**Associations of maternal type 1 diabetes with childhood adiposity and metabolic health in the offspring**

Anitha Pitchikaa,b, Manja Jolinka,b, Christiane Winklera,b, Jan Krumsiekc,d, Gabi Kastenmüllere, Jennifer Raaba,b, Olga Kordonourif, Anette-Gabriele Zieglera,b,g\*, Andreas Beyerleina,b\*

a Institute of Diabetes Research, Helmholtz Zentrum München, Neuherberg, Germany

b Forschergruppe Diabetes, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany

c Institute of Computational Biology, Helmholtz Zentrum München, Neuherberg, Germany

d German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany

e Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany

f Kinder- und Jugendkrankenhaus auf der Bult, Hannover Germany

g Forschergruppe Diabetes e.V., Neuherberg, Germany

\* shared last authorship

**Corresponding author**

Anette-Gabriele Ziegler

Helmholtz Zentrum München - German Research Center for Environmental Health

Institute of Diabetes Research

Ingolstädter Landstraße 1

85764 Neuherberg, Germany

Email: anette-g.ziegler@helmholtz-muenchen.de

Word count: 4,252

No of tables: 5

No of figures: 2

No of supplementary tables: 2

No of supplementary figures: 3**Abstract:**

**Background:** Exposure to intrauterine hyperglycemic environment has been suggested to increase the offspring’s later overweight and metabolic risk, but conclusive evidence for pregnancies affected by maternal type 1 diabetes (T1D) is still lacking. Further, it is unknown whether changes in the offspring’s metabolome are in the potential pathway.

**Methods:** We analyzed data from 2,169 and 610 offspring having a first-degree relative with T1D from the TEENDIAB and BABYDIAB/BABYDIET cohorts, respectively. Associations of maternal T1D with anthropometric and metabolic outcomes in the offspring, assessed longitudinally at 0.3-18 years of age, were investigated using mixed regression models. Non-targeted metabolomics measurements were carried out in 500 fasting serum samples from TEENDIAB and associated with maternal T1D and offspring overweight.

**Results:** Offspring of T1D mothers had a higher body mass index standard deviation score (SDS) and an increased risk for overweight than offspring of non-diabetic mothers (e.g. odds ratio for overweight in TEENDIAB: 2.40 (95% confidence interval: 1.41; 4.06)). Further, waist circumference SDS, fasting levels of insulin and C-peptide, as well as insulin resistance and abdominal obesity were significantly increased in offspring of T1D mothers, even when adjusted for potential confounders and birth weight. Metabolite patterns related to androgenic steroids and branched-chain amino acids were found to be associated with offspring’s overweight, but no significant associations were observed between maternal T1D and metabolite concentrations in the offspring.

**Conclusion:** Maternal T1D is associated with offspring’s overweight and metabolic health in later life, but this is not likely due to alterations in the offspring’s metabolome.

**Introduction:**

Obesity and overweight in children and adolescents remain a major public health problem due to its increasing prevalence[1](#_ENREF_1). It is also linked to other metabolic complications such as impaired glucose metabolism, insulin resistance, type 2 diabetes and cardiovascular disease[2](#_ENREF_2). It has been suggested that these metabolic conditions have their origins early during their fetal life. A growing body of evidence supports the concept of fuel-mediated teratogenesis, according to which intrauterine hyperglycemic exposure may lead to excess fetal glucose and insulin, and thus overgrowth of the fetus.[3](#_ENREF_3) These exposures during fetal life have been reported to extend beyond the neonatal period and influence metabolic complications in later life.

A number of studies have shown evidence associating gestational diabetes and type 2 diabetes with later adiposity, increased body mass index (BMI), insulin resistance, impaired glucose tolerance, higher cholesterol, hypertension and type 2 diabetes in the offspring[4-7](#_ENREF_4), but much less evidence to support the effects of maternal type 1 diabetes (T1D) on offspring health. However, it appears relevant to differentiate between T1D, gestational diabetes and type 2 diabetes, because the latter two are associated with maternal obesity, while T1D is rather not. Studies which reported a positive association of maternal T1D with BMI or metabolic outcomes in the offspring [8-11](#_ENREF_8) were cross-sectional in design and limited with respect to their sample size (n<600 each). Furthermore, two of these studies were based on children born as early as 1978-1985[8](#_ENREF_8) and 1982-1991[11](#_ENREF_11), respectively, when diabetes care in pregnant women was likely worse than nowadays[12](#_ENREF_12). Previous analyses of our own data indicated that children from T1D and non-diabetic mothers follow different growth patterns[13](#_ENREF_13),[14](#_ENREF_14), but also that a potential association between maternal T1D and overweight risk in the offspring was not independent of birth weight and breastfeeding duration[15](#_ENREF_15).

Here, we analyzed data from two prospective cohort studies containing over 2,770 children of which more than 1,500 were exposed to maternal T1D during pregnancy. These data cover longitudinal measurements of various anthropometric and metabolic variables in the offspring from shortly after birth up to age 18 years. A subset of 500 children was also characterized for non-targeted metabolomics, which are of particular interest, because recent studies showed significant associations between metabolic concentrations and childhood obesity[16-18](#_ENREF_16), while the associations between maternal T1D and metabolic profile in the offspring have not been investigated yet. The aims of this study were to investigate 1) whether there are differences in anthropometric and metabolic outcomes between offspring of T1D and non-diabetic mothers and 2) whether birth weight and/or changes in the offspring’s metabolome may be in the potential pathway.

**Methods:**

**1. Study population and clinical measurements:**

Our analysis was based on the prospective German cohorts TEENDIAB and BABYDIAB/BABYDIET. These cohorts include children with a familial background of T1D and have already been combined for other research questions[19](#_ENREF_19),[20](#_ENREF_20).

*1.1 TEENDIAB Study*

The TEENDIAB study is a prospective cohort study, conducted in the cities of Munich and Hannover, Germany. This study recruited 610 children aged 6-16 years, who were resident in Germany, and had at least one parent or sibling with T1D[21](#_ENREF_21). Recruitment began in 2009 and ended in 2015. Children were followed on an average of every six months from six to 18 years of age until 2016. All parents gave written informed consent for participation. The study was approved by the ethical committees of the Technische Universität München (No. 2149/08) and of the Hannover Medical School (No. 5644).

*1.1.1 Maternal characteristics and offspring measurements*

At the first visit, information on sociodemographic status including T1D and smoking status of the mother was obtained via self-administered questionnaires. Information on birth weight was taken from health records collected during the well-baby preventive health program which is routinely offered to all children in Germany. During each visit, anthropometric measurements and Tanner’s staging which indicates pubertal development were assessed and venous blood samples were collected by the study doctors or local pediatricians using standardized protocols. Weight was measured digitally or using a beam scale with a precision of ± 100g in light clothing and without shoes. Height was measured using a stadiometer with a precision of ± 1 mm and without shoes. 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 using a caliper at precisely defined measuring points. Subscapular skinfold was measured three times at the inferior angle of the right scapula. Triceps skinfold was measured three times at the posterior right upper arm located via an imaginary horizontal line extending from the inferior scapula angle. Skinfold thickness was calculated as the average readings of the three measurements. Tanner’s staging was assessed by the study doctor or local pediatricians using validated questionnaires.

Venous blood samples were collected for the determination of fasting blood glucose, insulin and C-peptide. All participants were asked to fast for at least 10 hours before blood collection. Fasting plasma glucoses were determined by the hospital laboratories of the two study sites. Insulin and C-peptide were determined using an automated immunoassay analyzer (AIA 360; Tosoh, San Francisco, CA). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR)[22](#_ENREF_22). Blood pressure was measured two times in the upper arm in sitting position after 3-5 mins of rest by means of auscultatory or oscillometric method following withdrawal of blood. Systolic blood pressure and diastolic blood pressure were calculated as the average readings of the first and second measurements. Lipids were measured by…..

*1.1.2 Metabolomic profiling*

Non-targeted metabolomic profiling was performed on 500 fasting serum samples collected at the first TEENDIAB visit using ultrahigh-performance liquid chromatography and mass spectrometry on the Metabolon platform (Metabolon, Inc., Durham, NC). All samples were stored at -80º C prior to analysis. Metabolites were identified following the Metabolomics Standardization Initiatives guidelines[23](#_ENREF_23). Quantification of metabolites was performed as outlined previously[24](#_ENREF_24). 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 normalized to account for run-day differences and log-transformed to bring them closer to a normal distribution. Missing data was then imputed using random forest imputation.

*1.2 BABYDIAB/BABYDIET studies*

The BABYDIAB and BABYDIET studies are two ongoing prospective German birth cohorts including 2,441 children born between 1989 and 2006 with a first degree relative with T1D. Between 1989 and 2000, a total of 1650 offspring of patients with T1D were recruited for the BABYDIAB study. Between 2000 and 2006, 791 additional offspring or siblings of patients with T1D were screened in the context of the BABYDIET study. Of those, 150 participated in the BABYDIET dietary intervention study; the intervention had no effect on islet autoimmunity development or on growth parameters[25](#_ENREF_25),[26](#_ENREF_26). Further details on the study design are described in detail elsewhere[25](#_ENREF_25),[27](#_ENREF_27),[28](#_ENREF_28). Data from these two cohorts were combined for longitudinal analyses of maternal T1D and anthropometric outcomes in the offspring. All parents gave written informed consent for participation. The studies were approved by the ethical committee of Bavaria, Germany (Bayerische Landesärztekammer No. 95357 and Ludwig-Maximilians University No. 329/00 respectively).

*1.2.1 Maternal characteristics and offspring measurements*

Information on T1D and smoking status of the mother during pregnancy was obtained via self-administered questionnaires. Height and weight measurements of the offspring were obtained from health records from the well-baby preventive health program 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. Measurements during the study visits 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 pediatrician or trained staff using validated questionnaires at every study visit.

*1.2.2 Exclusions*

We excluded the data of all BABYDIAB/BABYDIET participants from our analysis who had no height and weight data (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=2,169. We further excluded all visits performed before 0.3 years of age because these measurements were likely to be highly correlated with birth weight, which we wanted to investigate separately.

**2. Statistical analysis**

BMI was calculated as weight (kg) / height (m)². Prior to analysis, height, weight, BMI, waist circumference, subscapular and triceps skinfold thickness and lipids were transformed into age- and sex-specific standard deviation scores (SDS), and blood pressure into age-, sex- and height-specific SDS according to German reference values[29-31](#_ENREF_29). Overweight was defined as a BMI at or above an SDS of 1.31, corresponding with the 90th percentile. Waist circumference SDS was calculated only in children above 10 years of age, since the respective reference percentiles were available only between 11 and 18 years. Abdominal obesity was defined as a waist circumference at or above the 90th percentile or the adult threshold of the International Diabetes Federation[32](#_ENREF_32). Birth weight was transformed into age- and sex-specific percentiles based on German reference values[33](#_ENREF_33), and categorized as small for gestational age (birth weight <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.

*2.1 Maternal type 1 diabetes and metabolic outcomes in the offspring*

Differences in anthropometric and metabolic outcomes including overweight and overall metabolic risk between offspring of T1D and non-diabetic mothers were examined separately in TEENDIAB and BABYDIAB/BABYDIET because the studies differed in the number of outcomes assessed and the timing of the respective measurements. Firstly, BMI, weight and height were visually compared in yearly time intervals between offspring of T1D and non-diabetic mothers. Secondly, linear and logistic mixed effect models accounting for repeated observations within subjects were performed. Fasting glucose, insulin and C-peptide as well as HOMA-IR were log-transformed due to non-normal residuals in the respective linear models. Associations were analyzed based on stepwise adjustment. In the first model, we performed univariate analysis for all outcomes. In consistence with other studies[9](#_ENREF_9), 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 maternal smoking which is known to be a proxy for maternal education and a potential risk factor for childhood overweight[34](#_ENREF_34). In order to investigate whether birth weight was in the causal pathway between maternal T1D and overweight and metabolic risk in the offspring, birth weight was added as a categorical variable in the third model.

*2.2 Analyses of metabolomic profiles*

We further explored the extent to which the offspring’s metabolomics profile may play a mediating role in the association between maternal T1D and overweight. We tested our hypothesis in three steps based on the subset from the TEENDIAB study with available metabolomics data from the first study visit. First, we examined associations between every single metabolite concentration and overweight in the offspring both cross-sectionally (i.e. with overweight at the first visit) and prospectively (i.e. with overweight at the last visit) using logistic regression models. In the prospective models, the data of 27 children who had only one visit were left out, and adjustment was done for the time difference between first and last visit. The Benjamini-Hochberg procedure was used to control the false discovery rate 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, and the associations between these 15 principal components and overweight in the offspring were analyzed. In our second step, we investigated whether maternal T1D was associated with principal components or metabolites that were significant for overweight, adjusted for age and sex. In our third step, associations between maternal T1D and overweight in the offspring were assessed after adjusting for metabolites or principal components which were significantly associated with overweight. In addition, metabolite concentrations were categorized into 68 sub- and 8 super-pathways[24](#_ENREF_24). For each super- and sub-pathway, the mean of the metabolites belonging to that particular pathway was calculated for all samples and associated with offspring overweight and maternal T1D.

Results were reported as absolute change with 95% confidence interval (CI) for SDS outcomes, percent change with 95% CI for log-transformed outcomes and as odds ratios (ORs) with 95% CI for overweight and metabolic risk between offspring of T1D and non-diabetic mothers. All analyses were carried out using SAS 9.4 (SAS Institute Inc, Cary, NC) and R 3.4.1 (http://cran.r-project.org).

**Results:**

The study participants had a median follow-up of 3.0 and 10.7 years resulting in 3,583 and 13,235 observations in the TEENDIAB and BABYDIAB/BABYDIET cohort, respectively. In total, 257 (42%) and 1,287 (59%) children had T1D mothers, respectively (Table 1). None of the other children was known to have been exposed to another form of diabetes during pregnancy.

1. *Maternal type 1 diabetes and metabolic outcomes in the offspring*

Mean BMI SDS was constantly higher in offspring of T1D mothers from ages 7-15 years in TEENDIAB (Fig. 1). Mean weight SDS was also higher, and mean height SDS lower, in most of the age groups in offspring of T1D mothers in TEENDIAB. In BABYDIAB/BABYDIET, these 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 T1D mothers had significantly higher BMI SDS (TEENDIAB: +0.35 (95% CI: +0.19; +0.52), Table 2; BABYDIAB/BABYDIET: +0.13 (95% CI: +0.06; +0.20), Table 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 to offspring of non-diabetic mothers. These associations did not change considerably when adjusted for Tanner’s staging and maternal smoking. However, after further adjustment for birth weight, the observed associations attenuated in TEENDIAB and were not significant any more in BABYDIAB/BABYDIET, while the negative associations for height SDS became stronger and significant in both cohorts. In TEENDIAB, other anthropometric outcomes such as weight SDS, waist circumference SDS, and subscapular and triceps skinfold thickness SDS were significantly higher in offspring of T1D mothers than of non-diabetic mothers, but only the estimates for waist circumference SDS remained significant when adjusted for Tanner’s staging, maternal smoking and birth weight. Offspring of T1D mothers showed significantly increased abdominal obesity risk (OR: 1.92 (95% CI: 1.15; 3.20)) and metabolic risk (composite endpoint, OR: 1.45 (95% CI: 1.10; 1.92)), and also after adjustment for Tanner’s staging, maternal smoking and birth weight. Further, offspring of T1D mothers showed increased levels of fasting insulin (8.32 (95% CI: 0.68; 16.55) %), fasting C-peptide (6.01 (95% CI: -0.23; 12.64) %) and HOMA-IR (8.36 (95% CI: 0.38; 16.99) %), which did not change after adjustment for age, sex, Tanner’s staging, maternal smoking and birth weight. Although the effect estimate for C-peptide was almost similar in unadjusted and adjusted analyses, significant associations were observed only after adjustment (5.85 (95% CI: 0.18; 11.84) %). Systolic blood pressure SDS was slightly higher in children from T1D mothers in unadjusted analyses (+0.16 (95% CI: +0.01, +0.31)), but not after adjustment, while no significant differences in diastolic blood pressure, lipids or fasting glucose were observed between offspring of T1D and non-diabetic mothers in unadjusted or adjusted models.

*2. Analyses of metabolomic profiles*

In the metabolomics subset used for analysis, 247/485 (51%) children were male. Their first TEENDIAB visit, when the metabolomics blood sample was taken, happened at a mean age of 10.2 years, and 48 subjects (10%) were overweight at that time. Their last TEENDIAB visit took place at a mean age of 13.4 years, when 55 subjects (12%) were overweight.

Out of the 441 analyzed metabolites, 28 showed significant associations with overweight in the cross-sectional models after multiple testing correction, and 19 of these were of known identity (Table 4). All these metabolites were up-regulated in overweight individuals, including four metabolites from the amino acid class (valine, kynurenate, tyrosine and alanine), eleven from the lipid class (androgenic steroids such as androsterone sulfate, epiandrosterone sulfate etc, carnitine and the short chain acyl-carnitine (butyryl carnitine (C4)), glycerol, thromboxane B2, stearidonate and 2-aminoheptanoate), and four metabolites from other classes (N1-methyl-4-pyridone-3-carboxamide, urate, gamma-glutamyltyrosine and piperine). In the models predicting overweight status at the last visit, only valine, kynurenate and thromboxane B2 showed significant associations. At the pathway level analysis, sub-pathways such as androgenic steroids, branched chain amino acid metabolism, glycerolipid metabolism, lysine metabolism, polypeptide and food component/plant were up-regulated in overweight individuals, as was the super-pathway nucleotide (Fig. 2). Similarly, three principal components, characterized by androgenic steroids, branched chain amino acids (BCAA) and related metabolites or composed of amino acid, lipid and acetylated peptides, were associated with overweight status (Suppl Fig. S1 and Table S1). The principal components related to androgenic steroids and BCAAs were also positively associated with HOMA-IR (p=1.7\*10-5 and p=0.002 respectively), fasting insulin (p=1.7\*10-5 and p=0.005) and fasting C-peptide (p=0.002 and p=5.8\*10-6).

In contrast, there was no significant association of any metabolite with maternal T1D when corrected for multiple testing, and there was even no significant association on the 5% level for any of the metabolites which had been found to be associated with overweight (supplementary table S2). No significant associations were observed between maternal T1D and any of the principal components (Suppl Fig. S2) or super- and sub-pathways (Suppl Fig. S3) after correcting for multiple testing.

Further, the associations between maternal T1D and overweight in the offspring remained significant and were not even considerably attenuated after adjustment for any potentially relevant single metabolite concentrations or principal components (Table 5), indicating that none of them is in the causal pathway.

**Discussion:**

Our findings suggest that offspring of T1D mothers show an increased risk for overweight and higher BMI as well as increased insulin resistance, fasting insulin and c-peptide levels compared to offspring of non-diabetic mothers in two independent prospective cohorts of children from T1D-affected families. The effect estimates for anthropometric outcomes were slightly attenuated in TEENDIAB and significantly in BABYDIAB/BABYDIET when adjusting for birth weight in the model. This shows that association between maternal T1D and later overweight in offspring could be at least partially explained by increased birth weight in the causal pathway. However, while we observed that patterns related to androgenic steroids and BCAAs were associated with overweight, no such associations were observed with respect to maternal T1D. Furthermore, maternal T1D was positively associated with offspring overweight, independent of metabolite concentrations. This implies that possible alterations in serum metabolite concentrations assessed within our study may not explain the association between maternal T1D and overweight in the offspring.

Previous studies which examined offspring of T1D mothers were generally in concordance with our results. Vlachova et al[9](#_ENREF_9) reported increased weight, BMI and risk for prediabetes and metabolic syndrome components in adolescent offspring of T1D mothers. Weiss et al[11](#_ENREF_11) reported an increased BMI, insulin resistance, fasting glucose, insulin and C-peptide in 5-15 year old offspring of T1D mothers compared to control subjects. Lindsay et al[10](#_ENREF_10) reported increased weight, BMI, waist circumference and skinfold thickness in 7 year-old offspring of T1D mothers, but no differences in relation to glucose tolerance. Clausen et al[8](#_ENREF_8) reported a higher risk for overweight and metabolic syndrome in adult offspring of gestational diabetic and T1D mothers compared to offspring of normal reference population. In contrast, Rijpert et al[35](#_ENREF_35) found a similar prevalence of overweight in 6-8 year old offspring of T1D mothers under adequate glycemic control compared to a reference population.

In previous analyses of the BABYDIAB data (without BABYDIET and with much shorter follow-up than here), Hummel et al[15](#_ENREF_15) reported that maternal T1D may not be an independent predictor of overweight during childhood but associated factors like breastfeeding or birth weight may predispose individuals to overweight risk. Indeed, the associations between maternal T1D and offspring overweight attenuated by 62% after adjustment for birth weight in the BABYDIAB/BABYDIET study, but only by 10% in the TEENDIAB study. Moreover, the effect estimates were generally weaker in BABYDIAB/BABYDIET compared to TEENDIAB. We assume that these differences come from the different age structures of both studies. The BABYDIAB/BABYDIET cohort followed predominantly younger age groups starting 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 for 30% of the participants after 6 years of age in BABYDIAB/BABYDIET study. We therefore consider it plausible that birth weight is of more relevance to offspring’s BMI in the BABYDIAB/BABYDIET than in the TEENDIAB data. Besides, it has been suggested that maternal diabetes may have a delayed influence on offspring’s adiposity that increases with age. Silverman et al[36](#_ENREF_36) observed an increased weight in offspring of diabetic mothers at birth and progressively after the ages of 4 years but not between 1-3 years of age. Similarly, Baptiste-Roberts et al[37](#_ENREF_37) reported a significantly increased BMI at age 7 years in offspring of gestational diabetic mothers, while no differences at ages 3 and 4 years. We consider it less likely that the differences observed between our two cohorts are due to different environmental conditions around the time of birth, as the median birth year in TEENDIAB was 2001 compared to 1997 in the BABYDIAB/BABYDIET data, and a significant association between maternal T1D and offspring overweight had been consistently observed in previous studies irrespectively of when the children had been born[8-11](#_ENREF_8).

We were able to replicate results from previous studies on metabolomics and overweight status in children and adolescents. Of the 19 metabolite concentrations associated with overweight in our data, 16 had previously been reported in the literature[22](#_ENREF_22),[23](#_ENREF_23). For example, our finding that elevated androgenic steroids are associated with overweight as well as increased insulin resistance, fasting insulin and C-peptide levels is consistent with other studies[22](#_ENREF_22),[23](#_ENREF_23). Androgenic steroids have been reported to induce premature adrenarche, which may be involved in worsening metabolic profile leading to higher insulin resistance, higher fasting insulin, dyslipidemia and lower adiponectin[38](#_ENREF_38),[39](#_ENREF_39). Further, we could also confirm a BCAA related metabolite patterns to be associated with overweight risk and increased insulin resistance[17](#_ENREF_17). Possible mechanisms include incomplete BCAA oxidation, increased levels of certain gut bacteria in overweight individuals which may promote BCAA synthesis or alterations in skeletal muscle degradation, all of which may contribute to increased BCAA release into circulation[17](#_ENREF_17).

Studies on the association of exposure to maternal diabetes in utero 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[17](#_ENREF_17). Similarly, we did not find any associations of maternal T1D with metabolite concentrations in the offspring. Nevertheless, we were able to identify differences between the metabolomes of overweight and normal-weight children, most of which were only observed in cross-sectional, but not prospective analyses, however. It may therefore be possible that these differences were observed as an effect, rather than a cause of overweight, and hence are not in the causal pathway of the association between maternal T1D and offspring overweight.

The main strength of our study is the prospective study design with multiple follow-ups for the offspring up to age 18 years and 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 were able to validate the results for overweight and BMI. Both cohorts were based on children with a first degree relative with T1D, who were at increased risk to develop T1D 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. Especially, we had no data on maternal pre-pregnancy BMI, which is known to play a major confounding role, although it should not be so relevant when comparing mothers with and without T1D as it would be in the context of other diabetes forms. Maternal smoking during pregnancy was not assessed in the TEENDIAB study, but available in the BABYDIAB/BABYDIET study. However, general smoking status of the mother was taken as a substitute for adjustment in the TEENDIAB analyses. To our knowledge this is the first study examining the influence of metabolomics profile on the association between maternal T1D and offspring overweight. A systematic untargeted metabolomic profiling was performed which identified a large number of metabolites. With 441 metabolites analyzed in 485 children, and a number of metabolites confirming previously reported associations with overweight, we believe that our study had adequate statistical power and that therefore the missing associations between maternal T1D and metabolites in our data are not likely to be false negative findings.

In summary, offspring of T1D mothers showed increased adiposity, insulin resistance, fasting insulin and C-peptide compared to offspring of non-diabetic mothers. Certain metabolite concentrations were positively associated with overweight in the offspring. However, metabolic changes seem unlikely to be in the causal pathway between maternal T1D and offspring overweight, as this association could not be explained by any of the potentially relevant metabolites.

**Funding:**

The work was supported by grants from the Kompetenznetz Diabetes mellitus (Competence Network for Diabetes mellitus) funded by the Federal Ministry of Education and Research (FKZ 01GI0805-07), Juvenile Diabetes Research Fund (JDRF-No 17-2012-16) and the European Union’s HORIZON 2020 research and innovation program (grant agreement no. 633595 DynaHEALTH).

**Author contributions**

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, 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.

**Acknowledgements**

We thank Lorenz Lachmann, Claudia Matzke, Joanna Stock, Stephanie Krause, Annette Knopff, Florian Haupt, Maren Pflüger, Marlon Scholz, Anita Gavrisan, Simone Schneider, Kerstin Remus, Sarah Bläsig, Evelin Sadeghian and Anika Bokelmann for data collection and expert technical assistance. We also thank all families participating in the BABYDIAB/BABYDIET and TEENDIAB studies and also all pediatricians, diabetologists and family doctors in Germany for recruitment and continuous support. This work is part of Anitha Pitchika’s PhD thesis at the medical department of the University of Munich (in preparation).

References:

1. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet.*384(9945):766-781.

2. Weiss R, Kaufman FR. Metabolic Complications of Childhood Obesity. *Diabetes Care.* 2008;31(Supplement 2):S310.

3. Freinkel N. Banting Lecture 1980: of Pregnancy and Progeny. *Diabetes.* 1980;29(12):1023.

4. Boerschmann H, Pfluger M, Henneberger L, Ziegler AG, Hummel S. Prevalence and predictors of overweight and insulin resistance in offspring of mothers with gestational diabetes mellitus. *Diabetes Care.* Aug 2010;33(8):1845-1849.

5. Buinauskiene J, Baliutaviciene D, Zalinkevicius R. Glucose tolerance of 2- to 5-yr-old offspring of diabetic mothers. *Pediatric diabetes.* Sep 2004;5(3):143-146.

6. Silverman BL, Metzger BE, Cho NH, Loeb CA. Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism. *Diabetes Care.* May 1995;18(5):611-617.

7. Manderson JG, Mullan B, Patterson CC, Hadden DR, Traub AI, McCance DR. Cardiovascular and metabolic abnormalities in the offspring of diabetic pregnancy. *Diabetologia.* Jul 2002;45(7):991-996.

8. Clausen TD, Mathiesen ER, Hansen T, et al. Overweight and the metabolic syndrome in adult offspring of women with diet-treated gestational diabetes mellitus or type 1 diabetes. *The Journal of clinical endocrinology and metabolism.* Jul 2009;94(7):2464-2470.

9. Vlachova Z, Bytoft B, Knorr S, et al. Increased metabolic risk in adolescent offspring of mothers with type 1 diabetes: the EPICOM study. *Diabetologia.* Jul 2015;58(7):1454-1463.

10. Lindsay RS, Nelson SM, Walker JD, et al. Programming of Adiposity in Offspring of Mothers With Type 1 Diabetes at Age 7 Years. *Diabetes Care.* 2010;33(5):1080-1085.

11. Weiss PA, Scholz HS, Haas J, Tamussino KF, Seissler J, Borkenstein MH. Long-term follow-up of infants of mothers with type 1 diabetes: evidence for hereditary and nonhereditary transmission of diabetes and precursors. *Diabetes Care.* Jul 2000;23(7):905-911.

12. Beyerlein A, Von Kries R, Hummel M, et al. Improvement in pregnancy-related outcomes in the offspring of diabetic mothers in Bavaria, Germany, during 1987–2007. *Diabetic Medicine.* 2010;27(12):1379-1384.

13. Beyerlein A, Thiering E, Pflueger M, et al. Early infant growth is associated with the risk of islet autoimmunity in genetically susceptible children. *Pediatric diabetes.* Nov 2014;15(7):534-542.

14. Yassouridis C, Leisch F, Winkler C, Ziegler AG, Beyerlein A. Associations of growth patterns and islet autoimmunity in children with increased risk for type 1 diabetes: a functional analysis approach. *Pediatric diabetes.* Mar 2017;18(2):103-110.

15. Hummel S, Pfluger M, Kreichauf S, Hummel M, Ziegler AG. Predictors of overweight during childhood in offspring of parents with type 1 diabetes. *Diabetes Care.* May 2009;32(5):921-925.

16. Butte NF, Liu Y, Zakeri IF, et al. Global metabolomic profiling targeting childhood obesity in the Hispanic population. *The American journal of clinical nutrition.* Aug 2015;102(2):256-267.

17. Perng W, Gillman MW, Fleisch AF, et al. Metabolomic profiles and childhood obesity. *Obesity (Silver Spring, Md.).* Dec 2014;22(12):2570-2578.

18. Wahl S, Yu Z, Kleber M, et al. Childhood obesity is associated with changes in the serum metabolite profile. *Obesity facts.* 2012;5(5):660-670.

19. Raab J, Giannopoulou EZ, Schneider S, et al. Prevalence of vitamin D deficiency in pre-type 1 diabetes and its association with disease progression. *Diabetologia.* May 2014;57(5):902-908.

20. Raab J, Haupt F, Kordonouri O, et al. Continuous rise of insulin resistance before and after the onset of puberty in children at increased risk for type 1 diabetes - a cross-sectional analysis. *Diabetes Metab Res Rev.* 2013/11// 2013;29(8):631-635.

21. Ziegler AG, Meier-Stiegen F, Winkler C, Bonifacio E, Teendiab Study Group. Prospective evaluation of risk factors for the development of islet autoimmunity and type 1 diabetes during puberty--TEENDIAB: study design. *Pediatric diabetes.* Aug 2012;13(5):419-424.

22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* Jul 1985;28(7):412-419.

23. The Metabolomics Standards Initiative. *Nat Biotech.* 08//print 2007;25(8):846-848.

24. Krumsiek J, Mittelstrass K, Do KT, et al. Gender-specific pathway differences in the human serum metabolome. *Metabolomics.* 2015;11(6):1815-1833.

25. Hummel S, Pflüger M, Hummel M, Bonifacio E, Ziegler A-G. Primary Dietary Intervention Study to Reduce the Risk of Islet Autoimmunity in Children at Increased Risk for Type 1 Diabetes. *Diabetes Care.* 2011;34(6):1301.

26. Beyerlein A, Chmiel R, Hummel S, Winkler C, Bonifacio E, Ziegler AG. Timing of gluten introduction and islet autoimmunity in young children: updated results from the BABYDIET study. *Diabetes Care.* Sep 2014;37(9):e194-195.

27. Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes.* 1999;48(3):460.

28. Hummel M, Bonifacio E, Schmid S, Walter M, Knopff A, Ziegler A. Brief communication: Early appearance of islet autoantibodies predicts childhood type 1 diabetes in offspring of diabetic parents. *Annals of Internal Medicine.* 2004;140(11):882-886.

29. Kromeyer-Hauschild K, Wabitsch M, Kunze D, et al. Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschrift Kinderheilkunde.* 2001;149(8):807-818.

30. Robert-Koch-Institut. Referenzperzentile für anthropometrische Maßzahlen und Blutdruck aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS) 2003-2006. Beiträge zur Gesundheitsberichterstattung des Bundes Berlin: Robert Koch-Institut. 2011.

31. Dathan-Stumpf A, Vogel M, Hiemisch A, et al. Pediatric reference data of serum lipids and prevalence of dyslipidemia: Results from a population-based cohort in Germany. *Clinical biochemistry.* Jul 2016;49(10-11):740-749.

32. Alberti KGMM, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic Medicine.* 2006;23(5):469-480.

33. Voigt M, Schneider KT, Jahrig K. [Analysis of a 1992 birth sample in Germany. 1: New percentile values of the body weight of newborn infants]. *Geburtshilfe und Frauenheilkunde.* Oct 1996;56(10):550-558.

34. Beyerlein A, Ruckinger S, Toschke AM, Schaffrath Rosario A, von Kries R. Is low birth weight in the causal pathway of the association between maternal smoking in pregnancy and higher BMI in the offspring? *European journal of epidemiology.* May 2011;26(5):413-420.

35. Rijpert M, Evers IM, de Vroede MA, de Valk HW, Heijnen CJ, Visser GH. Risk factors for childhood overweight in offspring of type 1 diabetic women with adequate glycemic control during pregnancy: Nationwide follow-up study in the Netherlands. *Diabetes Care.* Nov 2009;32(11):2099-2104.

36. Silverman BL, Rizzo T, Green OC, et al. Long-term prospective evaluation of offspring of diabetic mothers. *Diabetes.* Dec 1991;40 Suppl 2:121-125.

37. Baptiste-Roberts K, Nicholson WK, Wang N-Y, Brancati FL. Gestational Diabetes and Subsequent Growth Patterns of Offspring: The National Collaborative Perinatal Project. *Maternal and child health journal.* 2012;16(1):125-132.

38. Idkowiak J, Lavery GG, Dhir V, et al. Premature adrenarche: novel lessons from early onset androgen excess. *European Journal of Endocrinology.* 2011;165(2):189-207.

39. Utriainen P, Laakso S, Liimatta J, Jääskeläinen J, Voutilainen R. Premature Adrenarche - A Common Condition with Variable Presentation. *Hormone Research in Paediatrics.* 2015;83(4):221-231.

**Table 1: Characteristics of study participants in the TEENDIAB and BABYDIAB/BABYDIET cohort**

| **Variable** |  | | | **TEENDIAB**  **(N= 610)** | |  | **BABYDIAB/BABYDIET (N= 2169)** | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | **N Obs** | **N (%)/ Mean±SD** |  | **N Obs** | **N (%)/ Mean±SD** |
| **Time constant variables** | | | | | | | | |
| **Sex** | | Males | | 610 | 313 (51.31) |  | 2169 | 1106 (49.01) |
| **Maternal T1D** | | Yes | | 610 | 257 (42.13) |  | 2169 | 1287 (59.34) |
| **Maternal smoking\*** | | Yes | | 581 | 75 (12.91) |  | 2128 | 228 (10.71) |
| **Birth weight** | | SGA | | 571 | 49 (8.58) |  | 2047 | 179 (8.74) |
|  | | AGA | | 571 | 407 (71.28) |  | 2047 | 1434 (70.05) |
|  | | LGA | | 571 | 115 (20.14) |  | 2047 | 434 (21.20) |
| **Time varying variables** | | | |  |  |  |  |  |
| **Age (years)** | | |  | 3583 | 11.93±2.16 |  | 13235 | 4.89±4.60 |
| **BMI SDS** | | |  | 3537 | 0.06±1.10 |  | 13235 | 0.09±1.06 |
| **Overweight** | | | Yes | 3537 | 476 (13.46) |  | 13235 | 1637 (12.37) |
| **Height SDS** | | |  | 3537 | 0.32±0.98 |  | 13235 | 0.12±1.02 |
| **Weight SDS** | | |  | 3537 | 0.21±1.04 |  | 13235 | 0.11±0.94 |
| **Waist circumference SDS** | | |  | 2418 | 0.01±1.08 |  | - | - |
| **Subscapular skinfold thickness SDS** | | |  | 765 | 0.05±0.98 |  | - | - |
| **Triceps skinfold thickness SDS** | | |  | 768 | -0.42±1.09 |  | - | - |
| **SBP SDS** | | |  | 2056 | -0.16±1.30 |  | - | - |
| **DBP SDS** | | |  | 2056 | 0.17±1.29 |  | - | - |
| **HDL SDS** | | |  | 590 | -0.76±1.25 |  |  |  |
| **LDL SDS** | | |  | 590 | -0.11±1.08 |  |  |  |
| **Triglyceride SDS** | | |  | 590 | 0.32±0.81 |  |  |  |
| **Cholesterol SDS** | | |  | 590 | -0.14±1.01 |  |  |  |
| **Metabolic risk**  **(Cut-off 1.5 SD)** | | | Yes | 3545 | 847 (23.89) |  | - | - |
| **Fasting glucose mg/dL** | | |  | 3346 | 86.57±11.06 |  | - | - |
| **Fasting insulin µU/mL** | | |  | 3314 | 9.06±8.18 |  | - | - |
| **Fasting C-peptide ng/mL** | | |  | 3130 | 1.58±0.88 |  | - | - |
| **HOMA-IR** | | |  | 3172 | 1.95±1.80 |  | - | - |
| N Obs: total number of observations available for the particular variable; N: Number; SD: standard deviation; T1D: type 1 diabetes; SGA: small for gestational age; AGA: appropriate for gestational age; LGA: large for gestational age; BMI: body mass index; SDS: standard deviation scores; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance  \*Maternal smoking refers to smoking during pregnancy in BABYDIAB/BABYDIET, and general smoking status in TEENDIAB.  Metabolic risk was defined as high risk when at least one of the SD score variables of BMI, waist circumference, subscapular and triceps skinfold thickness, blood pressure and lipids were higher than 1.5 | | | | | | | | |

**Table 2: Effect estimates for anthropometric and metabolic outcomes in offspring born to a mother with compared to without type 1 diabetes in the TEENDIAB cohort. Significant associations (p<0.05) are shown in bold.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Outcomes** | **Model 1** | |  |  | **Model 2** | |  | |  | **Model 3** | |  |
| **N.Sub (N.Obs)** | **Estimates (95% CI)** |  | **N.Sub (N.Obs)** | | **Estimates (95% CI)** |  | **N.Sub (N.Obs)** | | | **Estimates (95% CI)** | | |
| **Absolute change in SD scores** | | | | | | | | | | | | | |
| Height SDS | 610 (3537) | -0.12 (-0.28; 0.03) |  | 578 (3203) | | -0.10 (-0.25; 0.06) |  | 541 (3027) | | | **-0.27 (-0.43; -0.10)\*\*** | | |
| Weight SDS | 610 (3537) | **0.20 (0.04; 0.36)\*** |  | 578 (3203) | | **0.22 (0.05; 0.38)\*** |  | 541 (3027) | | | 0.08 (-0.18; 0.31) | | |
| BMI SDS | 610 (3537) | **0.35 (0.19; 0.52)\*\*** |  | 578 (3203) | | **0.35 (0.19; 0.52)\*\*** |  | 541 (3027) | | | **0.28 (0.10; 0.46)\*\*** | | |
| Waist circumference SDS | 489 (2418) | **0.29 (0.12; 0.46)\*\*** |  | 463 (2211) | | **0.28 (0.10; 0.46)\*\*** |  | 436 (2109) | | | **0.24 (0.05; 0.42)\*** | | |
| Subscapular skinfold SDS | 570 (765) | **0.19 (0.03; 0.35)\*** |  | 513 (679) | | **0.18 (0.02; 0.33)\*** |  | 483 (641) | | | 0.14 (-0.03; 0.30) | | |
| Triceps skinfold SDS | 572 (768) | **0.19 (0.02; 0.37)\*** |  | 514 (680) | | 0.15 (-0.03; 0.33) |  | 484 (642) | | | 0.09 (-0.10; 0.28) | | |
| SBP SDS | 597 (2056) | **0.16 (0.01; 0.31)\*** |  | 559 (1872) | | 0.15 (-0.01; 0.31) |  | 541 (1768) | | | 0.13 (-0.05; 0.30) | | |
| DBP SDS | 597 (2056) | 0.12 (-0.03; 0.26) |  | 559 (1872) | | 0.13 (-0.02; 0.29) |  | 541 (1768) | | | 0.16 (-0.01; 0.32) | | |
| HDL SDS | 590 | 0.06 (-0.14; 0.27) |  | 514 | | -0.06 (-0.16; 0.28) |  | 481 | | | 0.09 (-0.14; 0.32) | | |
| LDL SDS | 590 | 0.10 (-0.07; 0.28) |  | 514 | | 0.09 (-0.10; 0.28) |  | 481 | | | 0.10 (-0.10; 0.30) | | |
| Triglyceride SDS | 590 | 0.06 (-0.07; 0.19) |  | 514 | | 0.07 (-0.07; 0.21) |  | 481 | | | 0.08 (-0.08; 0.23) | | |
| Cholesterol SDS | 590 | 0.10 (-0.06; 0.27) |  | 514 | | 0.11 (-0.07; 0.28) |  | 481 | | | 0.12 (-0.06; 0.31) | | |
| **Percent change in metabolic outcomes** | | | | | | | | | | | | | |
| Fasting glucose | 606 (3346) | 1.00 (-0.32; 2.34) |  | 574 (3010) | | 1.18 (-0.21; 2.58) |  | 541 (2849) | | | 1.41 (-0.08; 2.92) | | |
| Fasting insulin | 608 (3314) | **8.32 (0.68; 16.55)\*** |  | 576 (2979) | | **9.18 (1.92; 16.95)\*** |  | 541 (2817) | | | **9.89 (2.10; 18.27)\*** | | |
| Fasting C-peptide | 601 (3130) | 6.01 (-0.23; 12.64) |  | 569 (2818) | | **5.85 (0.18; 11.84)\*** |  | 541 (2668) | | | **6.80 (0.68; 13.29)\*** | | |
| HOMA-IR | 606 (3172) | **8.36 (0.38; 16.99)\*** |  | 574 (2850) | | **9.53 (1.93; 17.71)\*** |  | 541 (2701) | | | **11.00 (2.75; 19.92)\*** | | |
| **Odds ratios** | | | | | | | | | | | | | |
| Overweight | 610 (3537) | **2.40 (1.41; 4.06)\*\*** |  | 578 (3203) | | **2.33 (1.35; 4.03)\*\*** |  | 541 (3027) | | | **2.14 (1.19; 3.85)\*** | | |
| Abdominal obesity | 498 (2564) | **1.92 (1.15; 3.20)\*** |  | 472 (2337) | | **2.01 (1.19; 3.40)\*** |  | 444 (2225) | | | **2.05 (1.17; 3.59)\*** | | |
| Metabolic risk | 610 (3545) | **1.45 (1.10; 1.92)\*** |  | 578 (3209) | | **1.46 (1.09; 1.96)\*** |  | 541 (3033) | | | **1.39 (1.01; 1.90)\*** | | |
| (Cut-off 1.5 SD) |  |  |  |  | |  |  |  | | |  | | |
| N.Sub: number of subjects, N.Obs: number of observations (only mentioned if different from number of subjects), CI: Confidence interval, SDS: standard deviation scores, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance  Model 1: Crude model ; Model 2: adjusted for age, sex (except for overweight, abdominal obesity, metabolic risk and SDS outcomes), Tanner’s staging and maternal smoking; Model 3: Model 2 + birth weight  \*indicates p-value < 0.05; \*\*indicates p-value < 0.005  Waist circumference SDS was calculated only in children > 11 years of age. Abdominal obesity was defined as waist circumference ≥ 90th percentile or the adult threshold based on International Diabetes Federation. Metabolic risk was defined as high risk when at least one of the SD score variables of BMI, waist, subscapular and triceps skinfold thickness, blood pressure and lipids were higher than 1.5, else defined as low risk | | | | | | | | | | | | | |

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

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Outcomes** | **Model 1** | |  |  | **Model 2** | |  |  | **Model 3** | |  |
| **N.Sub (N.Obs)** | **Estimates**  **(95% CI)** |  | **N.Sub (N.Obs)** | | **Estimates**  **(95% CI)** |  | **N.Sub (N.Obs)** | | **Estimates**  **(95% CI)** | |
| **Absolute change in SD scores** | | | | | | | | | | | |
| Height SDS | 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 SDS | 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 SDS | 2169 (13235) | **0.13 (0.06; 0.20)\*\*** |  | 2128 (11757) | | **0.14 (0.07; 0.21)\*\*** |  | 2010 (11374) | | 0.04 (-0.04; 0.11) | |
| **Odds ratios for overweight** | | | | | | | | | | | |
| 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) | |
| N.Sub: number of subjects, N.Obs: number of observations, CI: Confidence interval, SDS: standard deviation scores  Model 1: Crude model; Model 2: adjusted for Tanner’s staging and maternal smoking during pregnancy; Model 3: Model 2 + birth weight | | | | | | | | | | | |

**Table 4: Cross-sectional and prospective associations between metabolite concentrations and overweight in the offspring presented as odds ratios (with 95% confidence intervals)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Odds ratio for overweight** |  | **Cross-sectional models** | |  | **Predictive models** | |
|  |
| **Exposures** |  | **OR (95% CI)** | **p-value** |  | **OR (95% CI)** | **p-value** |
| **Amino acid** |  |  |  |  |  |  |
| alanine\* |  | **9.23 (2.42; 35.23)** | **0.0011** |  | 5.16 (1.45; 18.36) | 0.011 |
| valine\* |  | **88.27 (7.79; 999.85)** | **0.0003** |  | **152.86 (14.25; 1640.3)** | **3.3\*10-5** |
| kynurenate\* |  | **9.32 (3.14; 27.64)** | **5.7\*10-5** |  | **6.03 (2.12; 17.11)** | **0.0007** |
| tyrosine\* |  | **37.21 (5.66; 244.55)** | **0.0002** |  | 15.55 (2.72; 88.80) | 0.0020 |
| **Lipid** |  |  |  |  |  |  |
| androsterone sulfate\* |  | **2.02 (1.37; 2.98)** | **0.0004** |  | 1.53 (1.08; 2.16) | 0.017 |
| androstenediol (3β,17β) disulfate (1)\* |  | **1.92 (1.33; 2.77)** | **0.0005** |  | 1.71 (1.2; 2.44) | 0.0031 |
| epiandrosterone sulfate\* |  | **1.96 (1.34; 2.88)** | **0.0005** |  | 1.53 (1.08; 2.17) | 0.017 |
| 5α-androstan-3β,17β-diol disulfate\* |  | **1.92 (1.31; 2.81)** | **0.0007** |  | 1.64 (1.12; 2.40) | 0.011 |
| dehydroisoandrosterone sulfate (DHEA-S)\* |  | **1.94 (1.26; 2.98)** | **0.0028** |  | 1.66 (1.1; 2.48) | 0.015 |
| carnitine\* |  | **139.11 (11.03; 1754)** | **0.0001** |  | 9.66 (1.07; 87) | 0.043 |
| thromboxane B2 |  | **2.32 (1.44; 3.73)** | **0.0005** |  | **2.33 (1.48; 3.67)** | **0.0003** |
| butyrylcarnitine (C4)\* |  | **2.90 (1.63; 5.17)** | **0.0003** |  | 1.67 (0.92; 3.00) | 0.090 |
| 2-aminoheptanoate\* |  | **4.32 (1.68; 11.11)** | **0.0024** |  | 1.73 (0.73; 4.10) | 0.213 |
| glycerol |  | **5.90 (2.11; 16.50)** | **0.0007** |  | 3.26 (1.23; 8.66) | 0.018 |
| stearidonate (18:4n3) |  | **3.40 (1.53; 7.54)** | **0.0026** |  | 1.68 (0.81; 3.45) | 0.162 |
| **Cofactors and Vitamins** |  |  |  |  |  |  |
| N1-Methyl-4-pyridone-3-carboxamide\* |  | **4.37 (1.85; 10.31)** | **0.0008** |  | 3.37 (1.52; 7.48) | 0.0027 |
| **Nucleotide** |  |  |  |  |  |  |
| urate\* |  | **35.05 (4.58; 268.08)** | **0.0006** |  | 6.87 (1.07; 44.16) | 0.042 |
| **Peptide** |  |  |  |  |  |  |
| gamma-glutamyltyrosine\* |  | **8.24 (2.29; 29.62)** | **0.0012** |  | 7.35 (2.11; 25.68) | 0.0018 |
| **Xenobiotic** |  |  |  |  |  |  |
| piperine |  | **1.81 (1.32; 2.47)** | **0.0002** |  | 1.52 (1.14; 2.04) | 0.0049 |
| N: number of subjects; OR: odds ratio; CI: confidence interval  Cross-sectional models: Crude associations between overweight status and metabolite concentrations at the 1st visit. Predictive models: Associations between overweight status at last visit and metabolite concentrations from the 1st visit adjusted for time difference between first and last visit. Only the metabolites significantly associated with overweight in the cross-sectional models after multiple testing correction are reported in the table. Estimates in bold indicate its significance after correction for multiple testing.  \*Reported in the literature[16](#_ENREF_16),[17](#_ENREF_17) to be associated with overweight in children | | | | | | |

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Odds ratio for overweight** | | | | |
| **Models** |  |  | **OR (95% CI)** | **p-value** |
| Model 1 |  | - | **2.44 (1.33; 4.50)** | **0.004** |
| Model 2 |  | - | **2.75 (1.37; 5.50)** | **0.004** |
| Model 2 | **+** | Birth weight | **2.48 (1.20; 5.13)** | **0.014** |
| **Further adjustment for metabolites significant for overweight** | | | | |
|  |  | **Amino acid** |  |  |
| Model 2 | **+** | kynurenate | **2.97 (1.44; 6.11)** | **0.003** |
| Model 2 | **+** | tyrosine | **2.74 (1.35; 5.57)** | **0.005** |
| Model 2 | **+** | valine | **2.93 (1.44; 5.95)** | **0.003** |
| Model 2 | **+** | alanine | **2.71 (1.34; 5.47)** | **0.006** |
|  |  | **Lipid** |  |  |
| Model 2 | **+** | androsterone sulfate | **2.78 (1.38; 5.62)** | **0.004** |
| Model 2 | **+** | androstenediol (3β,17β) disulfate (1) | **2.74 (1.36; 5.51)** | **0.005** |
| Model 2 | **+** | epiandrosterone sulfate | **2.83 (1.4; 5.72)** | **0.004** |
| Model 2 | **+** | 5α-androstan-3β,17β-diol disulfate | **2.59 (1.28; 5.23)** | **0.008** |
| Model 2 | **+** | dehydroisoandrosterone sulfate (DHEA-S) | **2.78 (1.38; 5.59)** | **0.004** |
| Model 2 | **+** | carnitine | **2.62 (1.29; 5.32)** | **0.008** |
| Model 2 | **+** | thromboxane B2 | **2.93 (1.45; 5.93)** | **0.003** |
| Model 2 | **+** | butyrylcarnitine (C4) | **2.93 (1.44; 5.95)** | **0.003** |
| Model 2 | **+** | 2-aminoheptanoate | **2.69 (1.34; 5.42)** | **0.006** |
| Model 2 | **+** | glycerol | **2.76 (1.36; 5.62)** | **0.005** |
| Model 2 | **+** | stearidonate (18:4n3) | **2.89 (1.43; 5.88)** | **0.003** |
|  |  | **Cofactors and Vitamins** |  |  |
| Model 2 | **+** | N1-Methyl-4-pyridone-3-carboxamide | **2.95 (1.45; 6.01)** | **0.003** |
|  |  | **Nucleotide** |  |  |
| Model 2 | **+** | urate | **2.64 (1.31; 5.35)** | **0.007** |
|  |  | **Peptide** |  |  |
| Model 2 | **+** | gamma-glutamyltyrosine | **2.75 (1.36; 5.54)** | **0.005** |
|  |  | **Xenobiotic** |  |  |
| Model 2 | **+** | piperine | **2.86 (1.41; 5.82)** | **0.004** |
| **Further adjustment for principal components significant for overweight** | | | | |
| Model 2 | **+** | PC3 | **2.73 (1.35; 5.55)** | **0.005** |
| Model 2 | **+** | PC5 | **3.01 (1.46; 6.21)** | **0.002** |
| Model 2 | **+** | PC9 | **2.75 (1.37; 5.50)** | **0.004** |
| N: number of subjects; OR: odds ratio; CI: confidence interval; PC: principal components  Model 1: Crude model; Model 2: Adjusted for Tanner’s staging and maternal smoking | | | | |

Y:\AnithaPitchika\analysis\Plots\Age plots\Ageplots_manuscript.tif

**Fig.1:** Mean and 95% confidence interval (CI) of BMI (plot A), weight (B) and height (C) standard deviation scores (SDS) stratified by maternal type 1 diabetes (T1D) and age in the TEENDIAB and BABYDIAB/BABYDIET cohort

D:\Users\anitha.pitchika\Downloads\Owt_pathway_fig_manuscript.tif

**Fig. 2** Association between super and sub-pathways of metabolites and overweight in the offspring

Pathways located to the right of the zero line indicate up-regulation, and left to the zero line down-regulation, in overweight individuals. Pathways lying beyond the dashed red line on both sides indicate associations with p<0.05 without adjustment for multiple testing. After multiple testing correction, the sub-pathways of androgenic steroids, BCAA metabolism, glycerolipid metabolism, lysine metabolism, polypeptide and food component/plant were up-regulated in overweight individuals. Similarly, the super-pathway nucleotide was also found to be up-regulated in overweight individuals. Pathways with (\*) indicates significance after correction for multiple testing.

**Supplementary figures and tables:**

**Table S1:** Description of the principal components (PC) significantly associated with overweight and its metabolites with higher loading values

|  |  |  |  |
| --- | --- | --- | --- |
| **PC significant for overweight** | **Metabolites with absolute loading values greater than 0.40** | **Eigen value** | **Variance explained (%)** |
| PC3 (Lipids: Androgenic, pregnenolone and progestin steroids) | Andro steroid monosulfate (2)  Androsterone sulfate  Androstenediol (3beta,17beta) disulfate (1)  Androstenediol (3beta,17beta) disulfate (2)  Epiandrosterone sulfate  5alpha-androstan-3beta,17beta-diol disulfate  Dehydroisoandrosterone sulfate (DHEA-S)  Thromboxane B2  21-hydroxypregnenolone disulfate  Pregnenolone sulfate  5alpha-pregnan-3beta,20alpha-diol disulfate  Pregn steroid monosulfate  Pregnen-diol disulfate | 16.50 | 3.74% |
| PC5 (Amino acid: BCAA, lysine, phenyalanine, tryptophan, tyrosine metabolism, urea cycle, acetylated peptides) | Creatine  2-methylbutyrylcarnitine C5  Valine  Isobutyrylcarnitne C4  Leucine  Isovalerylcarnitine C5  Isoleucine  N6-acetyllysine  Urea  N1-Methyl-4-pyridone-3-carboxamide  N1-Methyl-2-pyridone-5-carboxamide  Propionylcarnitine C3  Gamma-glutamylvaline  Gamma-glutamylphenylalanine  Gamma-glutamylleucine  Gamma-glutamylisoleucine | 10.53 | 2 .39% |
| PC13 (Amino acid, lipid, acetylated peptides and xenobiotics) | 3-indoxyl sulfate  Glycolithocholate sulfate  Phenylacetylcarnitine  Phenylacetylglutamine  4-methylcatechol sulfate  P-cresol sulfate  3-phenylpropionate hydrocinnamate | 5.75 | 1.30% |

**Table S2:** Percent change in metabolomics outcomes in offspring of type 1 diabetic mothers compared to offspring of non-diabetic mothers

|  |  |  |  |
| --- | --- | --- | --- |
| **Outcomes** |  | **Adjusted analysis\* (N=485)** | |
|  | **% change (95% CI)** | **p-value** |
| **Amino acid** |  |  |  |
| alanine |  | 2.35 (-1.88; 6.76) | 0.28 |
| valine |  | -1.15 (-3.32; 1.06) | 0.30 |
| kynurenate |  | 0.04 (-4.48; 4.77) | 0.99 |
| tyrosine |  | 1.06 (-1.92; 4.12) | 0.49 |
| **Lipid** |  |  |  |
| androsterone sulfate |  | -4.2 (-17.74; 11.57) | 0.58 |
| androstenediol (3β.17β) disulfate (1)\* |  | -4.34 (-16.53; 9.62) | 0.52 |
| epiandrosterone sulfate |  | -5.38 (-18.34; 9.63) | 0.46 |
| 5α-androstan-3β.17β-diol disulfate |  | 7.56 (-4.26; 20.83) | 0.22 |
| dehydroisoandrosterone sulfate (DHEA-S) |  | -5.86 (-16.65; 6.31) | 0.33 |
| carnitine |  | 1.41 (-1.18; 4.06) | 0.29 |
| thromboxane B2 |  | -4.77 (-15.04; 6.74) | 0.40 |
| butyrylcarnitine (C4) |  | -0.96 (-8.4; 7.09) | 0.81 |
| 2-aminoheptanoate |  | 1.24 (-4.56; 7.38) | 0.68 |
| glycerol |  | 3.32 (-1.8; 8.72) | 0.21 |
| stearidonate (18:4n3) |  | -0.25 (-7.18; 7.2) | 0.95 |
| **Cofactors and Vitamins** |  |  |  |
| N1-Methyl-4-pyridone-3-carboxamide |  | -2.59 (-9.04; 4.32) | 0.45 |
| **Nucleotide** |  |  |  |
| urate |  | 0.68 (-2.22; 3.66) | 0.65 |
| **Peptide** |  |  |  |
| gamma-glutamyltyrosine |  | 2.11 (-2.2; 6.61) | 0.34 |
| **Xenobiotic** |  |  |  |
| piperine |  | -2.99 (-18.83; 15.94) | 0.74 |
| \*Adjusted for age and sex | | | |

Y:\AnithaPitchika\analysis\Plots\PC plots\Owt_PC_fit1.TIF

**Fig. S1:** Association between principal components (PC) of metabolites and overweight presented as odds ratios (blue circles) with 95% confidence intervals (bars). Pathways with (\*) indicates significance after correction for multiple testing. After multiple testing correction, three principal components of metabolites were associated with offspring overweight.

Y:\AnithaPitchika\analysis\Plots\PC plots\Matdiab_PC_fit1.TIF

**Fig. S2:** Association between principal components (PC) of metabolites and maternal type 1 diabetes presented as absolute change in PC scores (blue circles) with 95% confidence intervals (bars). After multiple testing correction, no principal components of metabolites were associated with maternal type 1 diabetes.

D:\Users\anitha.pitchika\Downloads\Matdiab_pathway_fig_manuscript.tif

**Fig. S3** Association between super and sub-pathways of metabolites and maternal type 1 diabetes

Pathways located to the right of the zero line indicate up-regulation, and left to the zero line down-regulation, in offspring of type 1 diabetic mothers. Pathways lying beyond the red dashed line indicate associations of p<0.05 without adjustment for multiple testing. However, after multiple testing correction, no super- and sub-pathways of metabolites were significantly associated with maternal type 1 diabetes.