sweden	1708 (35.9)	35 (15.6)	54 (16.6)	3 (21.4)	-	-	-	-
Socioeconomic status	Low	-	-	-	-	5 (1.5)	7 (2.8)	-	-
	Middle	-	-	-	-	148 (43.0)	149 (59.6)	-	-
	High	-	-	-	-	191 (55.5)	94 (37.6)	-	-
Maternal pre-pregnancy BMI (Kg/m^2)		24.5±5.0	25.3±4.7	28.3±6.4	35.0±7.5	-	-	-	-
Maternal smoking[a]	Yes	426 (9.0)	30 (13.3)	40 (12.3)	1 (7.1)	43 (12.8)	32 (13.1)	68 (7.9)	160 (12.7)
Maternal alcohol drinking during pregnancy	Yes	1,623 (34.1)	95 (42.2)	109 (33.4)	4 (28.6)	-	-	-	-
Gestational weight gain	Inadequate	754 (16.1)	34 (15.2)	117 (36.7)	4 (28.6)	-	-	-	-
	Adequate	1,725 (36.8)	78 (34.9)	92 (28.8)	4 (28.6)	-	-	-	-
	Excessive	2,205 (47.1)	112 (50.0)	110 (34.5)	6 (42.9)	-	-	-	-
Breastfed > 6 months	Yes	3,150 (66.2)	121 (53.8)	193 (59.2)	5 (35.7)	-	-	-	-
Birthweight z scores		-0.1±1.0	0.9±1.3	0.1±1.1	0.2±1.0	0.0±1.0	0.8±1.4	-0.1±1.0	0.6±1.3
Birthweight	SGA	-	-	-	-	37 (11.3)	12 (4.9)	90 (10.5)	89 (7.5)
	AGA	-	-	-	-	252 (76.8)	155 (63.8)	689 (80.1)	745 (62.8)
	LGA	-	-	-	-	39 (11.9)	76 (31.3)	81 (9.4)	353 (29.7)

Data are number (%) or mean±SD. [a]Smoking during pregnancy in TEDDY and BABYDIAB/BABYDIET and general smoking status in TEENDIAB. AGA, appropriate for gestational age; LGA, large for gestational age; OGDM, offspring of gestational diabetic mothers, OnonDM, offspring of non-diabetic mothers; OT1DM, offspring of type 1 diabetic mothers; OT2DM, offspring of type 2 diabetic mothers; SGA, small for gestational age

Table 5: Cross-sectional analysis of anthropometric outcomes between 5.5 year old offspring of mothers with and without diabetes of different types during pregnancy (reference: no diabetes) from the TEDDY study.

Outcomes	Exposure (Maternal diabetes)	Model 1 Estimate (95% CI)	Model 2 Estimate (95% CI)	Model 3 Estimate (95% CI)	Model 4 Estimate (95% CI)
Absolute change in SD scores					
BMI SDS	OGDM	0.19 (0.07, 0.29) *	-0.02 (-0.13, 0.10)	0.03 (-0.08, 0.14)	0.001 (-0.11, 0.11)
	OT1DM	0.22 (0.08, 0.35) *	0.18 (0.04, 0.31) *	0.17 (0.04, 0.30) *	-0.007 (-0.14, 0.13)
	OT2DM	0.75 (0.23, 1.27) *	0.24 (-0.27, 0.74)	0.28 (-0.22, 0.78)	0.32 (-0.18, 0.83)
Height SDS	OGDM	-0.02 (-0.13, 0.09)	-0.07 (-0.18, 0.04)	-0.04 (-0.16, 0.07)	-0.08 (-0.19, 0.04)
	OT1DM	-0.07 (-0.20, 0.07)	-0.08 (-0.21, 0.06)	-0.08 (-0.22, 0.05)	-0.27 (-0.40, -0.13) *
	OT2DM	-0.06 (-0.57, 0.45)	-0.20 (-0.71, 0.32)	-0.20 (-0.71, 0.31)	-0.21 (-0.73, 0.31)
Weight SDS	OGDM	0.10 (-0.01, 0.21)	-0.06 (-0.17, 0.05)	-0.01 (-0.12, 0.10)	-0.05 (-0.16, 0.06)
	OT1DM	0.12 (-0.02, 0.25)	0.08 (-0.05, 0.21)	0.07 (-0.06, 0.20)	-0.16 (-0.29, -0.03) *
	OT2DM	0.51 (-0.01, 1.02)	0.08 (-0.42, 0.58)	0.11 (-0.39, 0.61)	0.13 (-0.37, 0.63)
Odds ratios					
Overweight	OGDM	1.48 (1.14, 1.92) *	1.05 (0.80, 1.38)	1.14 (0.86, 1.51)	1.10 (0.82, 1.46)
	OT1DM	1.60 (1.16, 2.20) *	1.52 (1.10, 2.11) *	1.50 (1.08, 2.09) *	1.15 (0.81, 1.62)
	OT2DM	7.39 (2.46, 22.23) *	3.36 (1.06, 10.70) *	3.68 (1.14, 11.81) *	4.92 (1.40, 17.30) *
Obesity	OGDM	1.98 (1.34, 2.93) *	1.23 (0.81, 1.86)	1.33 (0.87, 2.04)	1.31 (0.85, 2.01)
	OT1DM	1.84 (1.09, 3.10) *	1.79 (1.05, 3.06) *	1.75 (1.02, 3.00) *	1.48 (0.85, 2.59)
	OT2DM	2.93 (0.65, 13.22)	0.95 (0.20, 4.57)	0.94 (0.19, 4.60)	1.02 (0.20, 5.09)

OGDM, offspring of gestational diabetic mothers; OT1DM, offspring of type 1 diabetic mothers; OT2DM, offspring of type 2 diabetic mothers; SDS, standard deviation scores. * indicates $p < 0.05$.

Model 1: Adjusted for sex, country; Model 2: Model 1 + maternal pre-pregnancy BMI; Model 3: Model 2 + breastfeeding, maternal smoking and drinking during pregnancy, gestational weight gain, maternal age and education ; Model 4: Model 3 + birth weight

Table 6: Longitudinal analysis of anthropometric and metabolic outcomes in offspring born to a mother with compared to without type 1 diabetes mellitus in the TEENDIAB and BABYDIAB/BABYDIET cohort.

Outcomes	Study	Model 1	Model 2
		Estimates (95% CI)	Estimates (95% CI)
Absolute change in SD scores			
Height SDS	TEENDIAB	-0.07 (-0.23, 0.08)	-0.27 (-0.43, -0.10) *
	BABYDIAB/BABYDIET	-0.06 (-0.14, 0.02)	-0.13 (-0.21, -0.06) *
Weight SDS	TEENDIAB	0.22 (0.06, 0.39) *	0.07 (-0.10, 0.25)
	BABYDIAB/BABYDIET	0.06 (-0.01, 0.13)	-0.05 (-0.12, 0.02)
BMI SDS	TEENDIAB	0.36 (0.19, 0.53) *	0.28 (0.09, 0.46) *
	BABYDIAB/BABYDIET	0.14 (0.07, 0.21) *	0.04 (-0.04, 0.11)
Waist circumference SDS	TEENDIAB	0.24 (0.06, 0.42) *	0.19 (0.00, 0.39) *
Odds ratio			
Overweight	TEENDIAB	2.28 (1.29, 4.01) *	2.06 (1.12, 3.78) *
	BABYDIAB/BABYDIET	1.45 (1.20, 1.74) *	1.15 (0.95, 1.40)
Abdominal obesity	TEENDIAB	1.91 (1.11, 3.30) *	1.97 (1.10, 3.55) *
% change in metabolic outcome			
Fasting glucose	TEENDIAB	1.71 (0.29, 3.16) *	2.05 (0.51, 3.62) *
Fasting insulin	TEENDIAB	8.45 (1.06, 16.38) *	9.70 (1.71, 18.31) *
Fasting C-peptide	TEENDIAB	5.18 (-0.59, 11.28)	6.61 (0.33, 13.27) *
HOMA-IR	TEENDIAB	9.49 (1.69, 17.88) *	11.55 (3.02, 20.79) *

SDS: standard deviation scores, HOMA-IR: homeostasis model assessment of insulin resistance.

* indicates $p < 0.05$.

Model 1: adjusted for age, sex (only for metabolic outcomes), Tanner's staging, maternal smoking and socioeconomic status (only in TEENDIAB); Model 2: Model 1 + birth weight

Waist circumference SDS was calculated only in children over 11 years of age.

Table 7: Cross-sectional associations between metabolite concentrations and overweight status in the offspring

Odds ratio for overweight	**Cross-sectional models (n=485)**	
Exposures	OR (95% CI)	p
Amino acid		
Alanine[a]	9.23 (2.42, 35.23) *	0.0011
Valine[a]	88.27 (7.79, 999.85) *	0.0003
Kynurenate[a]	9.32 (3.14, 27.64) *	$5.7*10^{-5}$
Tyrosine[a]	37.21 (5.66, 244.55) *	0.0002
Lipid		
Androsterone sulfate[a]	2.02 (1.37, 2.98) *	0.0004
Androstenediol (3β,17β) disulfate (1)[a]	1.92 (1.33, 2.77) *	0.0005
Epiandrosterone sulfate[a]	1.96 (1.34, 2.88) *	0.0005
5α-androstan-3β,17β-diol disulfate[a]	1.92 (1.31, 2.81) *	0.0007
Dehydroisoandrosterone sulfate (DHEA-S)[a]	1.94 (1.26, 2.98) *	0.0028
Carnitine [a]	139.11 (11.03, 1754) *	0.0001
Thromboxane B2	2.32 (1.44, 3.73) *	0.0005
Butyrylcarnitine (C4)[a]	2.90 (1.63, 5.17) *	0.0003
2-aminoheptanoate[a]	4.32 (1.68, 11.11) *	0.0024
Glycerol	5.90 (2.11, 16.50) *	0.0007
Stearidonate (18:4n3)	3.40 (1.53, 7.54) *	0.0026
Cofactors and Vitamins		
N1-Methyl-4-pyridone-3-carboxamide[a]	4.37 (1.85, 10.31) *	0.0008
Nucleotide		
Urate[a]	35.05 (4.58, 268.08) *	0.0006
Peptide		
Gamma-glutamyltyrosine[a]	8.24 (2.29, 29.62) *	0.0012
Xenobiotic		
Piperine	1.81 (1.32, 2.47) *	0.0002

Only the metabolites significantly associated with overweight after multiple testing correction are reported in the table.

* indicate its significance after correction for multiple testing.

[a] Reported in the literature[39,40] to be associated with overweight in children

4 Discussion

4.1 Iron markers and type 2 diabetes mellitus/ metabolic syndrome

The results based on the population-based SHIP cohort suggest that baseline serum ferritin concentrations were associated with a greater prevalence of T2DM and MetS in the total population and men independently of inflammatory markers and hepatic enzymes. These findings were in accordance with previous results from other cross-sectional studies[10-12,62]. However, longitudinal analyses are essential to understand whether elevated iron stores play a role in the development of T2DM and MetS or just reflect the presence of the condition. Upon further evaluation, our longitudinal analyses revealed that baseline serum ferritin concentrations were associated with higher risk of developing T2DM only in women and higher risk of developing MetS in the total population which were attenuated considerably after adjustment for hepatic enzymes. Serum transferrin was not associated with any of the outcomes.

The association between ferritin and increased risk of developing T2DM only in women is consistent with a meta-analysis of 15 prospective studies[63], but few studies have reported significant associations in men as well as in total populations, which is in contrast to our findings[62,64]. It is possible that rather healthy subjects participated in the follow-up examinations, biasing the estimates towards the null in the prospective analysis. It could also be speculated that the harmful effect of ferritin on T2DM might be masked by the presence of other risk factors in men who tended to be frequent smokers, hypertensive and diabetic and had higher lipid, ferritin and ALT concentrations (data not shown).

Higher ferritin concentrations may also indicate systemic inflammation or hepatic dysfunction apart from increased iron stores. Thus, it seems important to distinguish independent effects of increased iron stores from inflammation and hepatic dysfunction to understand the causal role on T2DM and MetS. While the association of ferritin with T2DM or MetS remained unchanged after adjustment for hs-CRP and leukocytes, the association with incident T2DM and MetS attenuated significantly after adjustment for ALT and GGT, consistent with other prospective studies[11,62,65,66]. This indicates that the association of ferritin with risk of developing T2DM and MetS in women and in total populations, respectively, may be partially explained by hepatic dysfunction but not inflammation.

4.2 Maternal diabetes and offspring adiposity/ metabolic health

The results based on three large prospective cohort studies- TEDDY, TEENDIAB and BABYDIAB/BABYDIET suggest that the offspring exposed to maternal diabetes in utero indeed has a higher BMI, fasting glucose, insulin and HOMA-IR, and increased risk for overweight and obesity, consistent with other studies[20,23-25,67]. Moreover, the results from the TEDDY study following children from birth to 6 years of age revealed that offspring of mothers with diabetes had a higher risk for overweight or obesity than offspring of mothers without diabetes, as children grow older, implying that the association may not be evident in the first few years of life. The increased overweight/obesity risk in offspring born to diabetic mothers was clearly evident at 5.5 years of age, a time point considered to be a strong predictor of overweight later in life[68].

Results from the TEENDIAB and BABYDIAB/BABYDIET cohort studies following children from birth to 18 years and 6 to 18 years of age, respectively, confirmed that the overweight risk was higher in offspring of type 1 diabetic mothers during late childhood and adolescence. Moreover, the risk estimates were higher in TEENDIAB study compared to TEDDY or BABYDIAB/BABYDIET studies probably because it included children only after school age. Therefore, it indicates that maternal diabetes may have a delayed influence on offspring overweight/obesity that increases with age.

In agreement with our results, most studies investigating offspring older than 5 years of age have shown positive associations between maternal diabetes and offspring adiposity and metabolic health[23,24,69], while studies on early childhood have been inconsistent[70,71]. In accordance, a recent meta-analysis suggested a higher risk for overweight/ obesity in offspring of diabetic mothers only during late childhood and adolescence[72]. However, two other studies which examined less than 6-year-olds[73,28] reported that maternal GDM was positively associated with offspring adiposity measured by the sum of skinfolds or fat mass but not by BMI. Therefore, it is possible that the risk may be subtle in early childhood and can be noticed in terms of BMI only after a certain age.

4.3 Potential pathways linking maternal diabetes and offspring adiposity

Apart from investigating the association between maternal diabetes and offspring adiposity, we also examined whether maternal pre-pregnancy BMI, birthweight or offspring metabolomics profile are in the potential pathway. The results from the TEDDY study showed that the positive association of maternal GDM with offspring overweight or obesity

was markedly attenuated after adjustment for maternal pre-pregnancy BMI, while the association of maternal T1DM remained unchanged after adjustment. Similarly, several GDM studies have reported significant attenuation of effects when adjusting for maternal pre-pregnancy BMI[24,29,31,74]. It could be because maternal obesity is closely associated with GDM and both together may contribute to shared intrauterine mechanisms that lead to fetal overnutrition[75], thereby causing offspring obesity. Our study also showed that there was no influence of maternal pre-pregnancy BMI on the association between maternal T1DM and offspring overweight/ obesity probably because maternal T1DM may not be as closely associated with maternal obesity as for maternal GDM.

In our studies, we observed that birthweight largely explains the positive associations between maternal T1DM and offspring overweight or obesity. However, birthweight seemed to not explain the association between GDM and offspring overweight or obesity, consistent with other studies[28,32,67]. In accordance, studies have reported higher rates of macrosomia and other adverse perinatal outcomes in OT1DM than with OGDM[76,77]. It could be because OT1DM mothers are exposed to hyperglycemia during the whole pregnancy period and high birthweight is possibly an adverse effect observed at a greater rate when exposed to hyperglycemia even in early developmental stages. In addition, we found that adjustment for birthweight had attenuated the associations between maternal T1DM and offspring overweight status by more than 60% in the TEDDY and BABYDIAB/BABYDIET studies, but only by 10% in the TEENDIAB study. Birthweight is more closely related to child's BMI in early childhood than later, which may justify the association being fully explained by birthweight in the TEDDY and BABYDIAB/BABYDIET studies which followed children from birth, whereas only partially in TEENDIAB study which followed children only from 6 years of age.

Within the metabolomics analyses of the TEENDIAB study, we found that elevated androgenic steroids and BCAA-related metabolite pattern were associated with offspring overweight, which was consistent with other studies based on children without a family history of T1DM[39,40]. It may be possible that the differences in metabolome between overweight and normal-weight children were observed as a consequence, more than a cause, of being overweight. Studies on the association between maternal diabetes and offspring's metabolome are scarce. While two studies have reported an association between maternal glycemia and fetal metabolome[78,79], a study which investigated metabolome of 6- to 10-year-olds found no significant association with GDM[40]. Similarly, we found no associations

between maternal T1DM and offspring's metabolome. Hence, our study adds that offspring metabolomics profile may not be in the causal pathway between maternal T1DM and offspring overweight.

4.4 Strengths and limitations

In the study assessing iron markers and T2DM/ MetS, some limitations have to be noted. First, the fasting blood samples were limitedly available. Thus, T2DM diagnosis was based on HbA1c values and self-reports instead of oral glucose tolerance test. However, HbA1c is recognized as an alternative diagnostic test by the American diabetes association and WHO[80,81]. Second, we do not know the exact date of T2DM or MetS diagnosis, but the time of occurrence was calculated by taking the midpoint between the visit in which it was first reported and the previous visit. Third, we could not perform mediation analyses to investigate the role of hepatic enzymes and inflammatory markers because of a relatively smaller sample size. Main strengths include the population-based study sample with a follow-up time of nearly 11 years and the availability of ferritin as well as transferrin as measures of iron markers.

In the studies assessing maternal diabetes and offspring adiposity/ metabolic health, some potential limitations need to be discussed. First, GDM diagnosis was based on maternal reports only; hence, could not be harmonized between countries in the TEDDY study. Second, offspring exposed to maternal T2DM were quite low (n=14) in the TEDDY study; thus we could not infer much about the association of maternal T2DM and offspring adiposity. Third, the participants of all three cohorts are at increased genetic or familial risk to develop T1DM themselves; thus, it may not be generalizable to the general population. Fourth, in the TEENDIAB study, we do not have clear information on whether the mother was diagnosed with T1DM before the pregnancy. However, since the onset of T1DM occurs most frequently at a younger age, we believe that most mothers had their diagnosis before the pregnancy. Some strengths of this dissertation have to be emphasized. First, data from three large study populations allowed us to validate our results for overweight status and BMI. Second, this is the first study to investigate the influence of the metabolomics profile on the association between maternal T1DM and offspring overweight. Third, the data allowed us to examine the effects of different forms of maternal diabetes on offspring overweight/ obesity at different time points from birth until 18 years of age, adjusting for potential confounders.

4.5 Conclusion

The evidence generated in this thesis can be summarized as follows:

1) Serum ferritin was significantly associated with the risk of developing T2DM and MetS, which might act through a pathway greatly overlapping with hepatic dysfunction.
2) Intrauterine exposure to maternal diabetes may accelerate offspring BMI growth, thereby increasing the risk for overweight and obesity. The offspring of diabetic mothers appear to be at particularly higher risk during late childhood and adolescence, which is not evident in early ages.
3) Intrauterine exposure to maternal T1DM also showed significantly higher levels of fasting glucose, insulin and HOMA-IR in the offspring when compared to offspring not exposed to maternal diabetes.
4) Exposure to maternal T1DM in utero may predispose children to later overweight via increased birthweight, while exposure to maternal GDM may contribute to overweight risk possibly via shared intrauterine mechanism with maternal BMI, thus suggesting different pathways.
5) Offspring's metabolomics profile was unlikely to be in the causal pathway between maternal T1DM and offspring overweight.

5 Summary

Obesity and diabetes have reached epidemic proportions and have emerged as massive public health problems globally. The etiology of both obesity and diabetes are related, multifactorial, highly complex, and involves interplay of genetic, environmental, socio-economic and physiological factors, which calls for a more extensive research in understanding the risk factors and biological pathways. Hence, this dissertation contributed in part to understanding the role of iron markers in the development of type 2 diabetes mellitus and the role of intrauterine hyperglycemia in influencing the risk of offspring obesity along with investigating potential pathways.

In the first part of my dissertation, the associations of iron markers (ferritin and transferrin) with type 2 diabetes mellitus and metabolic syndrome were investigated using the population-based Study of Health in Pomerania. The present analyses were based on 3,232 participants aged 20-81 years with a follow-up time of nearly 11 years. The results suggest that serum ferritin concentrations were associated with a higher prevalence of type 2 diabetes mellitus and metabolic syndrome in the total population as well as in men. However, the effects of serum ferritin on incident type 2 diabetes mellitus were observed only in women, while the effects on incident metabolic syndrome were seen in the total population. Serum ferritin is also known to reflect systemic inflammation or hepatic dysfunction in addition to increased iron stores. Hence, upon further analyses, the associations were found to be attenuated after adjustment for hepatic enzymes but not after adjustment for inflammation. Transferrin was not associated with any of the outcomes. Thus, our study provides evidence for a link between the iron marker ferritin and type 2 diabetes mellitus and metabolic syndrome, although the association seemed to vary by sex. Moreover, hepatic dysfunction seems likely to be in the pathway between ferritin and type 2 diabetes mellitus and metabolic syndrome.

In the second part of my dissertation, the association between maternal hyperglycemia and the risk of offspring overweight and obesity were investigated using three different cohorts: TEDDY, TEENDIAB and BABYDIAB/BABYDIET. The present analyses were based on a total of 8,103 children who were followed until 6 years of age in TEDDY study and until 18 years of age in TEENDIAB and BABYDIAB/BABYDIET studies. The dissertation revealed that maternal hyperglycemia in general may be associated with increased risk for childhood overweight and obesity, and that the association gets stronger as children grow older, with the risk being clearly evident at late childhood and adolescence. Moreover, this dissertation adds

that this association can be driven by different pathways based on the type of maternal diabetes to which the offspring was exposed. The association of maternal gestational diabetes mellitus with offspring overweight can be largely explained by the confounding influence of maternal BMI, whereas the association of maternal type 1 diabetes mellitus with offspring overweight can be substantially explained by birthweight in all three studies. In our attempt to understand biological pathways at a cellular level, we found that the offspring metabolome was unlikely to be in the causal pathway between maternal type 1 diabetes mellitus and overweight, because this association could not be explained by any of the potentially relevant metabolites.

To conclude, this dissertation acknowledges the fact that prevention and early intervention of obesity and diabetes is of paramount importance to lessen the impact of these public health problems. Thus, our findings of the role of ferritin in increasing the risk of type 2 diabetes mellitus/ metabolic syndrome and the role of intrauterine hyperglycemia in increasing the risk of offspring overweight helped to identify particular risk groups who may need closer attention with respect to prevention of obesity and diabetes.

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6 Scientific papers

The present dissertation is based on the following three scientific papers arranged in the given order.

1. **Pitchika A**, Schipf S, Nauck M, Dörr M, Lerch MM, Felix SB, Markus MRP, Völzke H, Ittermann T. Associations of iron markers with type 2 diabetes mellitus and metabolic syndrome: Results from the prospective SHIP study. Diabetes Res Clin Pract. 2020;163:108149. Epub 2020 Apr 15.

2. **Pitchika A**, Vehik K, Hummel S, Norris JM, Uusitalo UM, Yang J, Virtanen SM, Koletzko S, Andrén Aronsson C, Ziegler AG, Beyerlein A; TEDDY study group. Associations of Maternal Diabetes During Pregnancy with Overweight in Offspring: Results from the Prospective TEDDY Study. Obesity (Silver Spring). 2018 Sep;26(9):1457-1466.

3. **Pitchika A**, Jolink M, Winkler C, Hummel S, Hummel N, Krumsiek J, Kastenmüller G, Raab J, Kordonouri O, Ziegler AG, Beyerlein A. Associations of maternal type 1 diabetes with childhood adiposity and metabolic health in the offspring: a prospective cohort study. Diabetologia. 2018 Nov;61(11):2319-2332

Table 8: Overview of the first author's contribution to the scientific papers

Scientific paper	Conception and design	Data acquisition	Data analysis and interpretation	Manuscript writing and revision
1. Pitchika, Schipf, Nauck et al (2020)	50%**	n.a.	95%***	80-90%***
2. Pitchika, Vehik, Hummel et al (2018)	50%**	n.a.	95%***	80-90%***
3. Pitchika, Jolink, Winkler et al (2018)	50%**	n.a.	95%***	80-90%***

Notes: % indicates my percentage of contribution to each part
***own responsibility; **conducted together with coauthors; n.a. not applicable

6.1 Pitchika A, Schipf S, Nauck M, Dörr M, Lerch MM, Felix SB, Markus MRP, Völzke H, Ittermann T

Associations of iron markers with type 2 diabetes mellitus and metabolic syndrome: Results from the prospective SHIP study.

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International Diabetes Federation

Associations of iron markers with type 2 diabetes mellitus and metabolic syndrome: Results from the prospective SHIP study

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ABSTRACT

Aims: To assess the role of serum ferritin and transferrin with prevalent and incident type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) and whether these associations are independent of inflammatory markers and hepatic enzymes.

Methods: We analyzed data from 3,232 participants aged 20–81 years of the population-based Study of Health in Pomerania (SHIP) from Northeast Germany with a median follow-up time of 10.6 years. Logistic and Cox regression analyses were performed.

Results: Serum ferritin concentrations were associated with a higher prevalence of T2DM (total population OR: 1.16 [95% CI: 1.07, 1.26]; men OR: 1.18 [95% CI: 1.08, 1.30) and MetS (total population OR: 1.27 [95% CI: 1.16, 1.38]; men OR: 1.26 [95% CI: 1.15, 1.38]) in the total population and men independently of inflammatory markers and hepatic enzymes. In longitudinal analyses, baseline ferritin concentrations were associated with a higher risk of incident T2DM in women (HR: 1.38 [95% CI: 1.10, 1.71]), but not in men or in the total population and also with a higher risk of incident MetS (HR: 1.09 [95% CI: 1.01, 1.17]) in the total population. These longitudinal associations attenuated considerably after adjustment for hepatic enzymes but not inflammatory markers. Transferrin was not associated with any of the outcomes.

Conclusions: Our results suggest a link between ferritin and T2DM and MetS, which might be partially explained by hepatic dysfunction.

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1. Introduction

Iron is an essential micronutrient for regulation of metabolic processes such as DNA synthesis and oxygen transport. Moreover, it is involved in the production of reactive oxygen species, thereby leading to high levels of oxidative stress and decreased insulin secretory capacity [1,2]. Thus, elevated body iron stores have been suggested to contribute to the pathogenesis of metabolic disorders.

Serum ferritin concentrations are widely used as an indicator of body iron stores. Several observational studies have shown associations between ferritin concentrations and increased risk of impaired glucose metabolism, impaired pancreatic beta cell function, decreased insulin sensitivity, metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) in both Eastern and Western countries [3–8]. Despite increasing evidence, the association remains inconclusive since ferritin is also an acute phase protein and its synthesis can be stimulated by acute or chronic inflammation, liver dysfunction and insulin resistance regardless of iron status. It has been previously reported that hepatic enzymes such as alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) are associated with ferritin concentrations as well as with incident T2DM or MetS independently of inflammatory processes [9–12]. Thus, it is not clear whether the association of ferritin concentrations with T2DM or MetS were observed due to the excess of body iron stores or as an effect of an increase in inflammation or hepatic dysfunction.

Metformin is a hypoglycemic medication, which is used to treat T2DM. It acts by inhibiting glucose production in the liver [13]. It has been proposed that metformin treatment may also decrease serum ferritin concentrations by improving insulin sensitivity [14]. However, it is unknown whether metformin influences the association between ferritin and prevalent T2DM. Additional investigations of transferrin, a further marker of iron metabolism, may help to understand the role of iron in the pathogenesis of metabolic disorders. Transferrin is the iron transport protein, which increases with the rise in iron requirements. Studies investigating the relationship between transferrin and T2DM or MetS, in addition to ferritin are sparse [5,15,16], which warrant further research to confirm the findings with appropriate adjustment for potential confounders, particularly liver function and inflammation. The aims of the study were 1) to evaluate the associations of ferritin and transferrin concentrations at baseline on prevalent and incident T2DM and MetS during a median follow-up period of 10.6 years in a population-based cohort study, and 2) to evaluate whether these associations are independent of inflammatory markers, hepatic enzymes and use of metformin medication.

2. Subjects, materials and methods

2.1. Study population

The Study of Health in Pomerania (SHIP) is a population-based cohort study conducted in West Pomerania, Germany. The sample was drawn in two steps: First, 32 communities in the region were selected. Second, within the communities a simple random sample was drawn from residence registries, stratified by gender and age. During 1997–2001, a total sample of 6,265 subjects were drawn from the target population aged 20–79 years, of which, 4,308 participated in the first examination of the SHIP study (response 68.8%). The follow-up examinations of the cohort were conducted during 2002–2006 (SHIP 1) and 2008–2012 (SHIP 2) among 3,300 and 2,333 participants after a mean follow-up time of 5 and 11 years, respectively. Details on the study design, protocols and sampling methods have been reported elsewhere [17,18]. All participants provided written informed consent and the study was approved by the medical ethics committee of the University of Greifswald.

2.2. Interview and physical examination

All participants underwent a standardized computer-assisted personal interview, during which they provided information on sociodemographic and lifestyle factors as well as medical histories and medication use. School education was categorized into three groups: <10 years, 10 years and >10 years, smoking status into current, former and never smokers and alcohol consumption into no (0 g/day), moderate (men 0.1–39.9 g/day and women 0.1–19.9 g/day), and high alcohol (men ≥40 g/day and women ≥20 g/day) consumption. Participants who exercised for less than an hour/week in their leisure time during summer or winter were classified as physically inactive. Menopausal status was defined as postmenopausal (natural or induced) and premenopausal. Participants were asked to bring all medications taken 7 days before the time of examination. Medication data were obtained online using the IDOM program (online drug database led medication assessment) and categorized according to the Anatomical Therapeutical Chemical (ATC) classification index. Hypoglycemic medication was defined by the ATC code A10, metformin intake by the ATC code A10BA02, and lipid lowering medication by the ATC code C10AB and C10AD.

During the physical examination, standardized measurements of height, weight, waist circumference, hip circumference and blood pressure were performed. BMI and waist/hip ratio were calculated. Blood pressure was measured three times on the right arm in a sitting position after at least 5-min at rest, using an oscillometric device (OMRON HEM 705-CP). Systolic and diastolic blood pressures were calculated as the average reading of the second and third measurements. Participants were classified as hypertensive based on blood pressure readings ≥140/90 mmHg or use of self-reported antihypertensive medication.

2.3. Definition of type 2 diabetes mellitus and metabolic syndrome

Known T2DM was based on self-reported physician diagnosed diabetes or use of hypoglycemic medication (ATC code A10) whereas newly diagnosed T2DM was based on HbA1c ≥6.5% [19]. During each visit, participants were classified as having T2DM based on both known and newly diagnosed T2DM. MetS was defined as the presence of at least three out of the following 5 criteria: 1) waist circumference ≥94 cm in men and ≥80 cm in women; 2) high-density

lipoprotein (HDL) cholesterol < 1.03 mmol/l in men and < 1.2 9 mmol/l in women; 3) blood pressure ≥ 130/85 mmHg or antihypertensive treatment (ATC code C02); 4) random serum glucose ≥ 8 mmol/l or antidiabetic medication (ATC code A10); and 5) non-fasting triglycerides ≥ 2.3 mmol/l or lipid lowering medication (ATC code C10AB or C10AD). It was defined according to NECP/ ATP III modified with AHA/NHLBI and IDF criteria based on non-fasting blood values [20].

2.4. *Laboratory measurements*

Non-fasting blood samples were taken and serum samples were analyzed within 1 h or stored at −80°C. Serum ferritin concentrations were determined by an immunoturbidimetric assay (Cobas Mira Plus, F. Hoffmann-La Roche Ltd, Basel, Switzerland) and transferrin by chemiluminescent assay (Siemens Vista, TRF Flex® reagent cartridge, Siemens Healthcare Diagnostics Inc., Newark, DE, USA). Total and HDL cholesterol were measured photometrically (Hitachi 704, Roche, Mannheim, Germany). Triglycerides and glucose in serum were determined enzymatically using reagents from Roche Diagnostics (Hitachi 717; Roche, Mannheim, Germany). Serum creatinine concentrations were determined with the Jaffé method (Hitachi 717, Roche, Mannheim, Germany). Serum ALT and GGT concentrations were measured photometrically (Hitachi 717, Roche, Mannheim, Germany). Urinary creatinine and albumin concentrations were determined using a Behring Nephelometer (Siemens BN albumin; Siemens Healthcare, Marburg, Germany) and a Hitachi 717 device (Roche Diagnostics, Mannheim, Germany), respectively. High-sensitive C-reactive protein (hs-CRP) was determined immunologically on a Behring Nephelometer II with commercially available reagents (Dade Behring, Eschborn, Germany). White blood cell count measured in whole blood was analyzed within 60 min either at the hospital laboratory in Greifswald with a Coulter Max M analyzer (Coulter Electronics, Miami, USA) or at the hospital laboratory in Stralsund with a Coulter T660 analyzer (Coulter Electronics, Miami, USA). Glycated hemoglobin (HbA1c) measured in whole blood were determined by high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany).

2.5. *Statistical analyses*

Baseline characteristics of study participants were expressed as median and interquartile range for continuous data and as absolute numbers and percentages for categorical data. Differences between the subjects with and without T2DM at baseline were tested by Mann-Whitney *U* test for continuous data and χ^2 test for categorical data. Partial correlations were calculated between iron markers (ferritin and transferrin) and other continuous covariates at baseline after adjusting for age and sex. Logistic and linear regressions were performed to test associations between each baseline iron markers (ferritin and transferrin) and T2DM, MetS, HbA1c % and random serum glucose concentrations at baseline, respectively. Further, cox-proportional hazards regression model was performed to test the association of baseline iron markers with incident T2DM and MetS, separately. For this analysis, we excluded subjects with T2DM or MetS at baseline. The time of occurrence of T2DM or MetS in an individual was assumed to be the midpoint at which it was first reported and the previous visit. The person-years were estimated by the sum of the follow-up time from baseline until the time point of occurrence of T2DM or MetS and until the final observation time in individuals who did not develop these conditions. Associations were analyzed based on stepwise adjustment for all outcomes. The first model was adjusted for age and sex, the second for further potential confounders such as education, smoking, alcohol intake, physical inactivity, BMI, waist/hip ratio, hypertension, triglycerides, total/ HDL cholesterol ratio, serum creatinine, urinary albumin/creatinine along with the inflammatory markers hs-CRP and leukocytes, and the third additionally for the hepatic enzymes ALT and GGT. For the outcome MetS, the covariates BMI, waist/hip ratio, triglycerides and total/ HDL cholesterol ratio, which are components of MetS, were not used for adjustment.

For sensitivity analyses we examined (i) the associations of sex-specific ferritin quintiles with baseline as well as incident T2DM and MetS (ii) the association of ferritin with the outcomes separately in men and women along with additional adjustment for menopausal status in women (iii) the associations of iron markers after excluding subjects who were fasting for at least 8 h from the baseline (n = 11), first follow-up (n = 202) and second follow-up (n = 208) data in the cross-sectional and longitudinal models (iv) the associations of iron markers with newly diagnosed T2DM and known T2DM in the cross-sectional models (v) the associations between iron markers and T2DM with and without metformin intake compared with subjects without T2DM to explore the effects of metformin on this association (vi) the effect modification of association between ferritin and T2DM and MetS by sex, hs-CRP concentrations (median cut-off:≥1.35 mg/L vs. < 1.35 m g/L) and ALT concentrations (median cut off: ≥0.38 μkat/L vs. < 0.38 μkat/L) by including an interaction term between the iron marker and the potential modifier in the cross-sectional and time-to-event models.

Effect estimates were reported as odds ratios (OR) with 95% CI for logistic regression, absolute change with 95% CI for linear regression and as hazard ratios (HR) with 95% CI for cox-proportional hazards regression per 100 μg/l increase in ferritin and per 1 g/l increase in transferrin. All analyses were carried out using STATA 14.2 (Stata Corporation, College Station, TX, USA).

3. Results

The present analyses included a maximum of 3,232 out of 4,308 individuals examined at baseline, but the sample size varied according to specific outcome and analysis. A detailed breakdown of participants used in the analyses with available data on iron markers, outcomes and covariates are shown in Fig. 1. For the analysis of transferrin on metabolic outcomes, we additionally excluded participants with missing values in transferrin measurements. Over 500 subjects were lost to follow-up from the participants used in the cross-sectional analyses. In general, subjects lost to follow-up had significantly lower alanine transaminase, HbA1c and glucose concentration, but did not differ significantly with respect to

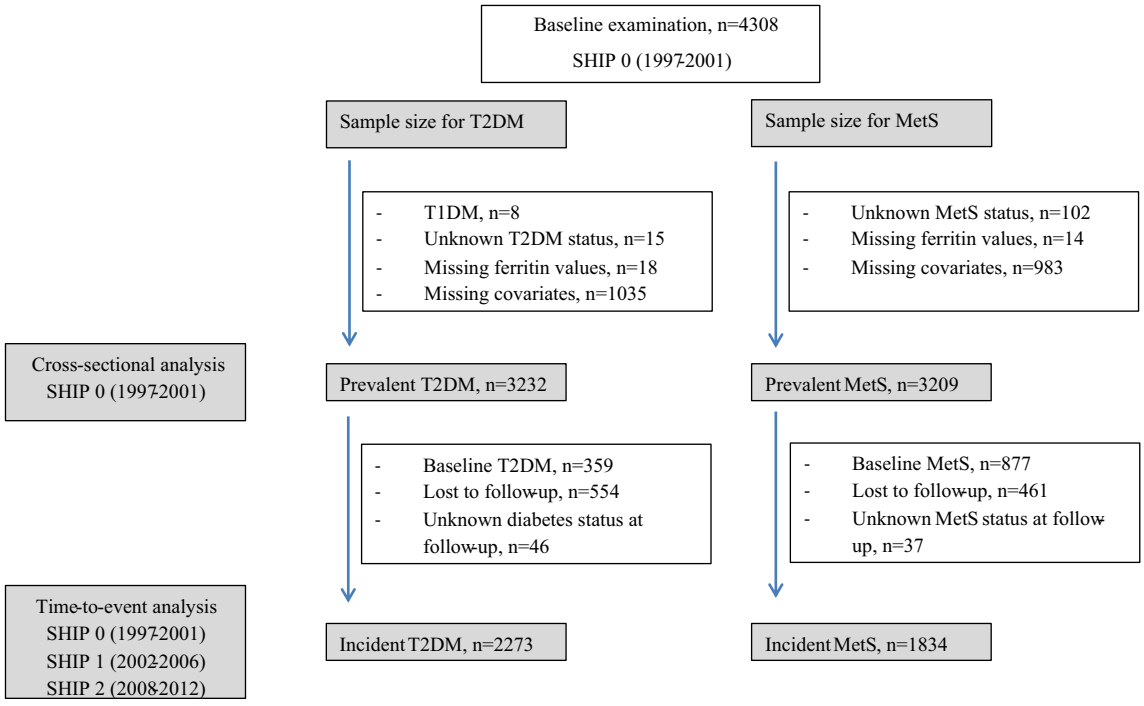

Fig. 1 – Flow chart of study participants used for the analyses. T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; MetS, metabolic syndrome.

sex, ferritin or transferrin concentrations compared to those used in the longitudinal analyses (data not shown). The prevalence of T2DM and MetS was 11% and 27% at baseline, respectively. Overall, subjects with T2DM tended to be older, were more often hypertensive, and exhibited significantly higher concentrations of ferritin, hepatic enzymes, inflammatory markers and metabolic parameters than individuals not having T2DM (Table 1). Ferritin concentrations were stronger correlated with ALT (r = 0.34) and GGT (r = 0.29) concentrations than with inflammatory markers, while transferrin showed only weak correlations with ALT, GGT and inflammatory markers (Supplementary Table 1).

3.1. *Cross-sectional analyses*

Associations between iron markers and metabolic outcomes at baseline in the total population are shown in Table 2. A higher value in serum ferritin concentration was associated with a greater prevalence of T2DM (OR: 1.16 [95% CI: 1.07, 1.26 per 100 μg/l]), MetS (OR: 1.27 [95% CI: 1.16, 1.38 per 100 μg/l]) and higher serum glucose concentrations (β: 0.08 mmol/l [95% CI: 0.03, 0.13 per 100 μg/l]) in the total population independently of known diabetes risk factors, renal function, inflammatory markers and hepatic enzymes but not with HbA1c. Similarly, in the analysis of sex-specific ferritin quintiles, the highest ferritin quintile was positively associated with prevalence of T2DM, MetS and serum glucose concentrations when compared to the lowest quintile (Supplementary Table 2). In the sex stratified analyses, serum ferritin concentrations were significantly associated with higher prevalence of T2DM (OR: 1.18 [95% CI: 1.08, 1.30]) and MetS (OR: 1.26 [95% CI: 1.15, 1.38]) only in men, independently of inflammatory markers and hepatic enzymes (Supplementary Table 3), however no significant effect modification by sex (Fig. 2). Transferrin was significantly associated with T2DM, MetS and HbA1c only in the age- and sex-adjusted models but attenuated considerably and became non-significant when adjusted for potential confounders mentioned above (Table 2). The results for ferritin and transferrin remained almost similar after excluding fasting subjects (data not shown). Further, serum ferritin concentrations were significantly associated with known T2DM (OR: 1.20 [95% CI: 1.09, 1.31]) but not newly observed T2DM defined by increased HbA1c or glucose concentrations (OR: 1.09 [95% CI: 0.94, 1.26]) at baseline in the fully adjusted model (Table 3). In addition, ferritin was significantly associated with T2DM in participants who did not take metformin (OR: 1.17 [95% CI: 1.07, 1.28]) but not in participants who took metformin (OR: 1.12 [95% CI: 0.96, 1.31]).

There was an effect modification of hs-CRP and ALT on the association of serum ferritin concentrations with T2DM (p-value of interaction < 0.10) but not with MetS (Fig. 2). We observed positive associations of serum ferritin concentrations with prevalence of T2DM in participants with hs-CRP (OR: 1.24 [95% CI: 1.12, 1.37]) and ALT (OR: 1.20 [95% CI: 1.10, 1.30]) concentrations above the median, but no associations in participants with hs-CRP or ALT concentrations below the median.

3.2. *Longitudinal analyses*

The median follow-up time was 10.62 years (range: 4.4–14.6 years). During a follow-up time of 20,692 person-years, 228 individuals (10%) developed T2DM. Similarly, during a

Table 1 – Baseline characteristics of SHIP population stratified by type 2 diabetes mellitus at baseline (N = 3232).

Characteristics	Overall cohort (N = 3232)	No diabetes (N = 2873)	Type 2 Diabetes (N = 359)	p-value[a]
Age (years)	50 (36–63)	47 (34–61)	66 (57–72)	<0.0001
Sex, males	1652 (51.11%)	1448 (50.40%)	204 (56.82%)	0.022
Education				
<10 yrs	1272 (39.36%)	1024 (35.64%)	248 (69.08%)	<0.0001
10 yrs	1408 (43.56%)	1334 (46.43%)	74 (20.61%)	
>10 yrs	552 (17.08%)	515 (17.93%)	37 (10.31%)	
Smoking				
Never smoker	1147 (35.49%)	1015 (35.33%)	132 (36.77%)	<0.0001
Ex-smoker	1100 (34.03%)	933 (32.47%)	167 (46.52%)	
Current smoker	985 (30.48%)	925 (32.20%)	60 (16.71%)	
Alchohol intake				
0 g/day	540 (16.71%)	424 (14.76%)	116 (32.31%)	<0.0001
0.01–39.99 g/day in men & 0.01–19.99 g/day in women	2409 (74.54%)	2196 (76.44%)	213 (59.93%)	
>40 g/day in men & >20 g/day in women	283 (8.76%)	253 (8.81%)	30 (8.36%)	
Physically inactive	1869 (57.83%)	1588 (55.27%)	281 (78.27%)	<0.0001
Hypertension	1705 (52.75%)	1400 (48.73%)	305 (84.96%)	<0.0001
Antidiabetic medication				
Metformin	79 (2.44%)		79 (22.01%)	
Other diabetic medication	280 (8.66%)		280 (77.99%)	
BMI (kg/m^2)	26.90 (23.74–30.05)	26.54 (23.47–29.62)	29.53 (26.86–33.16)	<0.0001
Waist to hip ratio	0.87 (0.80–0.94)	0.86 (0.79–0.93)	0.92 (0.86–0.98)	<0.0001
Triglycerides (mmol/l)	1.48 (1.02–2.28)	1.42 0.98–2.14)	2.19 (1.46–3.02)	<0.0001
Total cholesterol/ HDL ratio	4.03 (3.19–5.1)	3.95 (3.14–4.97)	4.87 (3.96–5.97)	<0.0001
Alanine aminotransferase (μkat/l)	0.39 (0.28–0.56)	0.38 (0.28–0.54)	0.46 (0.33–0.67)	<0.0001
Serum creatinine (μmol/l)	84 (75–93)	83 (75–92)	85 (77–97)	0.0007
Urinary albumin/creatinine (mg/g)	8.28 (5.11–16.84)	7.74 (4.93–14.96)	16.94 (8.40–44.09)	<0.0001
Random serum glucose (mmol/l)	5.3 (4.88–5.86)	5.23 (4.81–5.7)	7.63 (5.8–9.92)	<0.0001
HbA1c %	5.3 (4.9–5.8)	5.2 (4.8–5.6)	6.9 (6.5–7.9)	<0.0001
Serum ferritin (μg/l)	74.6 (38–134.3)	70.9 (35.4–127.2)	110.4 (64–196.6)	<0.0001
Serum transferrin (g/l)*	2.5 (2.2–2.7)	2.5 (2.2–2.7)	2.4 (2.2–2.7)	0.15
γ-glutamyl transferase (μkat/l)	0.34 (0.23–0.57)	0.33 (0.23–0.55)	0.43 (0.31–0.75)	<0.0001
hs-CRP (mg/l)	1.36 (0.65–3.14)	1.28 (0.62–2.95)	2.09 (1.04–5.11)	<0.0001
Leucocytes (10^9/l)	6.4 (5.4–7.8)	6.4 (5.3–7.7)	6.7 (5.6–8.1)	0.0004
.				

Data are median (interquartile range) or number (%). hs-CRP, high-sensitive C-reactive protein.

[a] Chi-square tests for categorical variables and Mann-Whitney *U* test for continuous variables.

* Based on 2162 individuals due to missing values in transferrin measurements.

Table 2 – Cross-sectional and longitudinal analysis of iron markers at baseline with metabolic outcomes.

Outcome	Iron marker	N	Model 1		Model 2		Model 3	
			Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p
Cross-sectional analysis								
Odds ratios								
T2DM at baseline	Ferritin	3232	1.26 (1.17, 1.35)‡	<0.0001	1.19 (1.10, 1.28)‡	<0.0001	1.16 (1.07, 1.26)‡	0.0001
	Transferrin	2162	1.60 (1.05, 2.42)*	0.027	1.11 (0.70, 1.75)	0.66	1.06 (0.67, 1.69)	0.81
MetS at baseline	Ferritin	3209	1.41 (1.30, 1.52)‡	<0.0001	1.39 (1.28, 1.51)‡	<0.0001	1.27 (1.16, 1.38)‡	<0.0001
	Transferrin	2144	1.60 (1.23, 2.08)‡	0.0001	1.27 (0.96, 1.68)	0.10	1.15 (0.85, 1.54)	0.37
Absolute change								
HbA1c	Ferritin	3231	0.05 (0.02, 0.07)‡	0.0001	0.01 (-0.01, 0.04)	0.26	0.02 (-0.01, 0.04)	0.20
	Transferrin	2162	0.11 (0.02, 0.19)*	0.01	0.02 (-0.07, 0.10)	0.69	0.02 (-0.07, 0.10)	0.73
Glucose	Ferritin	3229	0.16 (0.11, 0.21)‡	<0.0001	0.09 (0.04, 0.14)‡	0.001	0.08 (0.03, 0.13)†	0.003
	Transferrin	2158	0.11 (-0.06, 0.28)	0.22	−0.07 (-0.24, 0.09)	0.39	−0.09 (-0.26, 0.08)	0.31
Longitudinal analysis								
Hazard ratios								
Incident T2DM	Ferritin	2273	1.14 (1.05, 1.23)†	0.003	1.06 (0.96, 1.17)	0.25	1.02 (0.92, 1.13)	0.69
	Transferrin	1562	1.28 (0.84, 1.95)	0.26	1.06 (0.67, 1.69)	0.79	1.06 (0.66, 1.68)	0.82
Incident MetS	Ferritin	1834	1.08 (1.00, 1.16)*	0.045	1.09 (1.01, 1.17)*	**0.036**	1.02 (0.94, 1.11)	0.59
	Transferrin	1246	1.00 (0.74, 1.35)	0.99	0.94 (0.69, 1.29)	0.72	0.91 (0.66, 1.24)	0.54

T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome. * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

Model 1: Adjusted for age and sex; Model 2: Model 1 + education, smoking, alcohol consumption, physical activity, BMI, waist /hip ratio, hypertension, triglycerides, total/HDL cholesterol ratio, serum creatinine, urinary albumin/creatinine, hs-CRP, leucocytes; Model 3: Model 2 + alanine aminotransferase, γ-glutamyl transferase. For MetS, the covariates triglycerides, total/HDL cholesterol, BMI and waist/hip ratio which are components of MetS, were not used for adjustment.

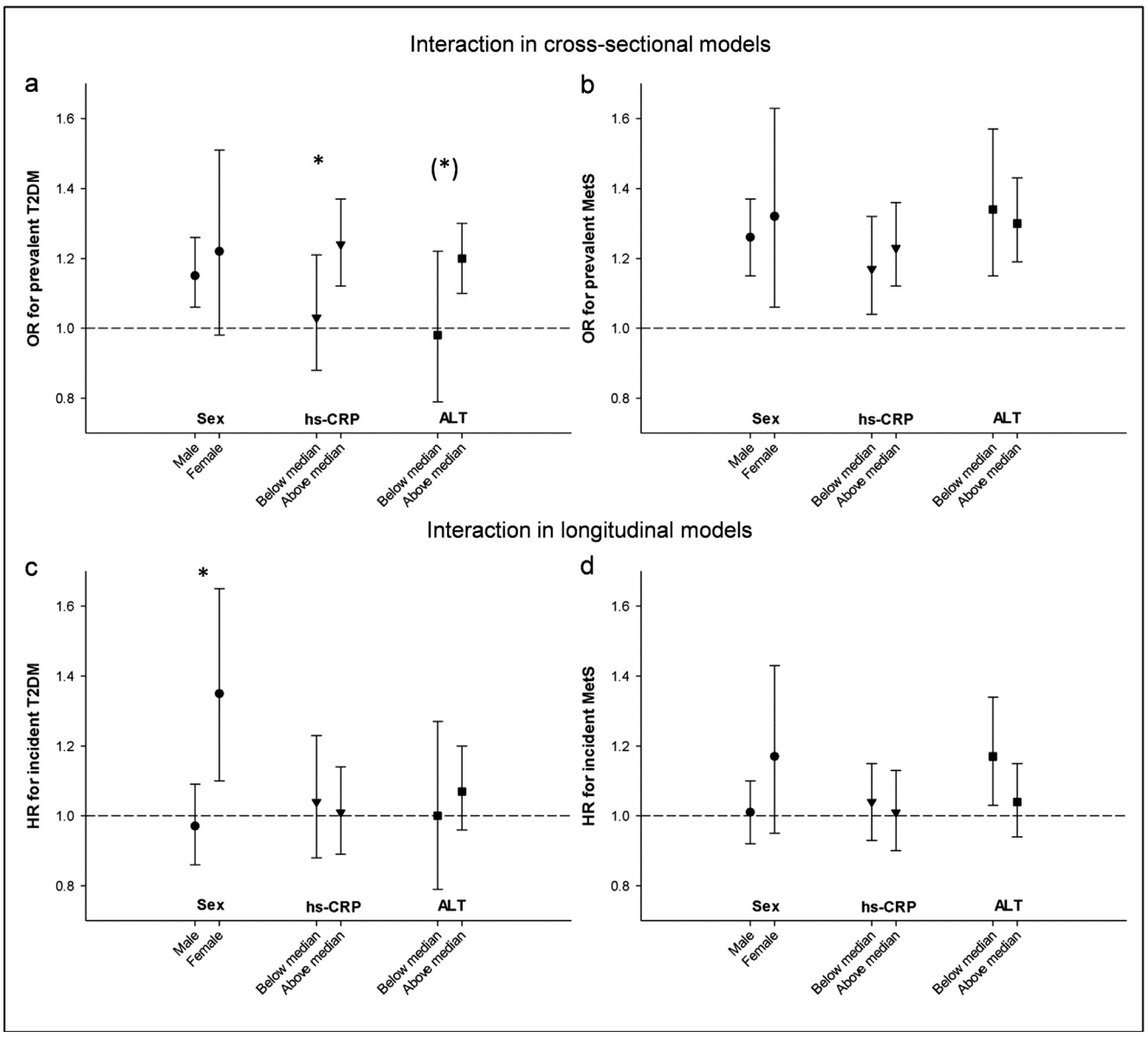

Fig. 2 – Modifications of association between ferritin and type 2 diabetes mellitus (a,c) and metabolic syndrome (b,d) by sex, hs-CRP and ALT in the cross-sectional (a,b) and longitudinal models (c,d). * p-value of interaction < 0.05; (*) p-value of interaction < 0.10; For type 2 diabetes mellitus, adjusted for age, sex, education, smoking, alcohol consumption, physical activity, BMI, waist/hip ratio, hypertension, triglycerides, total/HDL cholesterol ratio, serum creatinine, urinary albumin/creatinine, high-sensitive C-reactive protein (hs-CRP), leucocytes, alanine amino transferase (ALT), and gamma-glutamyl transferase except for stratifying factors; For metabolic syndrome, adjusted for all previously mentioned covariates except BMI, waist/hip ratio, triglycerides and total/HDL cholesterol.

follow-up time of 14,859 person-years, 479 individuals (26.12%) developed MetS. Incidence of T2DM and MetS were significantly higher in males than in females (Supplementary Table 4). In the total population, baseline serum ferritin concentrations were positively associated with incident T2DM (HR: 1.14 [95% CI: 1.05, 1.23]) and incident MetS (HR: 1.08 [95% CI: 1.00, 1.16]) in the age- and sex-adjusted model (Table 2). When adjusted for known diabetes risk factors, renal function and inflammatory markers, the associations attenuated considerably and became non-significant for incident T2DM (HR: 1.06 [95% CI: 0.96, 1.17]), but not for incident MetS (HR: 1.09 [95% CI: 1.01, 1.17]). Further adjustment for hepatic enzymes (ALT and GGT), reduced the HR estimates for incident MetS (HR: 1.02 [95% CI: 0.94, 1.11]). The results remained almost similar after excluding fasting subjects (data not shown). In the analysis of sex-specific ferritin quintiles, the highest ferritin quintile was positively associated with incident MetS when compared to the lowest quintile in all three models (Supplementary Table 2).

In women, higher serum ferritin concentrations were associated with an increased risk for developing T2DM(HR: 1.37 [95% CI: 1.10, 1.72]), after adjustment for known diabetes

Table 3 – Cross-sectional association between iron markers and known (n = 262) or newly diagnosed diabetes (n = 97) versus no diabetes (n = 2873) as well as diabetes with (n = 79) or without metformin intake (n = 280) versus no diabetes (n = 2873).

Outcome	Iron marker	Category	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Known and newly diagnosed T2DM	Ferritin	No T2DM	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
		Known T2DM	**1.29 (1.19, 1.39)**‡	**1.22 (1.12, 1.33)**‡	**1.20 (1.09, 1.31)**‡
		Newly diagnosed T2DM	**1.18 (1.05, 1.34)**†	1.10 (0.95, 1.27)	1.09 (0.94, 1.26)
	Transferrin	No T2DM	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
		Known T2DM	**1.85 (1.12, 3.06)***	1.27(0.73, 2.22)	1.20 (0.68, 2.10)
		Newly diagnosed T2DM	1.24 (0.63, 2.40)	0.90 (0.44, 1.84)	0.90 (0.44, 1.84)
T2DM with and without metformin intake	Ferritin	No T2DM	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
		T2DM with metformin intake	**1.24 (1.10, 1.41)**‡	1.13 (0.97, 1.31)	1.12 (0.96, 1.31)
		T2DM without metformin intake	**1.26 (1.16, 1.36)**‡	**1.20 (1.11, 1.30)**‡	**1.17 (1.07, 1.28)**‡
	Transferrin	No T2DM	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
		T2DM with metformin intake	**2.42 (1.06, 5.53)***	1.92 (0.73, 5.07)	1.90 (0.72, 5.04)
		T2DM without metformin intake	1.43 (0.90, 2.27)	1.00 (0.61, 1.65)	0.95 (0.57, 1.58)

OR, odds ratio; T2DM, type 2 diabetes mellitus; * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.
Model 1: Adjusted for age and sex; Model 2: Model 1 + education, smoking, alcohol consumption, physical activity, BMI, waist/hip ratio, hypertension, triglycerides, total/HDL cholesterol ratio, serum creatinine, urinary albumin/creatinine, hs-CRP, leucocytes; Model 3: Model 2 + alanine aminotransferase, gamma-glutamyl transferase.

risk factors, renal function, inflammatory markers and menopausal status but the associations attenuated considerably after additional adjustment for hepatic enzymes (HR: 1.25 [95% CI: 0.98, 1.60]). In males, no such association was observed before (HR: 0.97 [95% CI: 0.86, 1.09]) or after (HR: 0.97 [95% CI: 0.86, 1.09]) adjustment for hepatic enzymes (Supplementary Table 3). Likewise, in interaction analyses, we observed significant interactions between ferritin and sex for incident T2DM (p-value for interaction = 0.005) (Fig. 2) indicating a higher risk in women but not in men. Positive associations of serum ferritin concentrations with incident MetS were observed only in women (HR: 1.27 [95% CI: 1.03, 1.57]) in the age- and menopausal status-adjusted model, which attenuated considerably after adjustment for confounders (Supplementary Table 3). We found no further significant interactions of sex, hs-CRP or ALT concentrations with serum ferritin concentrations on the outcomes (Fig. 2).

4. Discussion

In this population-based sample, serum ferritin concentrations were significantly associated with prevalent T2DM and MetS in the total population and men, independent of inflammatory markers and hepatic enzymes, whereas serum transferrin concentrations showed no significant associations with these outcomes. The association between serum ferritin concentrations and prevalent T2DM was more pronounced in individuals with high hs-CRP and ALT concentrations. However, longitudinally, serum ferritin concentrations at baseline were significantly associated with incident T2DM only in women and incident MetS in the total population after adjustment for known diabetes risk factors and inflammatory markers but attenuated considerably after adjustment for hepatic enzymes.

4.1. *Iron markers and T2DM*

Previous cross-sectional and prospective epidemiological studies [3–5,21] have demonstrated that serum ferritin concentrations are associated with an increased risk for T2DM. Our findings, which were adjusted for a range of potential confounders including inflammatory markers and hepatic enzymes, were generally in line with the results of the cross-sectional studies. Further, our study showed stronger associations of serum ferritin concentrations with known T2DM but not with newly diagnosed T2DM. These results challenge the directionality of the association and whether increased iron stores play a role in the pathogenesis of T2DM or whether ferritin just reflects the progression of T2DM.

In our prospective analysis, we found strong associations of baseline ferritin concentrations with an increased risk for developing T2DM in women, consistent with a recent *meta*-analysis of 15 prospective studies [22]. However, we found no significant associations in the total population and men, which is in contrast to findings from other studies [21,23–26]. In our study, one cannot exclude the possibility that in general, a rather healthy part of the population is more likely to participate in the follow-up examinations, which may have

biased the estimates towards the null in the prospective analysis. Besides that, differences in biomarker distributions, genetics, lifestyle characteristics or underlying comorbidities between men and women might explain the results. Women were less likely to be frequent smokers, hypertensive and diabetic, had lower lipid, ferritin and ALT concentrations but higher hs-CRP concentrations (Supplementary Table 4). It is possible that the detrimental effect of ferritin on T2DM might be masked by the presence of other risk factors in men, and hence, seen only in women. The iron transport protein transferrin, which has been less investigated, was not associated with prevalent or incident T2DM in our study, although positive associations have been reported elsewhere [5,15,21].

4.2. *Iron markers and MetS*

MetS represents a cluster of metabolic abnormalities, which are collectively associated with an increased risk for cardiovascular disease [27]. Previous cross-sectional studies [28–30] support our positive findings of an association between ferritin and prevalent MetS. However, only few longitudinal studies [16,31,32] are available to understand the causality of this association. Results from existing prospective studies are conflicting. A study from a Swiss population based sample reported no independent positive associations of ferritin with incident MetS [16]. However, we found a positive association between baseline serum ferritin concentrations and risk of incident MetS in the total population, consistent with other prospective studies [31,32]. Similar to our T2DM results, transferrin was not associated with prevalent or incident MetS, contrary to positive associations observed in two other studies [16,33]. To our knowledge, only two studies [16,33] have analyzed the effects of transferrin on Mets, thus the evidence remains inconclusive.

4.3. *Role of inflammation and hepatic dysfunction*

Elevated ferritin concentrations are known to reflect systemic inflammation or hepatic dysfunction in addition to increased iron stores. We found stronger associations of ferritin with prevalent T2DM in individuals with high hs-CRP and ALT concentrations. However, the association of ferritin with T2DM or MetS remained unchanged after adjustment for hs-CRP and leukocytes in our study, similar to other previous studies [7,34]. This might indicate that the effect of ferritin on T2DM might be augmented by the coexistence of inflammation but not explain the pathway between ferritin and increased risk of developing T2DM observed in women.

ALT and GGT are markers of hepatic dysfunction, which have also been linked to T2DM and MetS [9,11,35]. Thus, adjustment for ALT and GGT seems important to differentiate independent effects of increased iron stores from hepatic dysfunction to understand the causal role on T2DM or MetS. Indeed, we observed a moderate correlation between ferritin and ALT concentrations (r = 0.34). Several prospective studies [15,24,31,33] lack adjustment for ALT in the analysis, and the studies [4,21,34,36] which adjusted for ALT have reported attenuation of their effects. Although the association between ferritin and prevalent T2DM and MetS showed only minimal attenuation of effects after adjustment for hepatic enzymes, the association between ferritin and incident T2DM observed among women attenuated significantly after adjustment for ALT and GGT with the hazard ratios decreasing from 1.37 to 1.25, and for incident MetS decreasing from 1.09 to 1.02 in the total population. This indicates that the association of ferritin with T2DM and MetS risk observed in women and the total population, respectively, might be partially explained by hepatic dysfunction. Indeed, the strength of the association was more strongly attenuated after adjustment for ALT than for GGT (data not shown). ALT concentrations are known to be associated with hepatic insulin resistance, which in turn is identified to play a major role in the pathophysiology of MetS and T2DM [9,37,38]. Thus, our study supports the role of ferritin on the risk of developing T2DM or MetS through a pathway greatly overlapping with hepatic dysfunction and insulin resistance. It could also be speculated that hepatic dysfunction and insulin resistance might be caused by iron deposits in the liver which reflects elevated ferritin concentrations in circulation [39,40]

4.4. *Role of metformin*

Our study suggests that serum ferritin concentrations were significantly associated with prevalent T2DM only in the participants not treated with metformin. Thus, it is possible that ferritin may have been influenced by metformin intake and thus, showed no significant associations with T2DM in the cross-sectional analysis. It should be mentioned, however, that the number of participants treated with metformin was quite limited (n = 79), and our findings should be interpreted with great caution. Thus, further studies are warranted to confirm our results.

4.5. *Strengths and limitations*

Main strengths of our study include the population-based study sample, prospective study design with a follow-up time of nearly 11 years, the availability of ferritin as well as transferrin as measures of iron markers and a lot of covariates for potential confounding. The main limitation of the study is the limited availability of fasting blood samples. Thus, diabetes assessment was based on HbA1c and physician diagnosed self-reports and not based on oral glucose tolerance test. However, the international expert committee followed by the American diabetes association and World Health Organization (WHO) have recommended the use of HbA1c as a diagnostic test for diabetes [19,41]. The definition of MetS was slightly modified according to non-fasting blood values, but it was considered to be robust and the prevalence of MetS was comparable to estimates from other population-based surveys conducted in Germany [20]. Also, the exact date of T2DM or MetS diagnosis was not known which may have occurred anywhere in between the follow-up. However, we accounted for it by taking the midpoint at which it was first reported and the previous visit to be the time of occurrence. Despite adjustment for some important covariates in our analyses, one cannot exclude the possibility of residual confounding in our study. Further, our study lacks information on insulin resistance and liver iron concentrations, which may have contributed further to understand the role of iron

and liver dysfunction in the pathogenesis of T2DM. Due to the relatively low number of participants in our study, we were not able to conduct a sufficiently powered mediation analyses to further investigate the role of hepatic enzymes and inflammatory markers on associations of serum ferritin concentrations with T2DM or MetS.

4.6. *Conclusion*

Overall, we found that ferritin was significantly associated with prevalent T2DM and MetS in the total population and in men, and with incident MetS in the total population, but effects on incident T2DM were only observed in women. The association was unchanged after adjustment for inflammatory markers but attenuated after adjustment for hepatic enzymes for incident T2DM and MetS. Transferrin was not associated with any of the metabolic outcomes. Thus, our study provides further evidence for a link between ferritin and T2DM and MetS, which might be partially explained by hepatic dysfunction. The causal role of iron metabolism needs to be further explored as it may play an integral role in the prevention and management of diabetes.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2020.108149.

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Associations of Maternal Diabetes During Pregnancy with Overweight in Offspring: Results from the Prospective TEDDY Study.

Original Article
PEDIATRIC OBESITY

Obesity

Associations of Maternal Diabetes During Pregnancy with Overweight in Offspring: Results from the Prospective TEDDY Study

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Objective: This study aimed to determine the relationship between different forms of, and potential pathways between, maternal diabetes and childhood obesity at different ages.
Methods: Prospective cohort data from The Environmental Determinants of Diabetes in the Young (TEDDY) study, which was composed of 5,324 children examined from 0.25 to 6 years of age, were analyzed. Cross-sectional and longitudinal analyses taking into account potential confounders and effect modifiers such as maternal prepregnancy BMI and birth weight *z* scores were performed.
Results: Offspring of mothers with gestational diabetes mellitus (GDM) or type 1 diabetes mellitus (T1DM) showed a higher BMI standard deviation score and increased risk for overweight and obesity at 5.5 years of age than offspring of mothers without diabetes. While these associations could be substantially explained by maternal prepregnancy BMI in offspring of mothers with GDM, significant associations disappeared after adjustment for birth weight *z* scores in offspring of T1DM mothers. Furthermore, overweight risk became stronger with increasing age in offspring of mothers with diabetes compared with offspring of mothers without diabetes.
Conclusions: Maternal diabetes is associated with increased risk of offspring overweight, and the association appears to get stronger as children grow older. Indeed, intrauterine exposure to maternal T1DM may predispose children to later obesity through increased birth weight, while maternal BMI is more important in children exposed to GDM.

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Introduction

The worldwide increase in the prevalence of childhood obesity in recent decades is alarming because it is also associated with other health consequences such as metabolic syndrome, diabetes, and cardiovascular disease in adulthood (1,2). Previous research has indicated that overweight at age 5 to 6 years is a strong predictor of overweight later in life (3), emphasizing the need to identify determinants of

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Author contributions: AP analyzed the data and wrote the first and final draft of the manuscript together with AB. KV, SH, UMU, JMN, JY, SMV, SK, CAA, and AGZ contributed to the interpretation of the results and to subsequent drafts of the manuscript.

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obesity in early life and even before birth (4). In particular, there is a growing body of literature that recognizes the role of maternal diabetes during pregnancy in the risk of offspring obesity (5–7). While several studies have shown that offspring of women with gestational diabetes mellitus (GDM), type 1 diabetes mellitus (T1DM), or type 2 diabetes mellitus (T2DM) have a higher risk for obesity during late childhood and adolescence (8–13), there is only weak and inconsistent evidence for an association between maternal diabetes and obesity during early childhood (14–18). Therefore, it is still not clear whether maternal diabetes has a delayed effect on offspring obesity.

In addition, most studies associating GDM with offspring obesity have shown that maternal obesity largely confounds this association (5,9,19,20). Only in one study did a positive association between GDM and overweight in 6-year-old offspring remain significant after adjustment for maternal BMI (21); therefore, it remains unclear whether this association is causal. Furthermore, high birth weight has been reported to be associated with maternal hyperglycemia in pregnancy regardless of the type of diabetes (22,23), potentially via exposure to excess fetal glucose and insulin and thus overgrowth of the fetus (4). However, the influence of birth weight on the pathway from maternal diabetes to childhood obesity has not been well investigated.

Therefore, this study aims to investigate (1) whether exposure to maternal diabetes during pregnancy (GDM, T1DM, or T2DM) is associated with subsequent offspring growth during early childhood, (2) whether this association varies by offspring age or maternal diabetes status, and (3) whether birth weight or maternal prepregnancy BMI is in the potential pathway.

Methods

The Environmental Determinants of Diabetes in the Young (TEDDY) study is an ongoing international, multicenter, prospective cohort study that seeks to identify the environmental factors triggering islet autoimmunity and T1DM. This large longitudinal cohort also offers the opportunity to investigate the factors influencing childhood overweight and obesity. The TEDDY study screened 424,788 newborns for T1DM-associated human leukocyte antigen genotypes between 2004 and 2010, and of these children, 8,676 were enrolled and followed up in six clinical research centers located in the United States, Finland, Germany, and Sweden. Children's study visits were scheduled every 3 months from birth until age 4 years and every 6 months thereafter. Further details on study design, eligibility, and data collection have been described elsewhere (24–26). Written informed consent for all participants was obtained separately from a parent or primary caretaker. The study is funded by the National Institutes of Health, was approved by local institutional review boards, and has been monitored by an external evaluation committee formed by the National Institutes of Health.

Maternal characteristics and offspring measurements

During each visit, children's height and weight were measured by trained TEDDY personnel at TEDDY clinics. Using a wall-mounted stadiometer, each child's height was measured as length before age 2 and as standing height to the nearest 0.1 cm after age 2 (27). Body weight was measured in kilograms using regularly calibrated electronic scales. For subjects who missed their study visit, anthropometric data were taken from their pediatricians' records collected near the TEDDY clinic visit date.

Information on maternal factors such as diabetes status during pregnancy, age, prepregnancy BMI, gestational weight gain, gestational age at delivery, education, and smoking or alcohol intake during pregnancy, as well as the child's birth weight, was obtained by self-administered questionnaires or structured interviews conducted during one of the follow-up visits in the first year of the study. Duration of both any and exclusive breastfeeding was assessed by giving a specific booklet to the parents at study entry, in which they recorded the age at weaning and age at introduction of all new foods.

Assessment of diet and physical activity

Dietary intake was assessed using a 3-day food record every 3 months until 12 months of age and every 6 months thereafter. Participating families were instructed to keep a consecutive 3-day record of their child's consumption of food and beverages, ideally for two weekdays and one weekend day, as described in detail elsewhere (27). To assess energy and nutrient intake, the food consumption data were entered and analyzed using country-specific food record databases that were harmonized for the TEDDY study (28). Average duration (in minutes) of moderate to vigorous physical activity per day was assessed using the ActiGraph GT3X accelerometer (ActiGraph, Pensacola, Florida) (29) on an annual basis, beginning at age 5. TEDDY staff provided demonstrations on how to wear and use the accelerometer for seven consecutive days, including two weekend days, during the study visit prior to the specific TEDDY visit targeted for activity data collection.

Data transformations

Children were classified into different groups according to maternal diabetic status during pregnancy: (1) offspring of mothers with GDM (O-GDM), (2) offspring of mothers with T1DM (O-T1DM), (3) offspring of mothers with T2DM (O-T2DM), and (4) offspring of mothers without diabetes (O-nonDM). BMI was calculated as weight in kilograms divided by height in meters squared. Prior to analysis, height, weight, and BMI were transformed to standard deviation scores (SDSs) using World Health Organization (WHO) reference values (30,31). SDS values less than −5 or greater than 5 were deemed implausible and excluded. BMI SDS values were also used to define overweight (including obesity; BMI SDS > 1) and obesity (BMI SDS > 2) according to WHO recommendations. Anthropometric outcomes at the age of 5.5 years were defined as those assessed at the 66-month visit if available (as in 86% of the children) or at the next closest visit between the ages of 54 and 72 months. Similarly, diet and physical activity at age 5 were defined as those outcomes assessed at the 60-month visit if available or at the next closest visit between the ages of 66 and 72 months. Gestational weight gain was classified as inadequate, adequate, or excessive according to Institute of Medicine guidelines (32). Birth weight was transformed to a *z* score adjusting for country, sex, gestational age, maternal height, and birth type (singleton or multiplet), similar to previous analyses of the TEDDY data (27,33).

Statistical analysis

To assess our main hypothesis that maternal diabetes was associated with offspring anthropometric measures, we performed several analyses. First, mean BMI, weight, and height were visually compared in yearly time intervals between O-GDM, O-T1DM, and O-nonDM. Second, cross-sectional associations between maternal diabetes and anthropometric outcomes (BMI, height, weight, overweight, and obesity) measured in the children at 5.5 years of age were investigated through linear and logistic regression models.

Third, longitudinal analyses between maternal diabetes and anthropometric outcomes measured between 0.25 and 6 years of age were performed through mixed-effects regression models with random intercepts for each subject in order to account for the correlation between repeated observations within subjects. Associations in both the cross-sectional and the longitudinal setting were analyzed based on stepwise adjustment. In the first model, we adjusted for age (only longitudinal analysis), sex, and country for all outcomes; in the second model, we additionally adjusted for maternal prepregnancy BMI. Furthermore, we included maternal age, gestational weight gain, maternal smoking during pregnancy (yes or no), maternal alcohol intake during pregnancy (any or none), maternal education (high school, less than high school, or more than high school), and duration of any breastfeeding (less than 6 months or more than 6 months) as potential confounders in the third model and, additionally, birth weight *z* scores in the fourth model to explore potential pathways. Furthermore, we explored interaction terms between maternal diabetes and child's age (in years) in the fully adjusted longitudinal model to explore whether the association changed with an increase in age.

Sensitivity analyses

We performed several sensitivity analyses. We added interaction terms between country and maternal diabetes in the cross-sectional and longitudinal models to explore whether association between maternal diabetes and anthropometric outcomes differed by country. Because the human leukocyte antigen DQ2/2 (HLA-DQ2/2) genotype was reported to be associated with increased risk for obesity at age 2 to 4 in a previous TEDDY study (33), we additionally adjusted for HLA-DQ2/2 genotype in the cross-sectional and longitudinal models. We further recalculated the cross-sectional analyses after the exclusion of children who had developed persistent islet autoantibodies or T1DM by 5.5 years of age. Furthermore, based on the subset of children with available energy intake and physical activity data at age 5 (54% of all children with available BMI measurements), we additionally adjusted for these two variables as potential confounders in cross-sectional models 3 and 4. We also assessed whether treatment with insulin compared with any other or no treatment during pregnancy was associated with anthropometric outcomes at 5.5 years of age in offspring of women with GDM and T2DM. All calculations were carried out with SAS 9.4 (SAS Institute, Cary, North Carolina).

Results

Of 8,676 children, 3,352 children with missing data on height and weight measurements after age 5 ($n=3,181$) or maternal diabetes status during pregnancy ($n=171$) were excluded (Figure 1). Our final study sample consisted of 5,324 children; of these, 2,746 (51.58%) were male, 326 (6.12%) and 225 (4.23%) were O-GDM and O-T1DM, respectively, and only 14 (0.26%) were O-T2DM (Table 1). Children who were excluded because of missing height and weight measurements were less likely to have a mother with diabetes (GDM: 4.94%; T1DM: 3.11%; χ^2 test: $P=0.02$). However, children who were excluded because of missing maternal diabetes status did not differ significantly from those included with respect to BMI SDS at 5.5 years of age (Mann–Whitney U test: $P=0.70$). Children had a mean BMI SDS of 0.35, with 1,154 (21.87%) and 303 (5.74%) children classified as having overweight and obesity, respectively, at 5.5 years of age. O-nonDM had a mean birth weight *z* score of −0.05, which was significantly lower than that in O-T1DM (0.87; $P<0.0001$) or O-GDM (0.13; $P=0.004$).

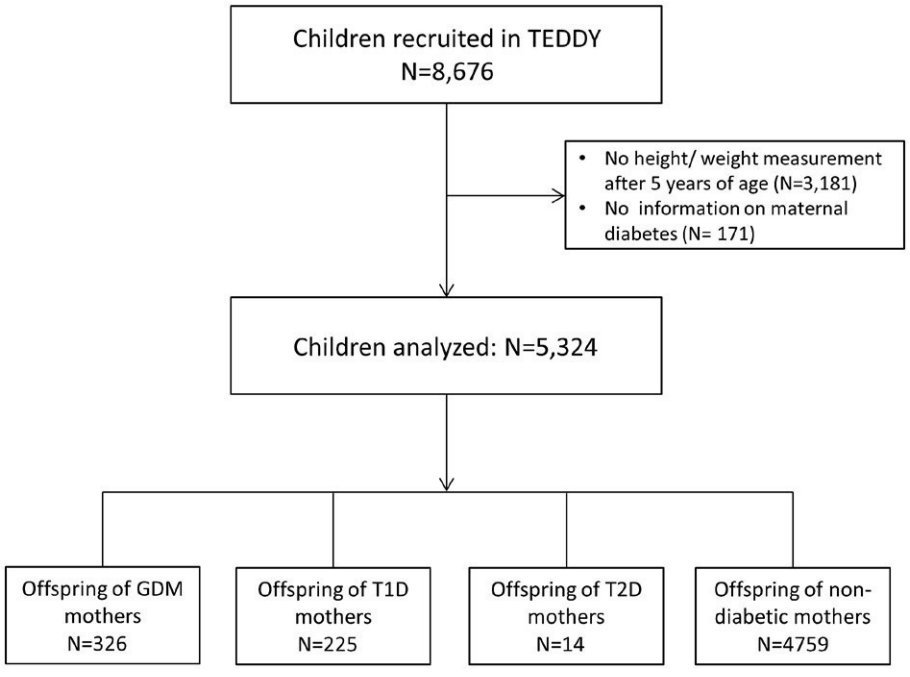

Figure 1 Flowchart of children analyzed. GDM, gestational diabetes mellitus; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

TABLE 1 Characteristics of the study population stratified according to maternal diabetes

Variable	Available n	Category	*n* (%) in each category	O-nonDM (*n* = 4,759) *n* (%)	O-GDM (*n* = 326) *n* (%)	O-T1DM (*n* = 225) *n* (%)	O-T2DM (*n* = 14) *n* (%)
Sex	5,324	Males	2,746 (51.6)	2,457 (51.6)	174 (53.4)	109 (48.4)	6 (42.9)
Country		US	2,013 (37.8)	1,807 (38.0)	117 (35.9)	78 (34.7)	11 (78.6)
	5,324	Finland	1,237 (23.2)	1,052 (22.1)	138 (42.3)	47 (20.9)	0
		Germany	274 (5.2)	192 (4.0)	17 (5.2)	65 (28.9)	0
		Sweden	1,800 (33.8)	1,708 (35.9)	54 (16.6)	35 (15.6)	3 (21.4)
Maternal smoking during pregnancy	5,320	Yes	497 (9.3)	426 (9.0)	40 (12.3)	30 (13.3)	1 (7.1)
Maternal alcohol drinking during pregnancy	5,322	Yes	1,831 (34.4)	1,623 (34.1)	109 (33.4)	95 (42.2)	4 (28.6)
Maternal education	5,251	High school	4,371 (83.2)	3,877 (82.6)	288 (89.2)	194 (87.4)	12 (85.7)
Gestational weight gain (according to Institute of Medicine guidelines)	5,241	Inadequate	909 (17.3)	754 (16.1)	117 (36.7)	34 (15.2)	4 (28.6)
		Adequate	1,899 (36.2)	1,725 (36.8)	92 (28.8)	78 (34.9)	4 (28.6)
		Excessive	2,433 (46.4)	2,205 (47.1)	110 (34.5)	112 (50.0)	6 (42.9)
Breastfed ≥ 6 months	5,324	Yes	3,469 (65.2)	3,150 (66.2)	193 (59.2)	121 (53.8)	5 (35.7)
Overweight at age 5.5	5,277	Yes	1,154 (21.9)	998 (21.2)	89 (27.6)	58 (25.9)	9 (64.3)
Obesity at age 5.5	5,277	Yes	303 (5.7)	252 (5.3)	32 (9.9)	17 (7.6)	2 (14.3)
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Maternal prepregnancy BMI (kg/m^2)	5,276		24.8 ± 5.2	24.5 ± 5.0	28.3 ± 6.4	25.3 ± 4.7	35.0 ± 7.5
Maternal age at delivery (y)	5,324		31.0 ± 4.9	30.9 ± 4.9	32.2 ± 5.3	30.8 ± 4.9	34.0 ± 5.6
Gestational age (wk)	5,318		39.5 ± 1.6	39.6 ± 1.6	39.2 ± 1.7	37.7 ± 1.8	38.1 ± 2.1
Birth weight *z* score	5,186		0.0 ± 1.0	−0.1 ± 1.0	0.1 ± 1.1	0.9 ± 1.3	0.2 ± 1.0
BMI SDS at age 5.5	5,277		0.4 ± 1.0	0.3 ± 1.0	0.5 ± 1.1	0.4 ± 1.1	1.1 ± 1.3
Height SDS at age 5.5	5,291		0.4 ± 1.0	0.4 ± 1.0	0.3 ± 1.0	0.3 ± 0.9	0.2 ± 0.7
Weight SDS at age 5.5	5,304		0.5 ± 1.0	0.5 ± 1.0	0.5 ± 1.0	0.5 ± 0.9	0.9 ± 1.2
Mean energy intake (kcal/d) at age 5	4,263		1,442.7 ± 362.2	1,443.6 ± 359.7	1,461.7 ± 428.5	1,402.1 ± 316.4	1,353.2 ± 317.3
MVPA (min/d) at age 5	3,276		68.0 ± 34.4	68.3 ± 34.4	63.0 ± 33.8	69.4 ± 36.8	54.4 ± 29.8

MVPA, moderate to vigorous physical activity; O-GDM, offspring of mothers with gestational diabetes mellitus; O-nonDM, offspring of mothers without diabetes mellitus; O-T1DM, offspring of mothers with type 1 diabetes mellitus; O-T2DM, offspring of mothers with type 2 diabetes mellitus; SDS, standard deviation score.

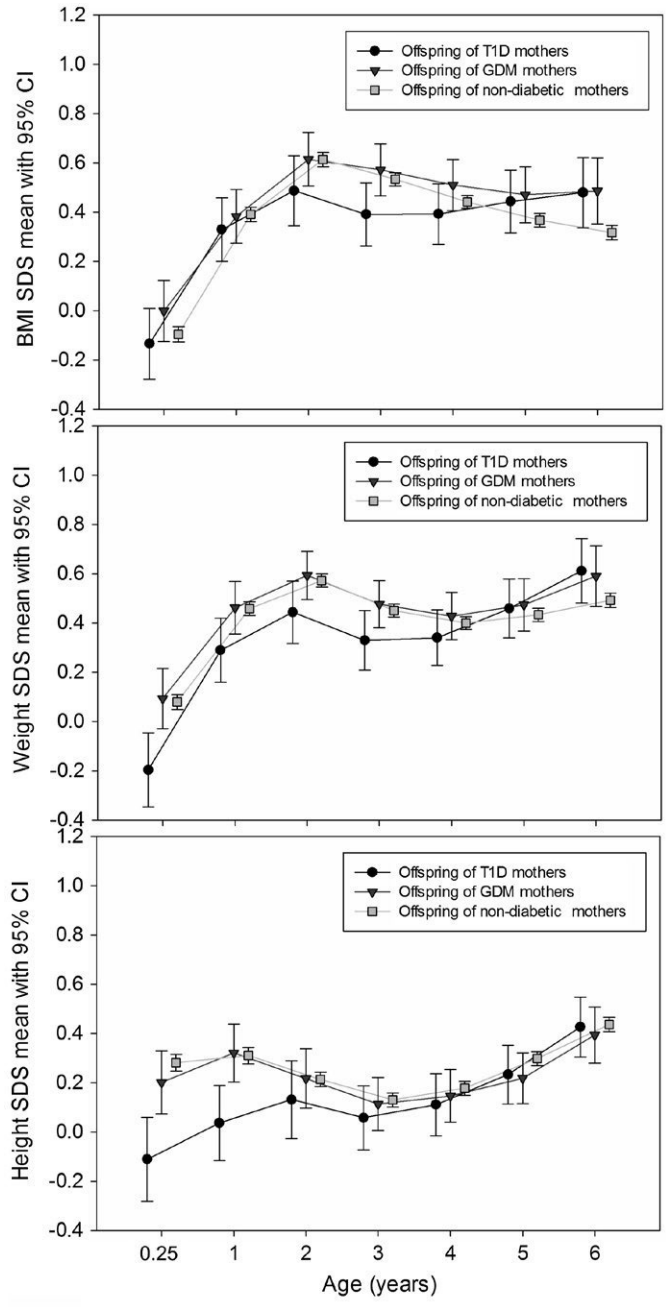

Figure 2 Comparison of mean BMI, weight, and height standard deviation scores (SDSs) with 95% CIs between offspring of mothers with gestational diabetes (GDM), offspring of mothers with type 1 diabetes mellitus (O-T1DM), and offspring of mothers without diabetes mellitus at different ages in the TEDDY study. This figure does not include trends for offspring of mothers with type 2 diabetes mellitus because of low numbers ($n = 14$) and wide CIs.

O-GDM had a similar SDS of both height and weight compared with O-nonDM from 3 months to 2 to 3 years of age; however, O-T1DM showed clearly lower values at this age but caught up with O-GDM until age 5 to 6 years of age (Figure 2). O-nonDM had similar mean BMI SDSs as O-GDM at age 2 but gradually declined afterward and had considerably lower values than O-GDM and O-T1DM at age 6. Accordingly, maternal diabetes was associated with higher BMI SDS (O-GDM: +0.19 [95% CI: 0.07-0.29]; O-T1DM: +0.22 [95% CI: 0.08-0.35]) and increased risk for overweight (O-GDM odds ratio [OR]: 1.48 [95% CI: 1.14-1.92]; O-T1DM OR: 1.60 [95% CI: 1.16-2.20]) and obesity (O-GDM OR: 1.98 [95% CI: 1.34-2.93]; O-T1DM OR: 1.84 [95% CI: 1.09-3.10]) at 5.5 years of age compared with O-nonDM when adjusted for sex and country (Table 2). After additional adjustment for maternal prepregnancy BMI, the respective associations for O-GDM were attenuated and became nonsignificant (e.g., OR for overweight: 1.05 [95% CI: 0.80-1.38]). In contrast, the O-T1DM estimates remained largely unaffected by adjustment for maternal BMI and also for further confounders such as breastfeeding, but they were attenuated considerably after adjustment for birth weight z scores (OR for overweight: 1.15 [95% CI: 0.81-1.62]). O-T2DM had a largely increased risk for overweight despite the small sample size (9 of the 14 O-T2DM children had overweight) and independently of birth weight z scores (OR in the full model: 4.92 [95% CI: 1.40-17.30]). No significant differences between offspring of mothers with diabetes and O-nonDM were observed for height SDS and weight SDS, with the exception of lower height and weight SDSs in O-T1DM after adjustment for birth weight z scores. The observed associations between maternal diabetes and offspring anthropometric outcomes remained similar even after adjusting for the HLA-DQ2/2 genotype or excluding children with islet autoantibodies or T1DM (data not shown). Sensitivity analyses on the reduced subset where physical activity and energy intake were available did not indicate a major confounding role for these two variables (Supporting Information Table S1).

In the longitudinal analysis, O-GDM was again not significantly associated with any outcome when adjusted for maternal prepregnancy BMI (Table 3). Similarly, O-T1DM showed no significant differences in any outcome except height SDS compared with O-nonDM in longitudinal models without birth weight z scores. After inclusion of birth weight z scores, maternal T1DM was associated with lower BMI, overweight, and obesity risk as well as lower height and weight SDS in the offspring.

After including an interaction term between child's age and maternal diabetes in the fully adjusted model, we observed that O-GDM, O-T1DM, and O-T2DM showed comparatively higher increases in BMI SDS per year compared with O-nonDM (Figure 3), indicating that the potential impact of maternal diabetes on childhood BMI becomes stronger with increasing age. For example, the average increase in BMI SDS per year increase in age was 0.06 (95% CI: 0.05 to 0.07) in O-T1DM compared with 0.02 (95% CI: 0.01 to 0.02) in O-nonDM. Therefore, a child with a BMI SDS of 0.00 at age 2 would be expected to have a BMI SDS of 0.08 at age 6 if O-nonDM compared with 0.24 at age 6 if O-T1DM. Similarly, a 1-year increase in age was associated with a higher risk for overweight or obesity in O-GDM, O-T1DM, and O-T2DM, while null or negative effects were found in O-nonDM. For example, the OR for overweight risk per year increase in age was 1.08 (95% CI: 1.02 to 1.14) in O-T1DM compared with 0.95 (95% CI: 0.94 to 0.96) in O-nonDM, implying a relative increase in risk of +13% per year in O-T1DM compared with O-nonDM. Furthermore, we observed no significant interaction terms between country and maternal diabetes in any of the cross-sectional and longitudinal models (data not shown). In addition, treatment with insulin ($n = 72$) compared with diet ($n = 243$), pills only ($n = 1$), or no treatment ($n = 24$) during pregnancy in women with GDM and T2DM was not associated with any of the anthropometric outcomes in offspring at 5.5 years of age (e.g., difference in BMI

TABLE 2 Cross-sectional analysis of anthropometric outcomes between 5.5-year-old offspring of mothers with and without diabetes of different types during pregnancy

Outcome	Exposure (maternal diabetes)	Model 1 (n = 5,277)		Model 2 (n = 5,232)		Model 3 (n = 5,119)		Model 4 (n = 4,994)	
		Estimate (95% CI)	P	Estimate (95% CI)	P	Estimate (95% CI)	P	Estimate (95% CI)	P
Absolute change in SD scores									
BMI SDS	O-GDM	**0.19 (0.07 to 0.29)**	**0.002**	−0.02 (−0.13 to 0.10)	0.78	0.03 (−0.08 to 0.14)	0.61	0.001 (−0.11 to 0.11)	0.99
	O-T1DM	**0.22 (0.08 to 0.35)**	**0.002**	**0.18 (0.04 to 0.31)**	**0.009**	**0.17 (0.04 to 0.30)**	**0.01**	−0.007 (−0.14 to 0.13)	0.91
	O-T2DM	**0.75 (0.23 to 1.27)**	**0.005**	0.24 (−0.27 to 0.74)	0.36	0.28 (−0.22 to 0.78)	0.27	0.32 (−0.18 to 0.83)	0.21
Height SDS	O-GDM	−0.02 (−0.13 to 0.09)	0.67	−0.07 (−0.18 to 0.04)	0.24	−0.04 (−0.16 to 0.07)	0.44	−0.08 (−0.19 to 0.04)	0.20
	O-T1DM	−0.07 (−0.20 to 0.07)	0.34	−0.08 (−0.21 to 0.06)	0.26	−0.08 (−0.22 to 0.05)	0.24	**−0.27 (−0.40 to −0.13)**	**0.0001**
	O-T2DM	−0.06 (−0.57 to 0.45)	0.82	−0.20 (−0.71 to 0.32)	0.45	−0.20 (−0.71 to 0.31)	0.44	−0.21 (−0.73 to 0.31)	0.44
Weight SDS	O-GDM	0.10 (−0.01 to 0.21)	0.08	−0.06 (−0.17 to 0.05)	0.32	−0.01 (−0.12 to 0.10)	0.84	−0.05 (−0.16 to 0.06)	0.36
	O-T1DM	0.12 (−0.02 to 0.25)	0.08	0.08 (−0.05 to 0.21)	0.23	0.07 (−0.06 to 0.20)	0.27	**−0.16 (−0.29 to −0.03)**	**0.02**
	O-T2DM	0.51 (−0.01 to 1.02)	0.05	0.08 (−0.42 to 0.58)	0.31	0.11 (−0.39 to 0.61)	0.67	0.13 (−0.37 to 0.63)	0.60
Odds ratios									
Overweight	O-GDM	**1.48 (1.14 to 1.92)**	**0.003**	1.05 (0.80 to 1.38)	0.75	1.14 (0.86 to 1.51)	0.38	1.10 (0.82 to 1.46)	0.52
	O-T1DM	**1.60 (1.16 to 2.20)**	**0.004**	**1.52 (1.10 to 2.11)**	**0.01**	**1.50 (1.08 to 2.09)**	**0.02**	1.15 (0.81 to 1.62)	0.44
	O-T2DM	**7.39 (2.46 to 22.23)**	**0.0004**	**3.36 (1.06 to 10.70)**	**0.04**	**3.68 (1.14 to 11.81)**	**0.03**	**4.92 (1.40 to 17.30)**	**0.01**
Obesity	O-GDM	**1.98 (1.34 to 2.93)**	**0.0007**	1.23 (0.81 to 1.86)	0.34	1.33 (0.87 to 2.04)	0.19	1.31 (0.85 to 2.01)	0.23
	O-T1DM	**1.84 (1.09 to 3.10)**	**0.02**	**1.79 (1.05 to 3.06)**	**0.03**	**1.75 (1.02 to 3.00)**	**0.04**	1.48 (0.85 to 2.59)	0.17
	O-T2DM	2.93 (0.65 to 13.22)	0.16	0.95 (0.20 to 4.57)	0.95	0.94 (0.19 to 4.60)	0.94	1.02 (0.20 to 5.09)	0.98

Significant associations ($P < 0.05$) shown in bold font.
Model 1: adjusted for sex and country; model 2: model 1 + maternal prepregnancy BMI; model 3: model 2 + breastfeeding, maternal smoking and drinking during pregnancy, gestational weight gain, maternal age, and education; model 4: model 3 + birth weight.
Reference: no diabetes.
O-GDM, offspring of mothers with gestational diabetes mellitus; O-T1DM, offspring of mothers with type 1 diabetes mellitus; O-T2DM, offspring of mothers with type 2 diabetes mellitus; SDS, standard deviation score.

TABLE 3 Longitudinal analysis of anthropometric outcomes assessed during study visits from 0.25 to 6 years of age in offspring of mothers with or without diabetes during pregnancy

Outcome	Exposure (maternal diabetes)	Model 1 (n = 95,162)		Model 2 (n = 94,330)		Model 3 (n = 92,782)		Model 4 (n = 90,569)	
		Estimate (95% CI)	P	Estimate (95% CI)	P	Estimate (95% CI)	P	Estimate (95% CI)	P
Absolute change in SD scores									
BMI SDS	O-GDM	**0.10 (0.01 to 0.19)**	**0.03**	−0.02 (−0.11 to 0.07)	0.70	0.03 (−0.06 to 0.12)	0.52	−0.003 (−0.09 to 0.09)	0.94
	O-T1DM	0.09 (−0.02 to 0.20)	0.12	0.06 (−0.05 to 0.17)	0.27	0.04 (−0.06 to 0.15)	0.42	**−0.15 (−0.26 to −04)**	**0.006**
	O-T2DM	**0.46 (0.04 to 0.87)**	**0.03**	0.15 (−0.26 to 0.56)	0.48	0.19 (−0.22 to 0.60)	0.36	0.19 (−0.22 to 0.60)	0.37
Height SDS	O-GDM	−0.002 (−0.10 to 0.10)	0.97	−0.03 (−0.14 to 0.07)	0.53	−0.001 (−0.11 to 0.11)	0.98	−0.04 (−0.15 to 0.06)	0.40
	O-T1DM	**−0.14 (−0.26 to −0.01)**	**0.03**	**−0.15 (−0.27 to −0.02)**	**0.02**	**−0.16 (−0.28 to −0.03)**	**0.01**	**−0.41 (−0.54 to −0.29)**	**<0.001**
	O-T2DM	−0.18 (−0.65 to 0.29)	0.46	−0.28 (−0.75 to 0.20)	0.25	−0.27 (−0.75 to 0.20)	0.26	−0.30 (−0.78 to 0.17)	0.21
Weight SDS	O-GDM	0.07 (−0.03 to 0.17)	0.15	−0.03 (−0.12 to 0.07)	0.56	0.02 (−0.07 to 0.12)	0.64	−0.02 (−0.12 to 0.07)	0.60
	O-T1DM	−0.02 (−0.14 to 0.09)	0.72	−0.05 (−0.16 to 0.07)	0.44	−0.06 (−0.18 to 0.05)	0.28	**−0.35 (−0.46 to −0.24)**	**<0.001**
	O-T2DM	0.23 (−0.21 to 0.67)	0.31	−0.05 (−0.48 to 0.39)	0.85	−0.01 (−0.44 to 0.42)	0.96	−0.03 (0.45 to 0.39)	0.88
Odds ratios									
Overweight	O-GDM	1.29 (0.99 to 1.68)	0.06	0.94 (0.72 to 1.23)	0.66	1.06 (0.76 to 1.30)	0.69	0.98 (0.75 to 1.28)	0.89
	O-T1DM	1.27 (0.92 to 1.75)	0.14	1.18 (0.86 to 1.62)	0.30	1.14 (0.83 to 1.57)	0.41	**0.69 (0.50 to 0.96)**	**0.03**
	O-T2DM	**3.84 (1.19 to 12.83)**	**0.03**	1.72 (0.54 to 5.48)	0.36	1.93 (0.61 to 6.11)	0.27	1.95 (0.60 to 6.34)	0.27
Obesity	O-GDM	**1.47 (1.11 to 1.95)**	**0.01**	1.08 (0.81 to 1.45)	0.60	1.19 (0.89 to 1.61)	0.24	1.14 (0.85 to 1.54)	0.38
	O-T1DM	0.99 (0.69 to 1.44)	0.97	0.94 (0.65 to 1.35)	0.72	0.91 (0.63 to 1.31)	0.61	**0.62 (0.42 to 0.91)**	**0.01**
	O-T2DM	2.53 (0.79 to 8.11)	0.12	1.14 (0.35 to 3.65)	0.83	1.19 (0.37 to 3.85)	0.77	1.39 (0.42 to 4.59)	0.59

Significant associations ($P < 0.05$) shown in bold font.
Model 1: adjusted for age, sex and country; model 2: model 1 + maternal prepregnancy BMI; model 3: model 2 + breastfeeding, maternal smoking and drinking during pregnancy, gestational weight gain, maternal age, and education; model 4: model 3 + birth weight.
Reference: no diabetes.
O-GDM, offspring of mothers with gestational diabetes mellitus; O-T1DM, offspring of mothers with type 1 diabetes mellitus; O-T2DM, offspring of mothers with type 2 diabetes mellitus; SDS: standard deviation score.

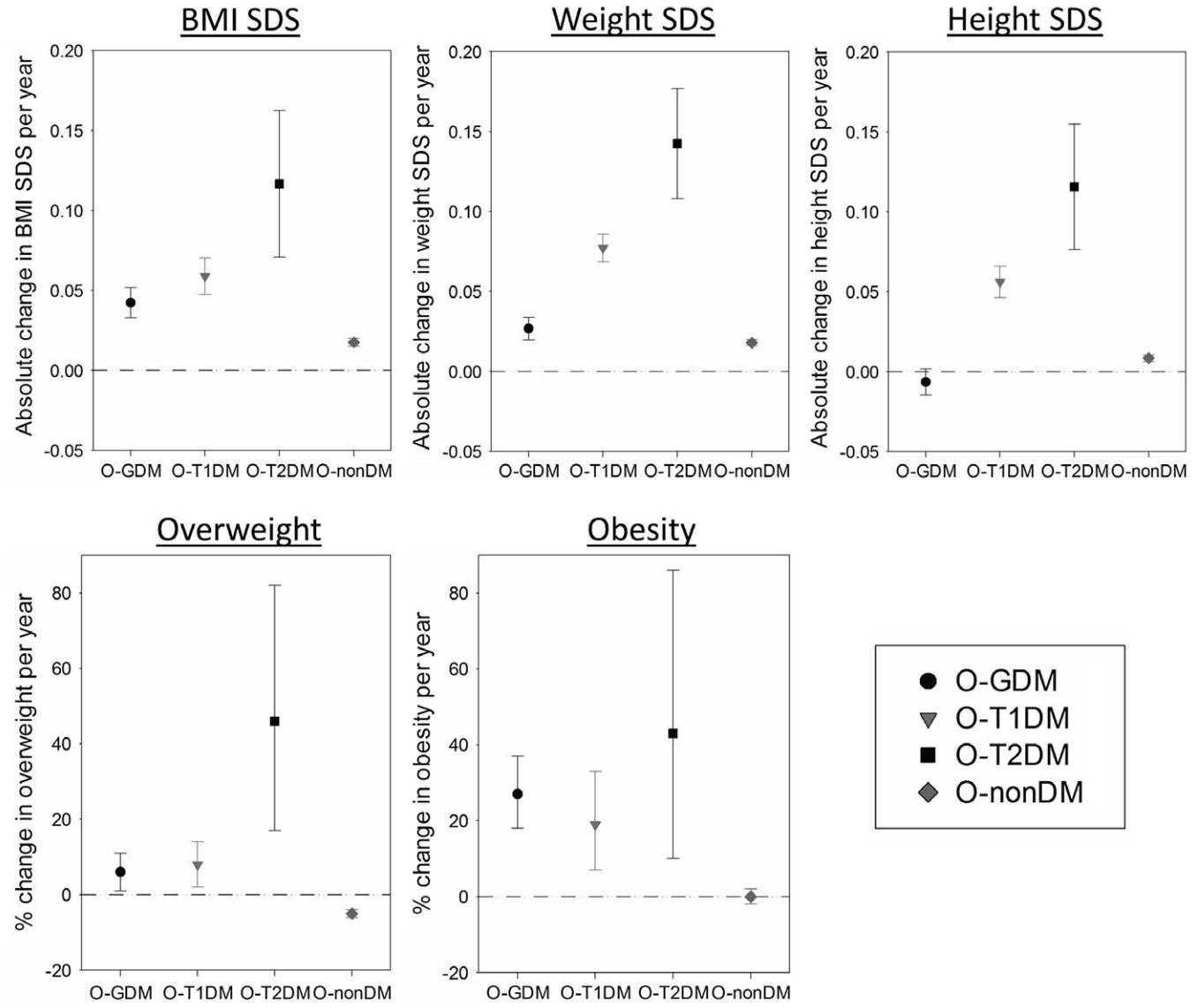

Figure 3 Modifications of association between child's age (per year) and anthropometric outcomes by maternal diabetes status presented as estimates (symbols) with 95% CIs (lines). O-GDM, offspring of mothers with gestational diabetes mellitus; O-nonDM, offspring of mothers without diabetes mellitus; O-T1DM, offspring of mothers with type 1 diabetes mellitus; O-T2DM, offspring of mothers with type 2 diabetes mellitus; SDS, standard deviation score.

SDS of insulin compared with no-insulin treatment: −0.05 [95% CI: −0.34 to 0.25]).

Discussion

In this large, prospective, multicenter cohort study, we observed that children with intrauterine exposure to diabetes had an increased risk for overweight and obesity at 5.5 years of age. This association was not clearly evident when the whole time span of 0.25 to 6 years of age was investigated in a longitudinal analysis. However, we observed that as children grew older, their overweight or obesity risk tended to increase when born to mothers with diabetes compared with when born to mothers without diabetes, implying that the association may not be evident in the first years of life. Furthermore, the observed associations were attenuated significantly after adjustment for prepregnancy BMI in O-GDM and for birth weight *z* scores in O-T1DM, indicating possible mediating effects of these two factors.

Our findings for exposure to maternal T1DM or GDM were generally in line with other studies indicating a positive association with offspring overweight or obesity. These positive associations have been predominantly seen in studies examining offspring older than 5 years (8–12,21,34). However, studies on early childhood offspring have shown inconsistent results. Silverman et al. (35) observed an increased weight in offspring of mothers with diabetes at birth and progressively after age 4 but not between ages 1 and 3. Similarly, Baptiste-Roberts et al. (36) reported a significantly increased BMI in O-GDM at age 7 but not at age 3 and 4. A recent meta-analysis that pooled studies according

to different age subgroups reported a higher risk for overweight and obesity in O-GDM or O-T1DM only during late childhood and adolescence (7). Accordingly, our study showed stronger effects as children grew older. Therefore, it may be possible that maternal diabetes has a delayed influence on offspring obesity that increases with age (37,38). However, two other studies, one of which examined 3-year-old children (15) and the other predominantly 3- to 6-year-old children (16), showed positive associations of GDM with offspring adiposity measured by the sum of skinfolds or fat mass but not by BMI SDS. Therefore, it could be speculated that the differences may be subtle in early ages and become evident with respect to BMI only after a certain age. Moreover, evidence has suggested that early catch-up growth may lead to obesity in later life (39). Accordingly, the associations between maternal diabetes and offspring obesity at 5.5 years of age may be partly attributable to early catch-up growth; Figure 2 indicates that O-T1DM seemed to have accelerated growth during early childhood compared with O-nonDM. These findings may further indicate that environmental factors may contribute to the association between maternal diabetes and offspring overweight. However, the associations in our data remained stable after adjustment for several of those variables, such as breastfeeding, parental education, or maternal age.

In addition, we found that the positive association of maternal GDM with offspring overweight or obesity was attenuated significantly after adjustment for maternal prepregnancy BMI. Several GDM studies have shown similar findings of maternal BMI playing a major confounding role in their analyses (5,9,37,40,41). Indeed, maternal obesity is clearly a risk factor for and often precedes GDM; therefore, it may be difficult to clearly separate the effects of GDM and maternal BMI on offspring obesity. Furthermore, birth weight seemed to substantially explain the positive association between maternal T1DM and offspring overweight or obesity in our data. Moreover, we found no considerable mediating effect of birth weight on the association between GDM and offspring obesity, in accordance with other studies (8,16,19,37). Rates of macrosomia as well as of other adverse outcomes have been reported to be higher in offspring of mothers with pre-GDM than with GDM (42,43). High birth weight may therefore be a proxy of poor glycemic control, which is possibly of greater importance in O-T1DM than O-GDM because the former are exposed to hyperglycemia during the whole pregnancy period. In that case, adding birth weight to the model might even lead to an overadjustment of the O-T1DM association, which might help to explain why we observed protective associations with respect to overweight in O-T1DM compared with O-nonDM in longitudinal analyses.

The main strengths of our study include the large sample size, the prospective study design with standardized protocols, multiple follow-up visits, and availability of many important covariates such as maternal prepregnancy BMI, gestational weight gain, birth weight, breastfeeding, and other postnatal influences such as children's diet and physical activity at age 5. These data allowed us to investigate the effects of different types of diabetes during pregnancy on offspring BMI and overweight at different ages from shortly after birth until age 6. It should be mentioned, however, that the number of children exposed to maternal T2DM during pregnancy was quite limited ($n = 14$), and therefore all associations for this subgroup showed large variability and should be interpreted with great caution. Furthermore, we were not able to assess such associations beyond age 6 because most subjects did not have sufficient follow-up after 6 years at the time these analyses were performed. GDM was defined based on maternal reports only and therefore could be neither confirmed by medical records, lab values, or similar nor harmonized between countries, unfortunately. This issue might have somewhat contributed to different prevalences of GDM between countries, but we do not expect that it has substantially biased our main results. A note of caution is due here with regard to generalizability of our results because these TEDDY cohort participants are all at increased genetic or familial risk to develop T1DM. We therefore cannot exclude that the associations were slightly overestimated, as all the children may generally have a higher background prevalence of overweight regardless of maternal diabetes status. We investigated several outcomes using different statistical models without formal adjustment for multiple testing. Although we cannot exclude that this approach yielded some false-positive results, we would not expect this to be a major limitation because the main findings were relatively consistent between the different models. Furthermore, exclusion because of missing height and weight measurements after age 5 was significantly associated with maternal diabetes status, indicating that families with mothers with diabetes were slightly less likely to drop out of the TEDDY follow-up. However, these differences were small, and we do not expect that they have biased our findings considerably.

In summary, maternal hyperglycemia seems to be associated with increased risk for childhood overweight and obesity. The strength of this association appears to increase as children grow older. Moreover, the association of maternal GDM with offspring obesity can be largely explained by confounding through maternal BMI, whereas the association of maternal T1DM with offspring overweight is substantially mediated by birth weight, possibly suggesting different pathways. Nevertheless, our study indicates that children exposed to maternal diabetes during pregnancy may need closer attention with respect to obesity and its consequences beyond early childhood.O

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ARTICLE

Associations of maternal type 1 diabetes with childhood adiposity and metabolic health in the offspring: a prospective cohort study

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Abstract

Aims/hypothesis Exposure to an intrauterine hyperglycaemic environment has been suggested to increase the offspring's later risk for being overweight or having metabolic abnormalities, but conclusive evidence for pregnancies affected by maternal type 1 diabetes is still lacking. This study aims to analyse the relationship between maternal type 1 diabetes and the offspring's metabolic health and investigate whether birthweight and/or changes in the offspring's metabolome are in the potential pathway.

Methods We analysed data from 610 and 2169 offspring having a first-degree relative with type 1 diabetes from the TEENDIAB and BABYDIAB/BABYDIET cohorts, respectively. Anthropometric and metabolic outcomes, assessed longitudinally at 0.3–18 years of age, were compared between offspring of mothers with type 1 diabetes and offspring of non-diabetic mothers but with fathers or siblings with type 1 diabetes using mixed regression models. Non-targeted metabolomic measurements were carried out in 500 individuals from TEENDIAB and analysed with maternal type 1 diabetes and offspring overweight status.

Results The offspring of mothers with type 1 diabetes had a higher BMI SD score (SDS) and an increased risk for being overweight than the offspring of non-diabetic mothers (e.g. OR for overweight status in TEENDIAB 2.40 [95% CI 1.41, 4.06]). Further, waist circumference SDS, fasting levels of glucose, insulin and C-peptide, and insulin resistance and abdominal obesity were significantly increased in the offspring of mothers with type 1 diabetes, even when adjusted for potential confounders and birthweight. Metabolite patterns related to androgenic steroids and branched-chain amino acids were found to be associated with offspring's overweight status, but no significant associations were observed between maternal type 1 diabetes and metabolite concentrations in the offspring.

Conclusions/interpretation Maternal type 1 diabetes is associated with offspring's overweight status and metabolic health in later life, but this is unlikely to be caused by alterations in the offspring's metabolome.

Keywords Birthweight · Maternal type 1 diabetes · Offspring metabolic health · Offspring metabolome · Offspring overweight

Anette-Gabriele Ziegler and Andreas Beyerlein are joint senior authors.

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Research in context

What is already known about this subject?

- Intrauterine exposure to hyperglycaemia is likely to influence the offspring's later risk for being overweight or having metabolic abnormalities, but conclusive evidence for pregnancies affected by maternal type 1 diabetes is still lacking
- Most previous studies on maternal type 1 diabetes were cross-sectional in design, limited in sample size and assessed children in different age groups; potential pathways were not investigated in any detail

What is the key question?

- Do offspring exposed to maternal type 1 diabetes in utero have an increased risk of being overweight or having metabolic abnormalities later in life, and by which pathways?

What are the new findings?

- Children exposed to maternal type 1 diabetes during pregnancy have a higher risk for being overweight and worse metabolic health during childhood and adolescence, independent of relevant confounders such as diet
- While increased birthweight partly explains this association, changes in the offspring's metabolomic profile are unlikely to be in the causal pathway

How might this impact on clinical practice in the foreseeable future?

- Although not yet recognised as a particular risk group, children whose mothers have type 1 diabetes might need closer attention with respect to excess weight gain and metabolic risk in later life

Abbreviations

BCAA	Branched-chain amino acid
DII	Dietary inflammatory index
SDS	Standard deviation score

Introduction

Obesity and excess weight in children and adolescents remains a major public health problem because it induces other metabolic disorders, such as diabetes and cardiovascular disease [1]. A growing body of evidence supports the concept of fuel-mediated teratogenesis, in which intrauterine exposure to hyperglycaemia leads to excess fetal glucose and insulin, and thus overgrowth of the fetus [2]. These exposures during fetal life have been reported to extend beyond the neonatal period and influence metabolic complications in later life.

Various studies have shown evidence associating gestational diabetes and type 2 diabetes with later adiposity, increased BMI, insulin resistance, impaired glucose tolerance, higher cholesterol, hypertension and type 2 diabetes in the offspring [3–6], but less evidence exists to support a similar effect of maternal type 1 diabetes on offspring health. However, it appears relevant to differentiate between type 1 diabetes, gestational diabetes and type 2 diabetes, because the last two are associated with maternal obesity, while type 1 diabetes is not. Studies which reported a positive association of maternal type 1 diabetes with BMI or metabolic outcomes in the offspring [7–10] were cross-sectional in design and limited with respect to their sample size ($n < 600$ in each). Furthermore, two of these studies were based on children born as early as 1978–1985 [7] and 1982–1991 [10], respectively, when diabetes care in pregnant women was probably less good than nowadays [11]. Previous analyses of our own data indicated that children with non-diabetic and type 1 diabetic mothers follow different growth patterns [12, 13], and also that a potential association between maternal type 1 diabetes and risk of being overweight in the offspring is not independent of birthweight and breastfeeding duration [14].

Here, we analysed data from two prospective cohort studies containing over 2770 children of whom more than 1500 were exposed to maternal type 1 diabetes during pregnancy. A subset of 500 children were also characterised for non-targeted metabolomics; these are of particular interest as recent studies have shown significant associations between metabolic concentrations and childhood obesity [15–17], while the associations between maternal type 1 diabetes and metabolic profile in the offspring have not yet been investigated. The aims of this study were to investigate: (1) whether there are differences in anthropometric and metabolic outcomes between offspring of mothers with type 1 diabetes and non-diabetic mothers; and (2) whether birthweight and/or changes in the offspring's metabolome may be in the potential pathway from maternal type 1 diabetes to later overweight status and poor metabolic health in the offspring.

Methods

Our analysis was based on the prospective German cohorts TEENDIAB and BABYDIAB/BABYDIET. These cohorts include children with a familial background of type 1 diabetes and have already been combined for other research questions [18, 19]. All parents gave written informed consent for participation. The studies were approved by the ethical committees of the Technische Universität München (number 2149/08) and Hannover Medical School (number 5644); the Bavarian General Medical Council (number 95357) and Ludwig-Maximilians University (number 329/00), respectively.

TEENDIAB study

The TEENDIAB study is a prospective cohort study conducted in the cities of Munich and Hannover, Germany. During 2009–2015, this study recruited 610 children aged 6–16 years who were resident in Germany and had at least one parent or sibling with type 1 diabetes [20]. Children were followed, on average, every 6 months from 6 to 18 years of age until 2016.

Maternal characteristics and offspring measurements At the first visit, information on type 1 diabetes, smoking status and education level of the parents as well as monthly family income was obtained via self-administered questionnaire. Birthweight information was taken from health records collected during the well-baby preventive health programme, which is routinely offered to all children in Germany. During each visit, weight was measured digitally or using a beam scale with a precision of ±100 g in light clothing. Height was measured using a stadiometer with a precision of ±1 mm. Waist circumference was measured using a measuring tape between the pelvic crest and the lower ribs while breathing with a precision of ±1 mm. Subscapular and triceps skinfold thickness were measured three times using a caliper at the inferior angle of the right scapula and at the posterior right upper arm, respectively, and were calculated as the average of the three measurements. Systolic and diastolic blood pressure were calculated as the average of two measurements, made using the auscultatory or oscillometric method and the upper arm, with the individual in a sitting position after 3–5 min of rest. Tanner's staging was assessed by the study doctor or local paediatrician using validated questionnaires [21]. Venous blood samples were collected to assess fasting blood glucose, insulin and C-peptide, and lipids (cholesterol and triacylglycerols). All participants were asked to fast for at least 10 h before blood collection.

Dietary intake was assessed in 330 children during their first study visit using two different methods. In 268 children, Diet Interview Software for Health Examination Studies Junior (DISHES Junior; Robert Koch Institute, Berlin, Germany), computer-assisted interview software, was used to assess retrospectively the frequency, type and quantity of foods and beverages consumed in the last 4 weeks. In the remaining 62 children, diet was assessed using a 3 day dietary record which was entered into PRODI (Nutri-Science, Stuttgart, Germany) nutrition software. Both software packages are linked to the German Nutrient Database (Bundeslebensmittelschluessel; Max Rubner Institut, Karlsruhe, Germany), which allows estimates to be made of the average daily intake of energy, macronutrients and micronutrients.

Metabolomic profiling Non-targeted metabolomic profiling was performed on fasting serum samples taken from 500 children at the first visit using ultra high-performance liquid chromatography and mass spectrometry on the Metabolon platform (Metabolon, Durham, NC, USA). All samples were stored at −80°C prior to analysis. Metabolites were identified following the metabolomics standardisation initiative guidelines [22]. Metabolites were quantified as outlined previously [23]. A total of 575 metabolites were quantified, of which 239 were unknown. Metabolites and samples which had more than 30% missing values were excluded, leaving a total of 441 metabolites, including 294 known and 147 unknown ones, and 485 samples. Metabolite concentrations in terms of raw ion counts were normalised to account for run-day differences and log-transformed to bring them closer to a normal distribution. Missing data were imputed using random forest imputation.

BABYDIAB/BABYDIET studies

The BABYDIAB and BABYDIET studies are two ongoing prospective studies of German birth cohorts; they include 2441 children born between 1989 and 2006 with a first-degree relative with type 1 diabetes. During 1989–2000, a total of 1650 offspring of individuals with type 1 diabetes were recruited for the BABYDIAB study. During 2000–2006, 791 additional offspring or siblings of individuals with type 1 diabetes were screened in the context of the BABYDIET study. Of those, 150 participated in the BABYDIET dietary intervention study randomising the timing of first gluten exposure; the intervention had no effect on islet autoimmunity development or on growth [24, 25]. Further details on the study design are described elsewhere [24, 26, 27]. Data from these two cohorts were combined for longitudinal analyses of maternal type 1 diabetes and anthropometric outcomes in the offspring.

Maternal characteristics and offspring measurements Information on the presence of type 1 diabetes within the family (mother, father or sibling) and smoking status of the mother during pregnancy was obtained via self-administered questionnaire. Height and weight measurements of the

offspring were obtained from health records from the well-baby preventive health programme visits, which were regularly conducted at birth and at the age of 3–10 days, 4–6 weeks and 3–4, 6–7, 10–12, 21–24, 46–48 and 60–64 months. Further height and weight measurements were assessed during study visits, which were scheduled at birth, age 9 months and at 2, 5, 8, 11, 14, 17 and 20 years of age in BABYDIAB, as well as 3-monthly from birth until the age of 3 years, and yearly until the age of 12 years in BABYDIET. These measurements were performed in the same way as described for the TEENDIAB study. From the age of 8 years, Tanner's staging was assessed by a paediatrician or trained staff using validated questionnaires at every study visit.

Exclusions We excluded from our analysis the data from BABYDIAB/BABYDIET participants who had no height and weight measurements ($n = 14$), were lost to follow-up after 0.3 years of age ($n = 44$), or who also participated in the TEENDIAB study ($n = 214$), leaving a final sample size of $n = 2169$. We further excluded all visits performed before 0.3 years of age because these measurements were likely to be highly correlated with birthweight, which we wanted to investigate separately.

Statistical analysis

Height, weight, BMI, waist circumference, subscapular and triceps skinfold thickness and lipids were transformed into age- and sex-specific SD scores (SDSs), and blood pressure into age-, sex- and height-specific SDSs according to German reference values [28–30]. Overweight was defined as a BMI at or above an SDS of 1.31, corresponding with the 90th percentile. For waist circumference SDS, the respective reference percentiles were available for only participants aged between 11 and 18 years. Abdominal obesity was defined as a waist circumference at or above the 90th percentile or the adult threshold set by the International Diabetes Federation [31]. Birthweight was transformed into age- and sex-specific percentiles based on German reference values [32], and categorised as small for gestational age (birthweight <10th percentile), appropriate for gestational age (10th–90th percentile) or large for gestational age (>90th percentile). Participants were classified as having high overall metabolic risk at a certain visit when at least one SDS of BMI, waist, skinfold thickness, blood pressure or lipids was greater than 1.5. Insulin resistance was estimated by HOMA-IR [33].

To adjust for potential confounders, categories of socioeconomic status (high, middle and low) were calculated based on parental education and family income as described previously [34]. Energy intake was adjusted for age and sex using the residual method [35]. Further, an energy-adjusted dietary inflammatory index (DII) score was calculated based on 27 out of a possible 45 food variables as described elsewhere [36]. A positive DII score indicates a proinflammatory diet, whereas a negative DII score indicates an anti-inflammatory diet.

Maternal type 1 diabetes and metabolic outcomes in the offspring In all our analyses, we compared offspring of mothers with type 1 diabetes with offspring who had mothers without diabetes, but fathers or siblings with type 1 diabetes. We did this separately for TEENDIAB and BABYDIAB/BABYDIET because the studies differed in the number of outcomes assessed and the timing of the respective measurements. First, anthropometric and metabolic outcomes were visually compared at yearly time intervals between offspring of mothers with and without type 1 diabetes. Second, linear and logistic mixed-effect models accounting for repeated observations within individuals were performed. Fasting glucose, insulin and C-peptide as well as HOMA-IR were log-transformed because of non-normal residuals in the respective linear models. Associations were analysed based on stepwise adjustment. In the first model, we performed univariate analysis for all outcomes. Consistent with other studies [8], we adjusted for age and sex (except for the SDS-corrected outcomes) as well as for Tanner's staging in the second model, and additionally for socioeconomic status and maternal smoking, which are known to be potential risk factors for excess weight gain in childhood [37, 38]. In order to investigate whether birthweight was in the causal pathway from maternal type 1 diabetes to overweight status and metabolic risk in the offspring, birthweight was added as a categorical variable in the third model.

Sensitivity analyses As a first sensitivity analysis, we excluded all children who developed type 1 diabetes during follow-up (8/610 in TEENDIAB and 100/2169 in BABYDIAB/BABYDIET), and reassessed the associations between maternal type 1 diabetes and offspring metabolic outcomes. Second, we compared anthropometric outcomes from the offspring of mothers with type 1 diabetes and fathers with type 1 diabetes separately from those for offspring whose parents did not have type 1 diabetes to see whether parental genetic transmission may also be a relevant factor in addition to intrauterine hyperglycaemia. Children who had both parents with type 1 diabetes were not considered in this analysis. Third, we further investigated cross-sectional associations after adjustment for daily energy intake and DII separately in two different models in addition to Tanner's staging, socioeconomic status and maternal smoking. Fourth, we analysed BMI, weight and height outcomes (not SDS transformed) by adding interaction terms between maternal type 1 diabetes status and child's age in the combined TEENDIAB and BABYDIAB/BABYDIET cohort data to explore whether the association changed with increasing age.

Analyses of metabolomic profiles We further explored the extent to which the offspring's metabolomic profile may play a mediating role in the association between maternal type 1 diabetes and being overweight. First, we examined associations between every single metabolite concentration and being overweight in the offspring assessed at the same visit using logistic regression models. The Benjamini–Hochberg procedure was used to control the false-discovery rate based on 441 tests in order to account for multiple comparisons. Further, principal components analysis with varimax rotation was performed on the 441 log-transformed metabolites to consolidate them into 15 principal components with eigenvalues >5, which accounted for 43% of the variance in metabolites; the associations between these 15 principal components and being overweight in the offspring were analysed. Second, we investigated whether maternal type 1 diabetes was associated with principal components or metabolites that were significant for overweight status, adjusted for age and sex. Third, associations between maternal type 1 diabetes and overweight status in the offspring were assessed after adjusting for metabolites or principal components which were significantly associated with being overweight. In addition, metabolite concentrations were categorised into 68 sub- and eight superpathways [23]. For each super- and subpathway, the mean of the metabolites belonging to that particular pathway was calculated for all samples and associated with offspring overweight status and maternal type 1 diabetes.

Results were reported as absolute change with 95% CI for SDS outcomes, per cent change with 95% CI for log-transformed outcomes and as OR with 95% CI for risk of being overweight and having metabolic abnormalities between offspring of type 1 diabetic and non-diabetic mothers. All analyses were carried out using SAS 9.4 (SAS Institute, Cary, NC, USA) and R 3.4.1 (http://cran.r-project.org).

Results

The study participants in TEENDIAB and BABYDIAB/BABYDIET had a median follow-up of 3.0 and 10.7 years, respectively, which corresponds to a median of six follow-up visits (TEENDIAB range 1–13; BABYDIAB/BABYDIET range 1–18) resulting in 3583 and 13,235 observations in the TEENDIAB and BABYDIAB/BABYDIET cohorts, including 257 (42%) and 1287 (59%) children of mothers with type 1 diabetes, respectively (Table 1). The age of enrolment and follow-up duration were not significantly different between offspring of type 1 diabetic and non-diabetic mothers in either cohort ($p > 0.90$ each; Mann–Whitney U test).

Maternal type 1 diabetes and metabolic outcomes in the offspring

In TEENDIAB, we observed a pattern of higher BMI SDS, weight SDS, fasting levels of glucose, insulin and C-peptide as well as insulin resistance, and of lower height SDS in offspring of mothers with type 1 diabetes in most age groups (Fig. 1 and electronic supplementary material [ESM] Fig. 1). In BABYDIAB/BABYDIET, the anthropometric associations were similar, but weaker and less consistent. However, in mixed models based on all longitudinal measurements significant associations were observed in both cohorts: offspring of mothers with type 1 diabetes had a significantly higher BMI SDS (TEENDIAB 0.35 [95% CI 0.19, 0.52]; BABYDIAB/BABYDIET 0.13 [95% CI 0.06, 0.20], Tables 2 and 3) and increased risk for being overweight (TEENDIAB OR 2.40 [95% CI 1.41, 4.06]; BABYDIAB/BABYDIET OR 1.44 [95% CI 1.20, 1.73]) compared with offspring of non-diabetic mothers. These associations did not change considerably when adjusted for Tanner's staging, socioeconomic status and maternal smoking. However, after further adjustment for birthweight, the observed associations were attenuated in TEENDIAB and were no longer significant in BABYDIAB/BABYDIET, while the negative associations for height SDS became stronger and significant in both cohorts. In TEENDIAB, weight SDS, waist circumference SDS and subscapular and triceps skinfold thickness SDSs were also significantly higher in offspring of mothers with type 1 diabetes compared with those whose mothers did not have type 1 diabetes, but only the estimates for waist circumference SDS remained significant when adjusted for potential confounders and birthweight. The offspring of type 1 diabetic mothers showed significantly increased abdominal obesity risk and metabolic risk, as well as significantly increased levels of fasting insulin and HOMA-IR, independent of potential confounders. Significant associations with fasting glucose and C-peptide were observed only after adjustment. Systolic blood pressure SDS was slightly higher in children with type 1 diabetic mothers in unadjusted analyses (+0.16 [95% CI +0.01, +0.31]), but not after adjustment, while no significant differences in lipids were observed between offspring of mothers with or without type 1 diabetes in unadjusted or adjusted models. The observed associations did not change considerably after excluding children who developed type 1 diabetes (data not shown). Also, the offspring of mothers with type 1 diabetes showed stronger anthropometric associations than offspring of fathers with type 1 diabetes when compared with offspring without parents with type 1 diabetes (ESM Table 1). Our sensitivity analyses based on 330 children indicated that the associations were independent of total energy intake or DII (ESM Table 2). Further, we observed that as children got older, BMI and weight increased at a greater rate in offspring of mothers with type 1 diabetes compared with offspring of

Table 1 Characteristics of study participants stratified by maternal type 1 diabetes in the TEENDIAB and BABYDIAB/BABYDIET cohort

Variable	TEENDIAB ($n = 610$)			BABYDIAB/BABYDIET ($n = 2169$)		
	No. obs	OT1DM ($n = 257$)	OnonDM ($n = 353$)	No. obs	OT1DM ($n = 1287$)	OnonDM ($n = 882$)
Time-constant						
Sex	610			2169		
Male		126 (49.03)	187 (52.97)		661 (51.36)	445 (50.45)
Maternal smoking[a]	581			2128		
Yes		32 (13.11)	43 (12.76)		160 (12.67)	68 (7.86)
Socioeconomic status[b]	594					
Low		7 (2.80)	5 (1.45)	–	–	–
Middle		149 (59.60)	148 (43.02)	–	–	–
High		94 (37.60)	191 (55.52)	–	–	–
Birthweight	571			2047		
SGA		12 (4.94)	37 (11.28)		89 (7.50)	90 (10.47)
AGA		155 (63.79)	252 (76.83)		745 (62.76)	689 (80.12)
LGA		76 (31.28)	39 (11.89)		353 (29.74)	81 (9.42)
Birthweight SDS	571	0.78 ± 1.39	−0.03 ± 0.99	2047	0.57 ± 1.32	−0.06 ± 1.00
Time-varying						
Age (years)	3583	11.90 ± 2.18	11.95 ± 2.15	13235	5.06 ± 4.69	4.63 ± 4.45
BMI SDS	3537	0.31 ± 1.09	−0.14 ± 1.08	13235	0.15 ± 1.08	0.01 ± 1.01
Overweight[c]	3537			13235		
Yes		282 (18.75)	194 (9.54)		1068 (13.70)	569 (10.46)
Height SDS	3537	0.27 ± 0.97	0.35 ± 0.99	13235	0.10 ± 1.01	0.15 ± 1.03
Weight SDS	3537	0.38 ± 1.06	0.09 ± 1.01	13235	0.14 ± 0.96	0.06 ± 0.91
Waist circumference SDS	2418	0.20 ± 1.10	−0.12 ± 1.05	–	–	–
Subscapular skinfold thickness SDS	765	0.18 ± 0.94	−0.04 ± 1.00	–	–	–
Triceps skinfold thickness SDS	768	−0.30 ± 1.09	−0.51 ± 1.09	–	–	–
SBP SDS	2056	−0.05 ± 1.31	−0.25 ± 1.29	–	–	–
DBP SDS	2056	0.27 ± 1.27	0.10 ± 1.31	–	–	–
HDL-cholesterol SDS	590	−0.72 ± 1.24	−0.78 ± 1.26	–	–	–
LDL-cholesterol SDS	590	−0.04 ± 1.04	−0.16 ± 1.11	–	–	–
Triacylglycerol SDS	590	0.36 ± 0.78	0.30 ± 0.83	–	–	–
Cholesterol SDS	590	−0.07 ± 0.95	−0.18 ± 1.05	–	–	–
Metabolic risk[d] (cut-off 1.5 SDS)	3545					
Yes		430 (28.51)	417 (20.47)	–	–	–
Fasting glucose (mmol/l)	3346	4.79 ± 0.62	4.74 ± 0.60	–	–	–
Fasting insulin (pmol/l)	3314	66.15 ± 59.39	60.61 ± 54.78	–	–	–
Fasting C-peptide (nmol/l)	3130	0.55 ± 0.31	0.51 ± 0.28	–	–	–
HOMA-IR	3172	2.06 ± 1.90	1.87 ± 1.72	–	–	–
Total energy intake (kJ)	330	9076.24 ± 2834.46	8634.60 ± 2489.59	–	–	–
DII score	330	0.20 ± 1.83	−0.11 ± 1.71	–	–	–

Data are number (%) or mean ± SD. Percentages were calculated based on the observations available for each variable

[a] Smoking during pregnancy in BABYDIAB/BABYDIET and general smoking status in TEENDIAB

[b] Based on the education level of parents and monthly net income of the family

[c] BMI at or above an SDS of 1.31, corresponding with the 90th percentile

[d] High risk when SDS >1.5 for at least one of BMI, waist circumference, subscapular and triceps skinfold thickness, BP and lipids

AGA, appropriate for gestational age; DBP, diastolic BP; LGA, large for gestational age; No. obs, total number of observations available for the variable; OnonDM, offspring of non-diabetic mothers; OT1DM, offspring of mothers with type 1 diabetes; SBP, systolic BP; SGA, small for gestational age; DII, dietary inflammatory index

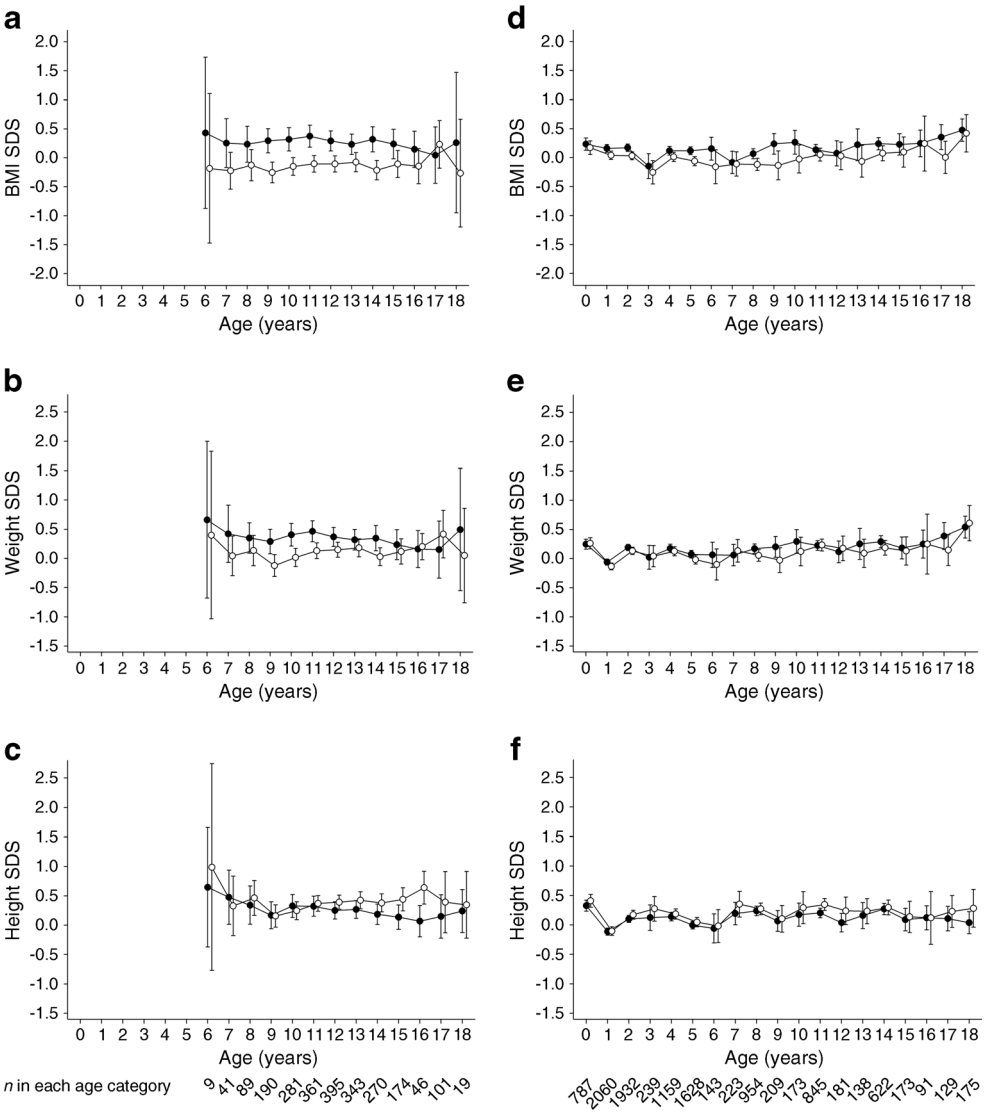

Fig. 1 Mean and 95% CI for BMI (**a**, **d**), weight (**b**, **e**) and height (**c**, **f**) SDSs stratified by age and maternal type 1 diabetes in the TEENDIAB (**a**–**c**) and BABYDIAB/BABYDIET (**d**–**f**) cohorts. Black circles, offspring of mothers with type 1 diabetes; white circles, offspring of non-diabetic mothers

non-diabetic mothers, whereas height increased at a greater rate in offspring of non-diabetic mothers (ESM Fig. 2 and 3).

Analyses of metabolomic profiles

The metabolomics blood samples were taken at a median age of 10 years (range 6–16 years), and 48 individuals (10%) were overweight at that time. Of the children included in the metabolomics analyses ($n = 485$), 247 (51%) were male and 197 (41%) had mothers with type 1 diabetes. Of the 441 metabolites analysed, 28 showed significant associations with being overweight after multiple testing correction, and 19 of these were of known identity (Table 4). All these metabolites were upregulated in overweight individuals, including four metabolites from the amino acid class (valine, kynurenate, tyrosine and alanine), 11 from the lipid class (androgenic steroids such as androsterone sulphate, epiandrosterone sulphate, carnitine and the short-chain acyl-carnitine [butyryl carnitine (C4)], glycerol, thromboxane B_2, stearidonate and 2-aminoheptanoate), and four metabolites from other classes (N1-methyl-4-pyridone-3-carboxamide, urate, γ-glutamyltyrosine and piperine). At the pathway level, several subpathways such as androgenic steroids and branched-chain amino acid (BCAA) metabolism were upregulated in overweight individuals, as was the superpathway nucleotide (Fig. 2). Similarly, three principal components, characterised by androgenic steroids, BCAAs and related metabolites or composed of amino acid, lipid and acetylated peptides, were associated with being overweight (ESM Fig. 4 and ESM Table 3). The principal components related to androgenic steroids and BCAAs were also positively associated with HOMA-IR ($p < 0.0001$ and $p = 0.002$ respectively), fasting insulin ($p < 0.0001$ and $p = 0.005$) and fasting C-peptide ($p = 0.002$ and $p < 0.0001$).

In contrast, there was no significant association of any metabolite with maternal type 1 diabetes when corrected for

Table 2 Effect estimates for anthropometric and metabolic outcomes in offspring born to a mother with vs without type 1 diabetes in the TEENDIAB cohort

Outcome	Model 1		Model 2		Model 3	
	No. participants (No. obs)	Estimates (95% CI)	No. participants (No. obs)	Estimates (95% CI)	No. participants (No. obs)	Estimates (95% CI)
Absolute change in SDS						
Height SDS	610 (3537)	−0.12 (−0.28, 0.03)	562 (3122)	−0.07 (−0.23, 0.08)	527 (2955)	−0.27 (−0.43, −0.10)**
Weight SDS	610 (3537)	0.20 (0.04, 0.36)*	562 (3122)	0.22 (0.06, 0.39)*	527 (2955)	0.07 (−0.10, 0.25)
BMI SDS	610 (3537)	0.35 (0.19, 0.52)**	562 (3122)	0.36 (0.19, 0.53)**	527 (2955)	0.28 (0.09, 0.46)**
Waist circumference SDS[a]	489 (2418)	0.29 (0.12, 0.46)**	452 (2152)	0.24 (0.06, 0.42)**	426 (2057)	0.19 (0.00, 0.39)*
Subscapular skinfold SDS	570 (765)	0.19 (0.03, 0.35)*	499 (662)	0.17 (0.01, 0.33)*	471 (626)	0.12 (−0.05, 0.30)
Triceps skinfold SDS	572 (768)	0.19 (0.02, 0.37)*	500 (663)	0.15 (−0.04, 0.33)	472 (627)	0.09 (−0.10, 0.29)
SBP SDS	597 (2056)	0.16 (0.01, 0.31)*	543 (1825)	0.13 (−0.03, 0.30)	510 (1727)	0.10 (−0.07, 0.28)
DBP SDS	597 (2056)	0.12 (−0.03, 0.26)	543 (1825)	0.14 (−0.01, 0.30)	510 (1727)	0.17 (0.00, 0.34)*
HDL-cholesterol SDS	590	0.06 (−0.14, 0.27)	502	0.04 (−0.18, 0.27)	471	0.06 (−0.19, 0.30)
LDL-cholesterol SDS	590	0.10 (−0.07, 0.28)	502	0.08 (−0.11, 0.28)	471	0.10 (−0.11, 0.31)
Triacylglycerol SDS	590	0.06 (−0.07, 0.19)	502	0.10 (−0.05, 0.24)	471	0.12 (−0.04, 0.27)
Cholesterol SDS	590	0.10 (−0.06, 0.27)	502	0.09 (−0.09, 0.27)	471	0.13 (−0.07, 0.32)
% change in metabolic outcome						
Fasting glucose	606 (3346)	1.00 (−0.32, 2.34)	558 (2937)	1.71 (0.29, 3.16)*	523 (2785)	2.05 (0.51, 3.62)*
Fasting insulin	608 (3314)	8.32 (0.68, 16.55)*	560 (2902)	8.45 (1.06, 16.38)*	525 (2749)	9.70 (1.71, 18.31)*
Fasting C-peptide	601 (3130)	6.01 (−0.23, 12.64)	553 (2744)	5.18 (−0.59, 11.28)	519 (2602)	6.61 (0.33, 13.27)*
HOMA-IR	606 (3172)	8.36 (0.38, 16.99)*	558 (2781)	9.49 (1.69, 17.88)*	523 (2641)	11.55 (3.02, 20.79)*
OR						
Overweight	610 (3537)	2.40 (1.41, 4.06)**	562 (3122)	2.28 (1.29, 4.01)**	527 (2955)	2.06 (1.12, 3.78)*
Abdominal obesity[a,b]	498 (2564)	1.92 (1.15, 3.20)*	460 (2273)	1.91 (1.11, 3.30)*	433 (2168)	1.97 (1.10, 3.55)*
Metabolic risk[c] (cut-off 1.5 SDS)	610 (3545)	1.45 (1.10, 1.92)*	562 (3128)	1.46 (1.07, 1.97)*	527 (2961)	1.37 (0.98, 1.90)

Model 1, crude model; model 2, adjusted for age, sex (except for overweight, abdominal obesity, metabolic risk and SDS outcomes), Tanner's staging, maternal smoking and socioeconomic status; model 3, model 2 + birthweight

[a] Calculated only in children ≥11 years of age

[b] Waist circumference ≥90th percentile or the adult threshold (International Diabetes Federation)

[c] High risk when SDS >1.5 for at least one of BMI, waist, subscapular and triceps skinfold thickness, blood pressure and lipids; otherwise defined as low risk

*$p < 0.05$ and **$p < 0.01$

No., number of; Obs, observations (if different from number of participants)

Table 3 Effect estimates for anthropometric outcomes in offspring born to a mother with vs without type 1 diabetes in the BABYDIAB/BABYDIET cohort

Outcome	Model 1		Model 2		Model 3	
	No. participants (No. obs)	Estimates (95% CI)	No. participants (No. obs)	Estimates (95% CI)	No. participants (No. obs)	Estimates (95% CI)
Absolute change in SDS						
Height	2169 (13235)	−0.06 (−0.13, 0.02)	2128 (11757)	−0.06 (−0.14, 0.02)	2010 (11374)	−0.13 (−0.21, −0.06)**
Weight	2169 (13235)	0.06 (−0.01, 0.13)	2128 (11757)	0.06 (−0.01, 0.13)	2010 (11374)	−0.05 (−0.12, 0.02)
BMI	2169 (13235)	0.13 (0.06, 0.20)**	2128 (11757)	0.14 (0.07, 0.21)**	2010 (11374)	0.04 (−0.04, 0.11)
OR						
Overweight	2169 (13235)	1.44 (1.20, 1.73)**	2128 (11757)	1.45 (1.20, 1.74)**	2010 (11374)	1.15 (0.95, 1.40)

Model 1, crude model; model 2, adjusted for Tanner's staging and maternal smoking during pregnancy; model 3, model 2 + birthweight

*$p < 0.05$ and **$p < 0.01$

No., number of; Obs, observations

multiple testing, and there was not even a significant association at the 5% level for any of the metabolites found to be associated with being overweight (ESM Table 4). No significant associations were observed between maternal type 1 diabetes and any of the principal components (ESM Fig. 5) or super- and subpathways (ESM Fig. 6) after correcting for multiple testing.

Further, the associations between maternal type 1 diabetes and offspring overweight status remained significant and were not markedly attenuated after adjustment for any potentially relevant single metabolite concentration or principal components (Table 5), indicating that none is in the causal pathway.

Discussion

Our findings suggest that the offspring of mothers with type 1 diabetes have a higher BMI and increased risk for being overweight as well as increased insulin resistance compared with offspring of non-diabetic mothers. The association between maternal type 1 diabetes and excess weight later in life could be substantially explained by birthweight in our birth cohort data, but only partially in our TEENDIAB data, perhaps because these did not include measurements before school age. Metabolic alterations, however, do not seem to be involved in the pathway. Although some metabolic patterns were found to be associated with being overweight, no such associations were observed with respect to maternal type 1 diabetes.

Previous studies that examined the offspring of mothers with type 1 diabetes reported similar findings with respect to excess weight gain, the metabolic syndrome and related outcomes at different ages [7–10]. However, one study [39] found that the prevalence of being overweight in 6–8-year-old offspring of mothers with type 1 diabetes under adequate glycaemic control was similar to that in a reference population, potentially pointing to a possible approach for the early prevention of excess weight gain in these children.

Our analysis indeed suggests that offspring of mothers with type 1 diabetes are more prone to worsening of metabolic profile than offspring of fathers with type 1 diabetes when compared with offspring whose parents did not have type 1 diabetes, thus providing evidence to support a potential role for intrauterine hyperglycaemia rather than for parental genetic transmission. Previous analyses of the BABYDIAB data (without BABYDIET and with much shorter follow-up than here) suggested that maternal type 1 diabetes may not be an independent predictor of overweight status during childhood but associated factors such as birthweight may predispose individuals to risk of being overweight [14]. Indeed, the associations between maternal type 1 diabetes and offspring overweight status were attenuated by 62% after adjustment for birthweight in the BABYDIAB/BABYDIET study, but only by 10% in the TEENDIAB study. Moreover, the effect estimates were generally weaker in BABYDIAB/BABYDIET compared with TEENDIAB. We assume that these differences come from the different age structures in the studies. The BABYDIAB/BABYDIET cohort followed children from birth, with most anthropometric measurements taken during the preschool period, whereas recruitment started at a minimum age of 6 years in TEENDIAB. Although both studies followed children until 18 years, anthropometric data were not available after 6 years of age for 30% of the BABYDIAB/BABYDIET participants. Birthweight is more strongly associated with a child's BMI in early childhood than later, which may explain the observed differences between the two studies. It has also been suggested that maternal diabetes may have a delayed influence on the offspring's adiposity that increases with age [40, 41]. We consider it less likely that the differences observed between our two cohorts are caused by different environmental conditions around the time of birth, as the median birth year in TEENDIAB was 2001 compared with 1997

Table 4 Cross-sectional associations between metabolite concentrations and overweight status in the offspring

Exposure	Cross-sectional models ($n = 485$)	
	OR[a] (95% CI)	p value
Amino acid		
Alanine[b]	9.23 (2.42, 35.23)*	0.0011
Valine[b]	88.27 (7.79, 999.85)*	0.0003
Kynurenate[b]	9.32 (3.14, 27.64)*	5.7×10^{-5}
Tyrosine[b]	37.21 (5.66, 244.55)*	0.0002
Lipid		
Androsterone sulphate[b]	2.02 (1.37, 2.98)*	0.0004
Androstenediol (3β,17β) disulphate (1)[b]	1.92 (1.33, 2.77)*	0.0005
Epiandrosterone sulphate[b]	1.96 (1.34, 2.88)*	0.0005
5α-Androstan-3β,17β-diol disulphate[b]	1.92 (1.31, 2.81)*	0.0007
Dehydroisoandrosterone sulphate (DHEA-S)[b]	1.94 (1.26, 2.98)*	0.0028
Carnitine[b]	139.11 (11.03, 1754)*	0.0001
Thromboxane B_2	2.32 (1.44, 3.73)*	0.0005
Butyrylcarnitine (C4)[b]	2.90 (1.63, 5.17)*	0.0003
2-Aminoheptanoate[b]	4.32 (1.68, 11.11)*	0.0024
Glycerol	5.90 (2.11, 16.50)*	0.0007
Stearidonate (18:4 *n*-3)	3.40 (1.53, 7.54)*	0.0026
Cofactor/vitamin		
N^1-methyl-4-pyridone-3-carboxamide[b]	4.37 (1.85, 10.31)*	0.0008
Nucleotide		
Urate[b]	35.05 (4.58, 268.08)*	0.0006
Peptide		
γ-Glutamyltyrosine[b]	8.24 (2.29, 29.62)*	0.0012
Xenobiotic		
Piperine	1.81 (1.32, 2.47)*	0.0002

Cross-sectional models: crude associations between overweight status and metabolite concentrations at the same visit. Only the metabolites significantly associated with being overweight in the cross-sectional models after multiple testing correction are reported in the table

[a] OR for overweight status

[b] Reported in the literature [15, 16] to be associated with overweight status in children

*Significant after correction for multiple testing

for BABYDIAB/BABYDIET, and a significant association between maternal type 1 diabetes and offspring being overweight has been consistently observed in previous studies irrespective of when the children were born [7–10].

Our findings are similar to previous studies on metabolomics and overweight status in children and adolescents without a type 1 diabetes background. Of the 19 metabolite concentrations associated with being overweight in our data, 16 have previously been reported in the literature [15, 16]. For example, our finding that elevated androgenic steroids and BCAA-related metabolite pattern are associated with being overweight and increased insulin resistance is consistent with other studies based on data from children without family history of type 1 diabetes [15, 16]. Studies on the association of exposure to maternal diabetes and changes in the offspring's metabolome are rare. We are aware of only one study which found no significant associations of gestational diabetes and offspring metabolites [16]. Similarly, we found no associations of maternal type 1 diabetes with metabolite concentrations in the offspring. Nevertheless, we were able to identify differences between the metabolomes of overweight and normal-weight children. It may be possible that these differences were observed as an effect, rather than a cause, of being overweight, and hence are not in the causal pathway between maternal type 1 diabetes and excess weight gain in offspring.

The main strength of our study is the prospective design with multiple follow-ups and the availability of a wide range of anthropometric and metabolic outcomes in addition to metabolomics data. As we had data available from two large study populations, we could validate the results for overweight status and BMI. Both cohorts were based on children with a first-degree relative with type 1 diabetes, who were at

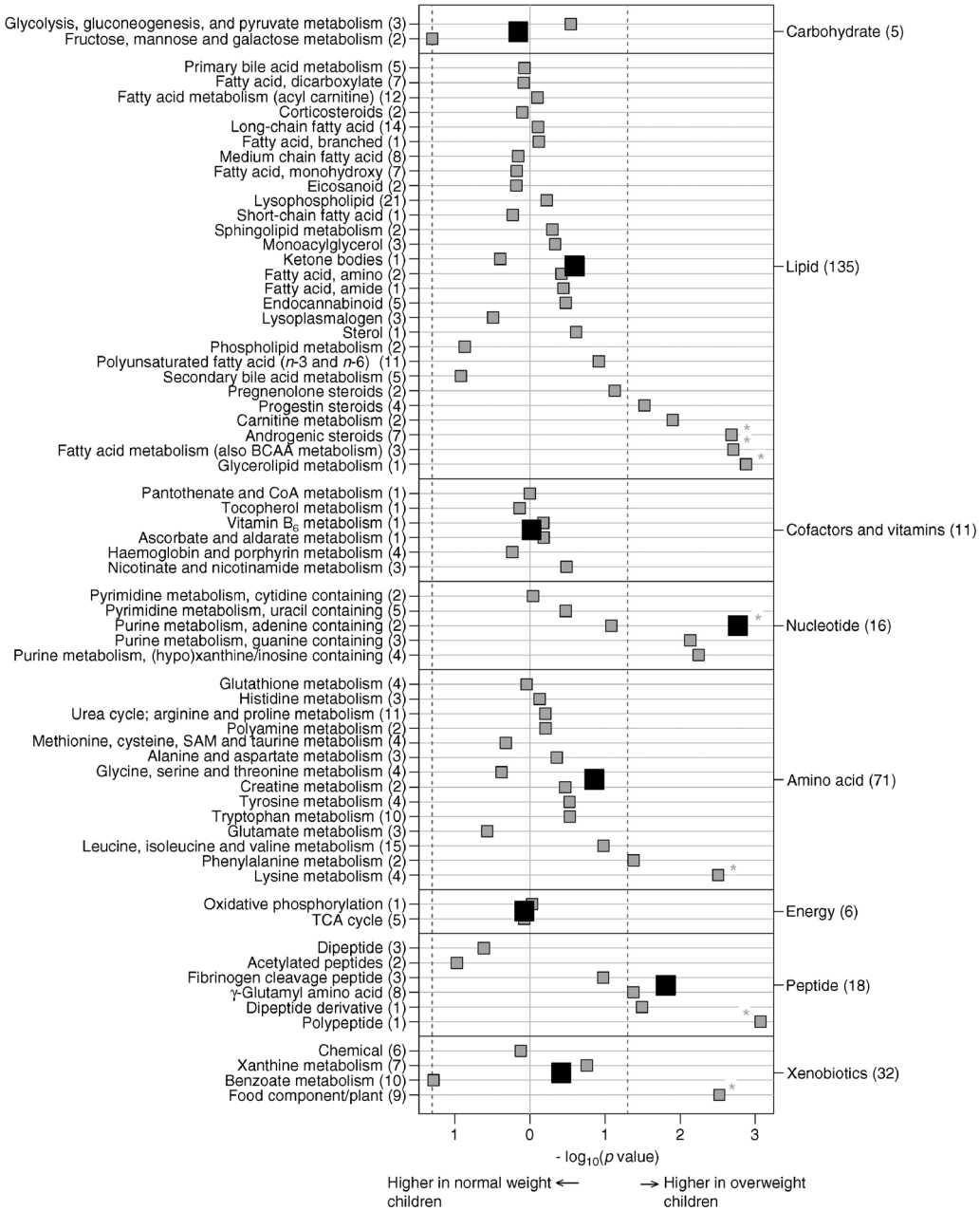

Fig. 2 Association between super- and subpathways of metabolites and overweight status in the offspring. Pathways located to the right of the zero line indicate upregulation, and left of the zero line indicate downregulation, in overweight individuals. Pathways lying beyond the dashed grey line on both sides indicate associations with $p < 0.05$ without adjustment for multiple testing. After multiple testing correction, the subpathways of androgenic steroids, fatty acid metabolism (also BCAA metabolism), glycerolipid metabolism, lysine metabolism, polypeptide and food component/plant were upregulated in overweight individuals. Similarly, the superpathway nucleotide was also found to be upregulated in overweight individuals. *Significant after correction for multiple testing. The numbers in brackets represent the number of metabolites in each super- or subpathway. Black squares, superpathway; grey squares, subpathway. SAM, *S*-adenosyl methionine; TCA, tricarboxylic acid

increased risk of developing type 1 diabetes themselves, but otherwise healthy. Despite adjustment for some important covariates in our analyses, we cannot rule out the possibility of unmeasured confounding in our study. In particular, we had no data on maternal pre-pregnancy BMI, which is known to play a major confounding role with respect to childhood excess weight gain. However, it should not be as relevant when comparing mothers with and without type 1 diabetes as it would be in the context of other diabetes forms. While the mothers of all BABYDIAB/BABYDIET children had been diagnosed with type 1 diabetes before the index pregnancy, we did not have this information available for the TEENDIAB children. Although we therefore cannot rule out that a small number of the TEENDIAB children had not been exposed to type 1 diabetes in utero, we believe that this is not a major concern as the onset of type 1 diabetes occurs most frequently

Table 5 Association between maternal type 1 diabetes and being overweight in the offspring adjusting for different covariates in the metabolomics subset (n = 485)

Model and adjustment	OR for overweight status (95% CI)	p value
Model 1	2.44 (1.33, 4.50)	0.004
Model 2	2.51 (1.23, 5.12)	0.004
Model 2[a]		
Birthweight	2.20 (1.04, 4.66)	0.040
Model 2[b]		
Amino acid		
Kynurenate	2.81 (1.34, 5.89)	0.006
Tyrosine	2.55 (1.23, 5.31)	0.012
Valine	2.76 (1.33, 5.70)	0.006
Alanine	2.51 (1.21, 5.21)	0.013
Lipid		
Androsterone sulphate	2.54 (1.23, 5.24)	0.012
Androstenediol (3β,17β) disulphate (1)	2.47 (1.20, 5.09)	0.014
Epiandrosterone sulphate	2.57 (1.24, 5.32)	0.011
5α-Androstan-3β,17β-diol disulphate	2.37 (1.15, 4.89)	0.020
Dehydroisoandrosterone sulphate (DHEA-S)	2.50 (1.22, 5.14)	0.013
Carnitine	2.52 (1.22, 5.20)	0.013
Thromboxane B_2	2.66 (1.29, 5.49)	0.008
Butyrylcarnitine (C4)	2.72 (1.32, 5.63)	0.007
2-Aminoheptanoate	2.47 (1.20, 5.07)	0.014
Glycerol	2.47 (1.19, 5.12)	0.015
Stearidonate (18:4 *n*-3)	2.58 (1.25, 5.34)	0.011
Cofactor/vitamin		
N^1-Methyl-4-pyridone-3-carboxamide	2.64 (1.27, 5.47)	0.009
Nucleotide		
Urate	2.45 (1.18, 5.08)	0.016
Peptide		
γ-Glutamyltyrosine	2.54 (1.23, 5.25)	0.011
Xenobiotic		
Piperine	2.66 (1.28, 5.51)	0.009
Model 2[c]		
PC3	2.50 (1.21, 5.18)	0.014
PC5	2.87 (1.37, 6.04)	0.005
PC13	2.59 (1.25, 5.37)	0.010

Model 1: crude model; Model 2: adjusted for Tanner's staging, maternal smoking and socioeconomic status

[a] Further adjusted for birthweight

[b] Further adjusted for metabolites significant for being overweight

[c] Further adjusted for principal components significant for being overweight

PC, principal components

at a young age and hence before women get pregnant for the first time. To our knowledge, this is the first study examining the influence of the metabolomics profile on the association between maternal type 1 diabetes and offspring overweight status. With 441 metabolites analysed in 485 children, and a number of metabolites confirming previously reported associations with being overweight, we believe that the missing associations between maternal type 1 diabetes and metabolites in our data are not likely to be false-negative findings.

In summary, offspring of mothers with type 1 diabetes showed increased adiposity, insulin resistance, fasting insulin and C-peptide compared with offspring of non-diabetic mothers. Certain metabolite concentrations were positively associated with being overweight in the offspring. However, metabolic changes seem unlikely to be in the causal pathway between maternal type 1 diabetes and excess weight in offspring, as this association could not be explained by any of the potentially relevant metabolites.

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Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement AP reviewed data, undertook statistical analysis, interpreted results and wrote the first and final draft of the manuscript together with AB. MJ contributed to data management and statistical analysis and reviewed the manuscript. CW, SH, NH, JR and OK acquired data and reviewed the manuscript. JK and GK interpreted results and reviewed the manuscript. A-GZ is the principal investigator of the BABYDIAB/BABYDIET and TEENDIAB studies, designed the studies and concept, interpreted the results and critically reviewed the manuscript for intellectual content. All authors approved the final version of the manuscript. A-GZ is the guarantor of this work.

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Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät und keiner anderen wissenschaftlichen Einrichtung vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Greifswald, den 23.10.2020 _____

Anitha Pitchika

Curriculum Vitae

Work Experience

Since 04/2018	Research associate Institut für Community Medicine, Universitätsmedizin Greifswald
09/2018 – 01/2020	Elternzeit
01/2017 – 03/2018	Research associate Institut für Diabetesforschung Typ 1, Helmholtz Zentrum München
11/2015 – 04/2016	Research assistant Institut für Gesundheitsökonomie und Management in Gesunheitswesen, Helmholtz Zentrum München
11/2015 – 01/2016 10/2016 – 12/2016	Tutor - SAS & biostatistics Institut für Medizinische Informationsverarbeitung, Biometrie und Epidemiologie, Ludwig-Maximilians-Universität München

Education

Since 01/2017	Doctoral student Institut für Community Medicine, Universitätsmedizin Greifswald
10/2014 – 09/2016	M.Sc. Epidemiology Institut für Medizinische Informationsverarbeitung, Biometrie und Epidemiologie, Ludwig-Maximilians-Universität München
10/2007 – 12/2012	Bachelor of Dental Surgery (B.D.S.) Ragas Dental College & Hospital, The Tamilnadu Dr. M.G.R. Medical University, India

Awards

03/2018	Best poster for "Associations of maternal type 1 diabetes with childhood adiposity and metabolic health in the offspring" at the International Society for Environmental Epidemiology conference
10/2016	"Münchner Epidemiologie-Preis" for the best master thesis
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List of own scientific publications

Pitchika A, Kühn JP, Schipf S, Nauck M, Dörr M, Lerch MM, Kromrey ML, Felix SB, Markus MRP, Rathmann W, Völzke H, Ittermann T. Hepatic steatosis and hepatic iron overload modify the association of iron markers with glucose metabolism disorders and metabolic syndrome. Liver Int. 2021 Aug;41(8):1841-1852. doi: 10.1111/liv.14868.

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List of presentations

Pitchika A, Jolink M, Winkler C, Hummel S, Hummel N, Krumsiek J, Kastenmüller G, Raab J, Kordonouri O, Ziegler AG, Beyerlein A. Associations of maternal type 1 diabetes with childhood adiposity and metabolic health in the offspring: a prospective cohort study. Poster presentation at the International Society for Environmental Epidemiology conference (ISEE Young), Freising 2018

Pitchika A, Jolink M, Winkler C, Hummel S, Hummel N, Krumsiek J, Kastenmüller G, Raab J, Kordonouri O, Ziegler AG, Beyerlein A. Maternal diabetes effects on childhood growth and metabolism. Paper presentation at the 11th Obtoberfest Symposium on Childhood Diabetes, Neuherberg 2017

Pitchika A, Hampel R, Wolf K, Kraus U, Cyrys J, Babisch W, Peters A, Schneider A. Long-term associations of modeled and self-reported measures of exposure to air pollution and noise at residence on prevalent hypertension and blood pressure. Poster presentation at the Health – Exploring Complexity conference, Munich 2016

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