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the EPICOM study

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Apolipoprotein D and transthyretin are reduced in female adolescent offspring of women with type 1 diabetes: the EPICOM study

Running title: Apolipoprotein D and transthyretin in metabolic memory

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Novelty:

- Intrauterine exposure to hyperglycemia increases the offspring risk of obesity, metabolic syndrome, and type 2 diabetes later in life
- Adolescent offspring of mothers with type 1 diabetes possess a higher BMI and frequency of prediabetes compared to offspring of normoglycemic mothers
- Apolipoprotein D and transthyretin are reduced in female offspring of women with type 1 diabetes
- Apolipoprotein D levels in female offspring of women with type 1 diabetes correlated with indices of insulin sensitivity and secretion
- Low apolipoprotein D may be regarded as an early risk marker of metabolic dysfunction and thus may play a role in identifying individuals that could benefit from preventive measures.

Abstract

Aims: Adolescent offspring exposed to maternal diabetes during intrauterine life show a less favourable metabolic profile than the background population. Here, we hypothesize that offspring of women with type 1 diabetes (T1D), possess sex specific alterations in the serum profile of proteins involved in lipid, metabolic and transport processes and that these alterations are associated with lipid profile and indices of insulin sensitivity and secretion.

Methods: A prospective nationwide follow-up study (EPICOM) in a Danish population. Blood samples were assessed from offspring of women with T1D (index offspring, $n = 267$, 13–20 years), and matched control offspring ($n = 290$). Serum proteins were analysed using a 25-plex cardio-metabolic targeted proteomics assay, which includes 12 apolipoproteins and 13 transport and inflammatory proteins.

Results: Apolipoprotein D (ApoD) and transthyretin (TTR) were reduced in index females as compared to female controls (-8.1%, $p < 0.001$ and -6.1%, $p = 0.006$, respectively), but not in index males (2.2%, $p = 0.476$ and -2.4%, $p = 0.731$, respectively). Sex-dependent inverse associations between exposure to maternal T1D in utero and ApoD and TTR were significant after adjusting for age, BMI-SDS and Tanner stage (OR = 0.252 [95% CI 0.085, 0.745], $p = 0.013$ and OR = 0.149 [95% CI 0.040, 0.553], $p = 0.004$). ApoD correlated to indices of insulin sensitivity and secretion in a similar sex specific pattern in crude and adjusted analyses.

Conclusions: Low ApoD may be regarded as an early risk marker of metabolic syndrome. A possible link between ApoD and cardiovascular disease needs further investigation.

Key words: Apolipoprotein D, transthyretin, type 1 diabetes, adolescent offspring, metabolic risk, EPICOM study

Introduction

Intrauterine exposure to hyperglycemia increases the offspring risk of obesity, metabolic syndrome, and type 2 diabetes (T2D) in adulthood independent of genetic factors [1, 2]. The concept of fetal origin of adult disease as proposed by Barker more than three decades ago [3], links intrauterine stress stimuli during the critical periods of embryonic and fetal development to permanent structural, physiological and metabolic changes in the fetus and thus predisposing the individual to disease in adult life. The molecular framework underlying developmental programming of adult disease is complex. However, follow-up studies of offspring of diabetic pregnancies have provided important insight into the epigenetic, phenotypic, and clinical characteristics that substantiates this phenomenon [4-6]. Adult and adolescent offspring of mothers with type 1 diabetes (T1D) were shown to have both a higher BMI and frequency of prediabetes [7, 8], linked to later development of T2D. Further studies of adolescents in the EPICOM cohort showed that index offspring possess a less favorable metabolic profile including a higher BMI and prevalence of the metabolic syndrome, reduced insulin sensitivity and relative insulin secretion deficiency as well as decreased HDL cholesterol and adiponectin and elevated leptin [7, 9]. These findings were more prominent among female offspring. Moreover, elevated levels of sCD163 in index offspring associated with indices of fatty liver [10] .

T2D and underlying manifestations such as dyslipidemia and fatty liver are associated with proteomic changes in blood proteins involved in key processes such as hormone and vitamin transport, regulation, lipoprotein structure and function and oxidative stress [11-13]. However, it remains to be elucidated whether early indices and signs of developing metabolic syndrome (i.e. obesity, glucose intolerance, hypertension and dyslipidemia) among adolescent offspring of mothers with T1D diabetes, may be associated with such key proteomic changes. Previously, we evaluated the predictive potential of early pregnancy

changes in key proteins involved in hormone -and lipid transport and metabolism for later development of gestational diabetes mellitus (GDM) in obese and non-obese women [14]. By the use of a 25-plex "cardio-metabolic" targeted mass spectrometry (MS) assay, we demonstrated reduced levels of apolipoproteins (Apo) ApoD, ApoM and ApoL1 in GDM. Apolipoproteins comprise a crucial portion of lipoproteins functioning as a structural and/or regulatory component of major lipoprotein species i.e. HDL, chylomicrons, VLDL and LDL as well as receptor ligands and cofactors for lipid-metabolizing enzymes [15]. A growing body of evidence suggests that apolipoproteins play a role in the development of dyslipidemia and T2D [16].

To gain further insight into the role of the cardio-metabolic protein profile in metabolic memory, we here hypothesize that the serum profile of lipid, metabolic and transport proteins is altered in adolescents exposed to a diabetic intrauterine environment.

Methods

Study population

This is a sub-study of the prospective nationwide follow-up study, EPICOM (EPIgenetic, genetic and environmental effects on growth, COgnitive functions and Metabolism in offspring of women with T1D) described previously [7]. In brief, the study group consists of adolescent offspring from The Danish Diabetes Association Birth Register born to mothers with T1D between the years 1993 and 1999 (index offspring). The registry contains detailed information on maternal demography, diabetes status and pregnancy outcome. Adolescent offspring (singletons, firstborn during the inclusion period) and their mothers were included; 278 index offspring with mean age 16.7 years (range 13.0–19.8) and 303 control offspring with mean age 16.8 years (range 13.5–20.4) (Figure 1). The control offspring were identified from the background population and matched on date of birth (within one month), sex and postal code. Clinical data including maternal HbA1c in pregnancy, gestational age at birth and birth weight was recorded as described [7]. The study was performed in accordance with the Declaration of Helsinki and approved by the Regional Ethical Review Committee (M-20110239). Written informed consent was obtained either from the parents (if the participants were below 18 years of age) or from the participants themselves. The EPICOM study is registered at Clinicaltrials.gov (ID: NCT01559181). A full STROBE Statement checklist for reports of observational studies is provided in Supplementary Table S6.

Clinical examinations

The adolescent participants were examined in one of three different university hospitals in Denmark (Copenhagen, Odense and Aarhus) from April 2012 until October 2013. Clinical examinations, 2-hour oral glucose tolerance testing (OGTT) and blood sampling were performed after an overnight fast. Anthropometric measurements (height, weight, and waist circumference) were performed in triplicate (except height) as previously described [7].

Standard deviation scores (SDS) of height, weight, and BMI were calculated using normal Danish reference material as previously described [9]. Birth weight (bw) SDS was calculated using intrauterine growth curves adjusted for gestational age and sex. Small for gestational age (SGA) and large for gestational age (LGA) were defined as birth weight > 90-percentile and < 10-percentile respectively of the expected birthweight according to sex and gestational age [7].

Pubertal development (Tanner stage) was evaluated by inspection and palpation based on breast development in girls and genital development and measurement of testicular volume in boys. Total body fat percentage was determined using dual-energy x-ray absorptiometry (DEXA) as previously described [9].

Glucose and lipid status

The oral glucose tolerance test (OGTT) was performed with a glucose load of 1.75 g/kg body weight up to a total of 75 g. Exception was made if an adolescent had already been diagnosed with diabetes. Diabetes was defined according to the World Health Organization 1999 criteria [17]. Venous blood was drawn at time 0, 30 and 120 min after the glucose administration to determine plasma glucose and serum insulin as well as lipids and HbA1c (time 0) [7]. Indices of insulin sensitivity was evaluated by the OGTT-derived model for assessment of insulin sensitivity index (BIGTT-S_I) [18]. To assess insulin secretion we calculated the OGTT-derived index of acute insulin response (BIGTT-AIR) and to evaluate insulin secretion corrected for the prevailing insulin sensitivity, the disposition index (DI) was calculated as BIGTT-S_I * BIGTT-AIR [18]. These data have previously been presented [7, 9]. Fasting serum and plasma was transferred to a biobank for long term storage at -80°C. Serum leptin, adiponectin and leptin/adiponectin ratio (LAR) was determined by Lohse and coworkers as previously described [9].

Targeted MRM-MS protein analysis

Serum protein levels were analysed using a 25-plex targeted multiple-reaction-monitoring (MRM)-MS assay which included 12 apolipoproteins; ApoA-I, A-II, A-IV, B-100, C-II, C-III, D, E, H, J, L1, M and 13 transport and inflammation proteins; alpha-1-antichymotrypsin (ACT), alpha-2-macroglobulin (alpha-2-M), ceruloplasmin, C-reactive protein (CRP), fibronectin, haptoglobin, lipocalin (NGAL), serum paraoxonase/arylesterase 1 (PON1), retinol-binding protein 4 (RBP-4), sex hormone-binding globulin (SHBG), transthyretin (TTR), serotransferrin (transferrin), vitamin D-binding protein (VDP) (Table 2). Sample preparation for MRM analysis was performed according to Ravnsborg et al [14]. Serum samples (1 μg) were analysed in selected-reaction-monitoring (SRM) mode using an Easy-nLC II system coupled to a TSQ Vantage triple quadruple mass spectrometer (Thermo Scientific) [14].

Standard curves were prepared in triplicates for each peptide to derive linearity and lower limit of quantitation (LOQ) for each peptide [14]. For calculations of intra- and inter-assay coefficient of variation (CV), serum pool quality controls (QCs) was prepared in triplicate and analyzed in duplicate on the LC-MS/MS system along with each batch of 20 patient samples. Mean intra-assay CV for all 25 proteins in the assay was 5.9% (range, 4.7–7.8). Mean inter-assay CV was 12.3% (range, 8.7–19.3).

Data analysis and statistics

MRM-MS raw files were processed using Pinpoint 1.4 (Thermo Scientific). Data from each peptide was presented as the peak area ratio between the endogenous light peptide and the heavy labelled spike-in peptide (L/H ratio). Data handling and statistical analysis were carried out using Microsoft Excel 2010 (Microsoft) and STATA version 16.0 (StataCorp). Data were presented as median and interquartile range or n (%). For univariate statistical

analysis, the Mann–Whitney U test was used for continuous variables and the Pearson's $\chi 2$ test for categorical variables. Benjamini-Hochberg correction was used to correct for multiple testing. Binary associations were established by pairwise Spearman correlation analyses of continuous variables. Multivariate analyses on binary outcomes (index versus control offspring) were performed using logistic regression, reporting odds ratios and 95% confidence intervals (CI). Analyses were grouped by sex and performed as crude and adjusted models, after correcting for age [19], BMI-SDS and Tanner stage. A significance level of $p < 0.05$ was applied for all statistical tests in this study.

Results

Maternal characteristics including female and male offspring data by index/control status are shown in Table 1 and Supplementary Table S1. Blood for assessment of cardio-metabolic protein levels were available in 267 (96.0%) index and 290 (96.0%) control offspring (Figure 1). Among 25 proteins profiled using MRM-MS, ApoD and TTR showed altered levels in a sex dependent manner (Table 2). Index females showed reduced levels of ApoD and TTR as compared to controls; median 0.91 (IQR 0.78–1.09) vs. median 0.99 (IQR 0.87–1.17), $p < 0.001$ and median 0.77 (IQR 0.67–0.90) vs. median 0.82, IQR (0.70–0.97), $p = 0.006$, respectively. ApoD remained significant when controlling for false discovery rate (Benjamini-Hochberg correction). There were no significant differences in median ApoD and TTR between male index and controls 0.93 (IQR 0.79–1.09) vs. 0.91 (IQR 0.78–1.09), $p = 0.476$ and 0.82 (IQR 0.73–0.92) vs. 0.84 (IQR 0.70–0.94), $p = 0.731$, respectively.

Multivariate logistic regression analysis showed a negative association between index female offspring and ApoD (OR = 0.186 [95% CI 0.069, 0.504], $p = 0.001$), which remained significant after adjustment for age, BMI-SDS and Tanner stage (OR = 0.252 [95% CI 0.085, 0.745], $p = 0.013$) (Table 3). In index female offspring we found a negative association to TTR (OR = 0.176 [95% CI 0.052, 0.598], $p = 0.005$), which remained significant in adjusted analyses (OR 0.149 [95% CI 0.040, 0.553], $p = 0.004$). Index male offspring showed no association to ApoD or TTR in crude or adjusted analyses (Table 3).

We found a significant positive correlation between ApoD and HDL cholesterol in female offspring (Spearman's Rho, $r_s = 0.47$, $p < 0.001$) but not in male offspring (Figure 2, Supplementary Table S2). In both sexes, ApoD correlated positively to total cholesterol whereas ApoD correlated positively to LDL cholesterol in male offspring only (Supplementary Table S2). ApoD was negatively correlated to triglycerides in females but not in males. Indices of insulin sensitivity, BIGTT-S_I and insulin secretion corrected for

insulin sensitivity, DI, were positively correlated with ApoD in females but not in males (Figure 2, Supplementary Table S2). Index of acute insulin response, BIGTT-AIR, was negatively correlated with ApoD in female and in male offspring. Adipose-derived insulin sensitizer adiponectin and leptin/adiponectin ratio (LAR) showed significant correlations to ApoD in a similar sex specific manner (Supplementary Table S2). All correlations, except for DI remained significant after adjusting for age, BMI-SDS and Tanner stage (Supplementary Table S4).

TTR was positively correlated to total cholesterol and triglyceride in both female and male offspring in unadjusted and adjusted analyses (Supplementary Tables S3, S5). Adiponectin and LAR showed no consistent correlation to TTR (Supplementary Tables S3, S5).

Discussion

Here, we demonstrate reduced ApoD and TTR levels in adolescent female offspring of women with T1D compared to control female offspring. In both crude and adjusted analyses low ApoD and TTR were associated with index offspring in a sex-dependent manner. HDL-cholesterol correlated strongly to ApoD, while the latter correlated to indices of insulin sensitivity and insulin secretion in female offspring.

ApoD is a widely expressed glycosylated protein of the lipocalin superfamily, a family of proteins that transport small hydrophobic ligands [20]. In contrast to other apoproteins, being mainly expressed in liver and intestine, ApoD is expressed in numerous tissues, including brain, intestine, liver, cardiac and skeletal muscle, adipose tissue and pancreas. Accordingly, ApoD is considered a multi-ligand, multi-function protein and has been shown to be involved in numerous important processes such as lipid trafficking, food intake, metabolic dysfunction, inflammation, antioxidative response and development [20]. There is increasing evidence that ApoD plays an important role in the regulation of triglyceride and glucose metabolism. Mice models showed impaired lipid metabolism and reduced ApoD in leptin receptor deficient db/db mice [21], whereas ApoD deficiency resulted in nonfasting hypertriglyceridemia and hyperinsulinemia in ApoD mice [22]. Furthermore, overexpression of human ApoD in transgenic mice induced insulin resistance and altered lipid metabolism [23]. Thus variations in the levels and/or sites of ApoD expression influence the lipid and glucose metabolism. A further link between low ApoD and cardiovascular disease was provided in mice models showing that ApoD confers cardioprotective properties after experimentally induced myocardial infarction [24].

Taken together, an increasing body of evidence from animal models corroborate with our present study results showing reduced ApoD in female index offspring and correlation to indices of insulin resistance and insulin secretion as well as fasting triglycerides.

Compelling evidence from clinical studies support an important role for ApoD as an early metabolic risk marker of dyslipidemia leading to development of metabolic syndrome and T2D later in life. In normal uncomplicated pregnancies ApoD levels decrease and were even lower in women with excessive weight gain [25], and in pregnancies complicated by GDM, ApoD was shown to be reduced in the first trimester of both obese and non-obese women [14]. In addition, a reduced cord blood ApoD in newborn offspring of women with diabetes was associated with postnatal problems relating to carbohydrate metabolism [26]. Finally, higher ApoD levels in fat depots in severely obese women were associated with improved metabolic health (i.e. lower fasting insulin levels, higher insulin sensitivity and improved inflammatory profile) [27], suggestive of a role for ApoD as a marker of metabolic health in obesity. In support of this, early genetic studies, provided evidence that genetic variants of APOD are associated with abnormal lipid metabolism and increased risk of metabolic syndrome [28]. Taken together ApoD may indeed be regarded as an early metabolic risk marker, however, it remains to be elucidated, whether the observed sex differences observed in our study, may be evident in related cohorts. Similar sex dependent differences for adiponectin levels were found in a previous study of the EPICOM cohort [9], supporting that adolescent index females have a more unfavorable metabolic profile than male offspring including higher BMI-SDS and total body fat (Table 1). In animal studies, ApoD was found to influence bone metabolism in a sex specific manner with the most prominent phenotypes in female mice [29], implying that sex differences may also be an inherent trait to ApoD function. It is presently unclear why female offspring seems to be more affected metabolically by an intrauterine milieu as the one encountered among pregnant women with T1D.

In the present study TTR levels paralleled that of ApoD showing a sex specific pattern (Tables 2–3). TTR is a plasma protein secreted by the liver that circulates bound to RBP-4 and its retinol ligand [30]. Previous studies have indicated that plasma TTR may be an

important determinant of plasma RBP-4, showing contrasting effects of glomerular filtration of RBP-4 through opposite TTR expression in T2D and T1D [31]. Newly diagnosed T1D patients had reduced TTR levels, which existed predominantly as a monomer that may interfere with its interaction with RBP-4, resulting in its loss through glomerular filtration, whereas in T2D conditions, plasma TTR levels remained high, thus reducing glomerular filtration of RBP-4 [31]. In our study, RBP-4 was reduced marginally by 3.3% (p = 0.146, Table 2) in index females, suggesting that the TTR/RBP-4 phenotype may be weakly attenuated in offspring of T1D mothers.

It is becoming increasingly evident, that exposure to maternal diabetes during intrauterine life lead to altered fetal lipid and lipoprotein metabolism [32] as well as increased prevalence of prediabetes in the offspring [7, 8]. In the EPICOM cohort of adolescent offspring of mothers with T1D, these manifestations of impaired biochemical profile includes decreased HDL cholesterol, ApoD, TTR and adiponectin (in females) and elevated leptin and sCD163 [7, 9, 10]. Combined this profile of index offspring includes early signs of impaired function in triglyceride metabolism, indices of fatty liver disease, decreased insulin sensitivity and insufficient compensatory insulin secretion compared with offspring in the control group. Thus, there is a need for future studies designed to test these findings and define a panel of predictive risk markers that could help identifying high-risk adolescents who will benefit the most by improved physical activity and a healthy diet.

Strengths and limitations apply to the present study. EPICOM is a large and well-characterized cohort and this is the first study to provide an extensive cardio-metabolic plasma protein profile in offspring exposed to an intrauterine diabetic environment. The cross-sectional design of the study is a limitation when comparing to a cohort with repeat measurements in index and control offspring (i.e. from childhood to adulthood). Although the EPICOM study is a prospective study, data for the control group was collected

retrospectively in medical birth records, resulting in a number of missing data for birth weight and maternal pre-pregnancy BMI (approximately 25% of control offspring). Other limitations pertaining to the EPICOM study was reported previously [7, 9, 33]. Some limitations apply to the sex specific differences in ApoD and TTR and correlations to glucose homeostasis. Firstly, the differences may have been less significant if the number of female subjects had matched that of the male groups. Secondly, correlations of ApoD with insulin sensitivity and insulin secretion in female offspring where weak but still significant after adjusting for relevant confounding variables. Thus, the clinical relevance of ApoD as a predictive marker of developing metabolic dysfunction may be modest. The use of an MS-based MRM assay in this study represents a strength since this methodology inherently measures total (bound and free) protein levels due to enzymatically protein digestion prior to quantitative peptide analysis [14]. Thus, the method is preferable for the selected panel of proteins as compared to immunological assays that may be flawed by binding proteins.

In conclusion, we show that ApoD and TTR are reduced in female adolescent offspring of mothers with T1D. ApoD correlated to indices of insulin sensitivity and insulin secretion. Low ApoD is associated with metabolic dysfunction and development of metabolic syndrome and T2D later in life. A possible direct link between low ApoD and cardiovascular disease needs further investigation.

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Declaration of interest: The authors have nothing to disclose.

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Author contribution statement

TR and MO developed the MRM assay and conducted the MRM blood sample analyses in this study. SK, BB and ZL performed the data collection for the EPICOM cohort. MO, TR, SK, ZL, BB, TDC, RBJ, PD, HJM, KH, CHG, and DMJ contributed substantially to the conception and design of the study. MO, TR and DMJ analyzed and interpreted the data. MO drafted the manuscript and designed the tables and figures. PD contributed to the establishment of the original registry. PD and DMJ contributed to the data collection for the original registry. All authors critically revised the manuscript and approved the final version for publication. MO is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1. Baseline and follow-up characteristics of mothers and adolescent offspring

Characteristic	Index females (n = 164)	Control females (n = 182)	p-value	Index males (n = 114)	Control males (n = 121)	p-value
Maternal and offspring data at birth						
Maternal age (years)	29 (26–32)	30 (27–32)	0.243	29 (26–31)	30 (27–32)	0.200
BMI (kg/m^2)	23.4 (21.5–25.3)	22.3 (20.6–24.4)	0.024	22.3 (20.6–24.4)	23.1 (20.8–25.0)	0.775
Gestational age at birth (days)	259 (273–287)	280 (273–287)	<0.001	261 (249–266)	281 (276–287)	<0.001
Birth weight (g)	3,700 (3,200–4,080)	3,533 (3,275–3,800)	0.023	3,630 (3,163–4,085)	3,620 (3,360–3,930)	0.702
SGA (n, %)	6 (3.9)	13 (9.5)	0.061	11 (10.4)	10 (12.0)	0.817
LGA (n, %)	94 (61)	10 (7.3)	<0.001	56 (53)	5 (6.0)	<0.001
Adolescent offspring						
Age (years)	17.0 (15.5–18.2)	17.3 (15.6–18.5)	0.280	16.4 (15.0–17.8)	16.2 (15.1–17.8)	0.941
BMI-SDS	0.90 (0.25–1.95)	0.52 (-0.17–1.28)	<0.001	0.10 (-0.51–0.92)	-0.13 (-0.79–0.50)	0.042
Weight-SDS	0.69 (-0.19–1.63)	0.19 (-0.52–1.12)	<0.001	0.28 (-0.36–1.07)	-0.11 (-0.66–0.78)	0.015
Height-SDS	0.13 (-0.89–1.70)	-0.03 (-0.70–0.63)	0.469	0.41 (-0.13–1.03)	0.18 (-0.50–0.97)	0.122
Birth weight SDS	2.04 (0.48–3.49)	-0.04 (-0.54–0.66)	<0.001	1.35 (0.37–2.75)	-0.04 (-0.60–0.61)	<0.001
Total body fat (%)	34.9 (29.7–40.4)	31.0 (27.0–35.3)	<0.001	17.4 (14.6–24.4)	16.8 (13.6–21.4)	0.159
Total cholesterol (mmol/L)	4.0 (3.6–4.5)	4.1 (3.6–4.6)	0.401	3.6 (3.2–4.1)	3.6 (3.2–3.9)	0.827
LDL-cholesterol (mmol/L)	2.2 (1.9–2.6)	2.2 (1.9–2.7)	0.671	2.0 (1.7–2.4)	1.9 (1.6–2.3)	0.371
HDL-cholesterol (mmol/L)	1.4 (1.2–1.6)	1.5 (1.3–1.7)	0.133	1.3 (1.1–1.5)	1.3 (1.2–1.5)	0.139
Triacylglycerol (mmol/L)	0.80 (0.61–1.07)	0.80 (0.60–1.08)	0.442	0.70 (0.56–0.97)	0.70 (0.58–0.80)	0.276
HbA1c (mmol/mol)	33 (32–36)	33 (31–35)	0.419	34 (32–36)	34 (32–36)	0.395
HbA1c (%)	5.2 (5.1–5.4)	5.2 (5.0–5.4)	-	5.3 (5.1–5.4)	5.3 (5.1–5.4)	-
BIGTT-S_I	8.1 (5.5–10.2)	9.4 (7.4–11.5)	<0.001	8.6 (6.1–10.6)	10.0 (7.5–11.7)	0.003
BIGTT-AIR	1,994 (1,574–2,624)	1,780 (1,464–2,212)	0.022	1,834 (1,504–2,408)	1,694 (1,457–2,083)	0.079
Disposition Index	15,515 (11,379–20,459)	17,236 (13,868–20,783)	0.039	15,726 (12,172–19,784)	16,425 (13,959–19,399)	0.205

Data in Table 1 were presented in previous EPICOM studies [7, 9, 10]

Data are presented as median and interquartile range, n (%)

P-values for differences between groups were calculated using the Mann-Whitney U test for continuous variables and the Pearson's $\chi 2$ test/Fisher`s exact test for categorical variables

BMI, body mass index before pregnancy; SDS, standard deviation score; BIGTT-S_I, OGTT-derived Index of Insulin Sensitivity; BIGTTAIR, OGTT-derived Index of Acute Insulin Response. Disposition Index; insulin secretion corrected for insulin sensitivity.

Table 2. Cardio-metabolic protein profile in adolescent offspring by sex/index/control status

Protein	Index females (n = 158)	Control females (n = 174)	p-value	Index males (n = 109)	Control males (n = 116)	p-value
ApoA-I	0.96 (0.86–1.09)	0.99 (0.88–1.11)	0.119	0.88 (0.78–0.97)	0.87 (0.79–1.00)	0.440
ApoA-II	0.89 (0.80–1.04)	0.93 (0.79–1.09)	0.222	0.88 (0.77–0.98)	0.86 (0.78–0.96)	0.597
ApoA-IV	2.42 (2.07–2.89)	2.39 (2.08–3.03)	0.649	2.79 (2.40–3.19)	2.74 (2.38–3.23)	0.931
ApoB-100	0.87 (0.75–1.08)	0.92 (0.78–1.12)	0.210	0.79 (0.71–0.99)	0.76 (0.67–0.97)	0.29
ApoC-II	1.98 (1.54–2.39)	2.11 (1.65–2.63)	0.086	1.97 (1.51–2.58)	2.09 (1.66–2.53)	0.491
ApoC-III	1.17 (0.96–1.41)	1.24 (0.97–1.53)	0.123	1.09 (0.87–1.23)	1.07 (0.82–1.23)	0.735
ApoD	0.91 (0.78–1.09)	0.99 (0.87–1.17)	<0.001[a]	0.93 (0.79–1.09)	0.91 (0.78–1.09)	0.476
ApoE	1.15 (0.96–1.42)	1.20 (0.97–1.47)	0.431	1.08 (0.95–1.37)	1.15 (0.96–1.35)	0.636
ApoH	1.73 (1.55–1.91)	1.80 (1.59–1.98)	0.060	1.78 (1.63–1.98)	1.78 (1.59–1.97)	0.728
ApoJ	1.18 (1.09–1.35)	1.22 (1.07–1.36)	0.476	1.14 (1.06–1.26)	1.11 (1.03–1.26)	0.354
ApoL1	1.26 (1.08–1.66)	1.32 (0.97–1.77)	0.764	1.12 (0.97–1.25)	1.10 (0.91–1.30)	0.568
ApoM	2.29 (2.01–2.60)	2.39 (2.08–2.70)	0.072	2.25 (2.02–2.53)	2.30 (2.07–2.57)	0.373
ACT	1.63 (1.43–1.85)	1.64 (1.48–1.85)	0.636	1.61 (1.39–1.85)	1.57 (1.42–1.78)	0.550
Alpha-2-M	1.63 (1.43–1.85)	1.64 (1.48–1.85)	0.057	1.61 (1.39–1.85)	1.57 (1.42–1.78)	0.282
Ceruloplasmin	1.36 (1.04–1.94)	1.30 (1.05–1.90)	0.937	1.02 (0.87–1.20)	1.01 (0.83–1.12)	0.262
CRP	0.47 (0.21–1.84)	0.41 (0.15–1.29)	0.105	0.22 (0.13–0.40)	0.22 (0.13–0.42)	0.942
Fibronectin	1.06 (0.86–1.23)	1.03 (0.87–1.21)	0.713	1.21 (1.10–1.34)	1.18 (1.02–1.34)	0.230
Haptoglobin	0.73 (0.52–0.94)	0.70 (0.52–0.91)	0.603	0.59 (0.35–0.76)	0.53 (0.35–0.77)	0.415
NGAL	1.48 (1.27–1.79)	1.60 (1.27–1.81)	0.056	1.62 (1.35–1.87)	1.56 (1.36–1.87)	0.516
Pon1	1.53 (1.32–1.78)	1.58 (1.37–1.90)	0.258	1.45 (1.21–1.72)	1.40 (1.22–1.69)	0.923
RBP-4	1.48 (1.22–1.75)	1.53 (1.27–1.89)	0.146	1.38 (1.20–1.61)	1.41 (1.17–1.66)	0.506
SHBG	1.66 (1.07–2.57)	1.76 (1.23–2.56)	0.121	0.82 (0.65–1.06)	0.90 (0.68–1.22)	0.089
Transferrin	0.94 (0.85–1.04)	0.97 (0.86–1.09)	0.225	0.89 (0.82–0.96)	0.85 (0.78–0.95)	0.066
TTR	0.77 (0.67–0.90)	0.82 (0.72–0.97)	0.006	0.82 (0.73–0.92)	0.84 (0.70–0.94)	0.731
VDB	0.78 (0.68–0.95)	0.81 (0.70–0.97)	0.352	0.72 (0.66–0.79)	0.70 (0.64–0.77)	0.144

[a]Statistical significance ($p < 0.05$) after controlling for false discovery rate by Benjamini-Hochberg correction

Data are presented as median and interquartile range of peptide light/heavy ratio

P-values for differences between groups were calculated using the Mann-Whitney U test

Table 3. Multivariate regression models of associations between apolipoprotein D, transthyretin and sex/index/control status.

	Crude				Adjusted[a]			
	n	*OR*	95% CI	*p*-value	*n*	*OR*	95% CI	*p*-value
Index females								
Apolipoprotein D	332	0.186	0.069–0.504	0.001	311	0.252	0.085–0.745	0.013
Transthyretin	332	0.176	0.052–0.598	0.005	311	0.149	0.040–0.553	0.004
Index males								
Apolipoprotein D	225	0.626	0.208–1.885	0.405	192	0.623	0.151–2.570	0.513
Transthyretin	225	2.071	0.447–9.596	0.352	192	2.037	0.370–11.20	0.414

[a]Adjusted for age, BMI-SDS and Tanner stage

OR, Odds ratio; BMI-SDS, BMI-standard deviation score

Figure 1

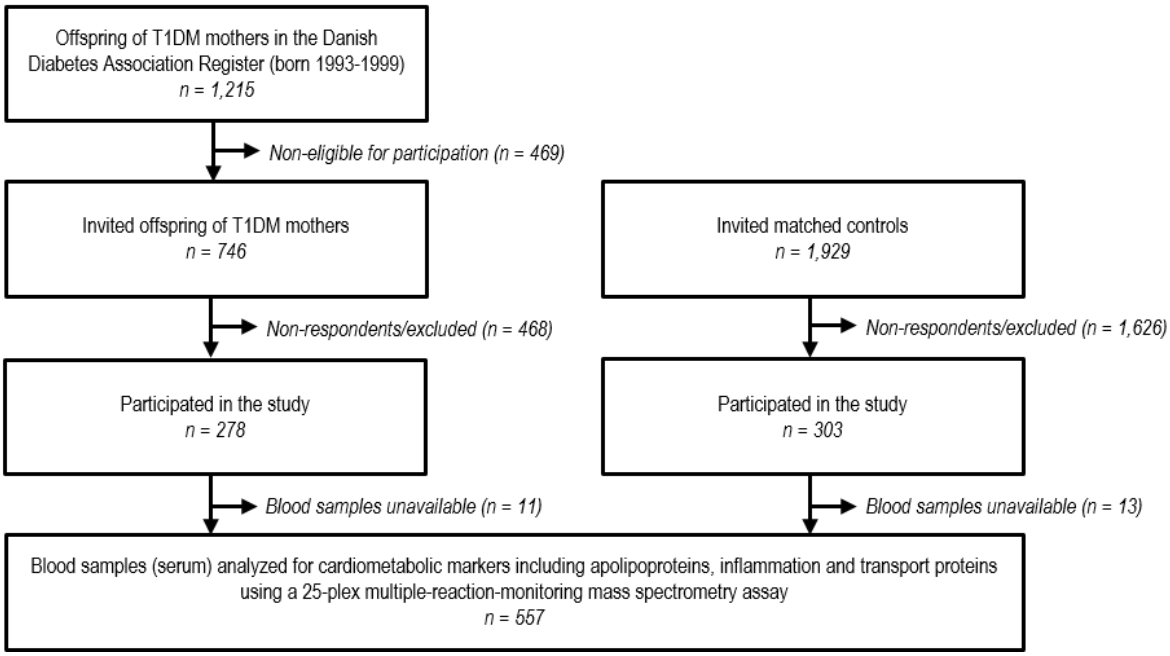
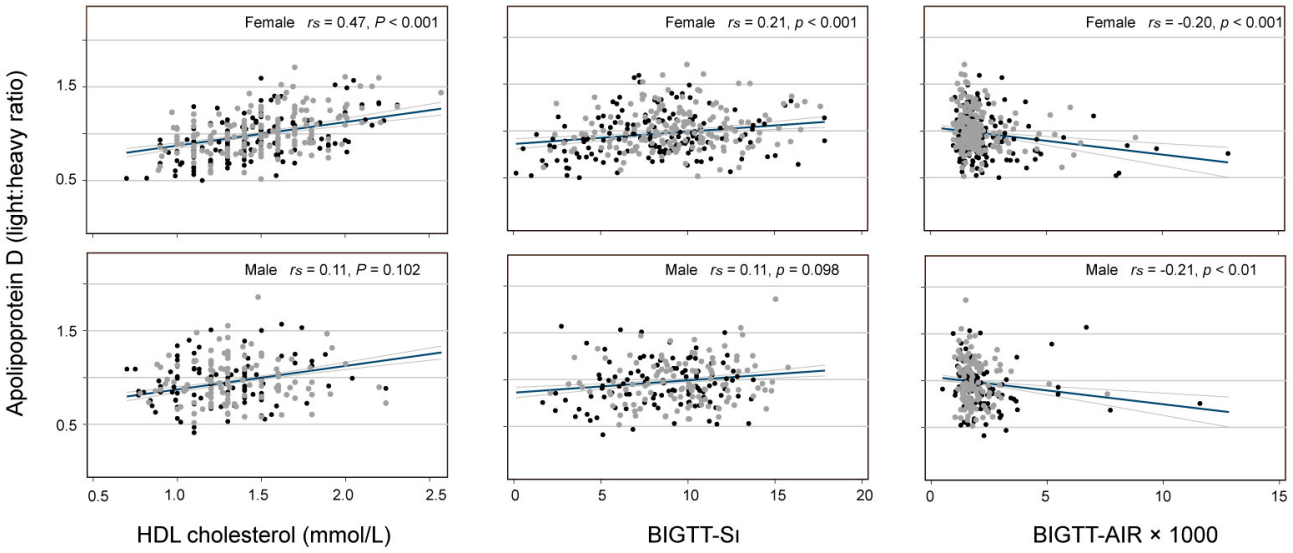

Offspring of T1DM mothers in the Danish Diabetes Association Register (born 1993-1999)
n = 1,215
Non-eligible for participation (n = 469)
Invited offspring of T1DM mothers
n = 746
Invited matched controls
n = 1,929
Non-respondents/excluded (n = 468)
Non-respondents/excluded (n = 1,626)
Participated in the study
n = 278
Participated in the study
n = 303
Blood samples unavailable (n = 11)
Blood samples unavailable (n = 13)
Blood samples (serum) analyzed for cardiometabolic markers including apolipoproteins, inflammation and transport proteins using a 25-plex multiple-reaction-monitoring mass spectrometry assay
n = 557

Figure 2


Female rs = 0.47, P < 0.001
Female rs = 0.21, p < 0.001
Female rs = -0.20, p < 0.001
Male rs = 0.11, P = 0.102
Male rs = 0.11, p = 0.098
Male rs = -0.21, p < 0.01
Apolipoprotein D (light:heavy ratio)
HDL cholesterol (mmol/L)
BIGTT-SI
BIGTT-AIR × 1000

Legends to figures

Figure 1. Flowchart of the Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes (EPICOM) study cohort, blood sampling and cardio-metabolic protein analyses.

Figure 2. Spearman correlation analyses between apolipoprotein D and HDL-cholesterol in female (left upper panel) and male offspring (left lower panel); apolipoprotein D and estimates of insulin sensitivity in female (middle upper panel) and male offspring (middle lower panel); apolipoprotein D and estimates of acute insulin response in female (right upper panel) and male offspring (right lower panel. Scatterplots show unadjusted correlations. Black circles represent index offspring and grey circles represent control offspring**.** BIGTT-S_I, OGTT-derived Index of Insulin Sensitivity; BIGTT-AIR, OGTT-derived Index of Acute Insulin Response.