Decreased Cord-Blood Phospholipids in Young Age–at–Onset Type 1 Diabetes

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Children developing type 1 diabetes may have risk markers already in their umbilical cord blood. It is hypothesized that the risk for type 1 diabetes at an early age may be increased by a pathogenic pregnancy and be reflected in altered cord-blood composition. This study used metabolomics to test if the cord-blood lipidome was affected in children diagnosed with type 1 diabetes before 8 years of age. The present case-control study of 76 index children diagnosed with type 1 diabetes before 8 years of age and 76 healthy control subjects matched for HLA risk, sex, and date of birth, as well as the mother's age and gestational age, revealed that cord-blood phosphatidylcholines and phosphatidylethanolamines were significantly decreased in children diagnosed with type 1 diabetes before 4 years of age. Reduced levels of triglycerides correlated to gestational age in index and control children and to age at diagnosis only in the index children. Finally, gestational infection during the first trimester was associated with lower cord-blood total lysophosphatidylcholines in index and control children. In conclusion, metabolomics of umbilical cord blood may identify children at increased risk for type 1 diabetes. Low phospholipid levels at birth may represent key mediators of the immune system and contribute to early induction of islet autoimmunity. ***Diabetes*** **62:3951–3956, 2013**

Type 1 diabetes is one of the most common chronic diseases among children and adolescents, with a worldwide increase in incidence (1,2). The clinical onset occurs when insulin secretion decreases due to autoimmune destruction of β-cells. Often overlooked is a prodrome of islet autoimmunity manifested by autoantibodies toward the islet autoantigens insulin, GAD65, islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8) (3–6). Type 1 diabetes is also strongly associated with specific HLA-DQ haplotypes (7).

Factors triggering the onset of islet autoimmunity and the subsequent transition to overt diabetes are largely unknown, thus making a prevention strategy still a challenge. A critical role of the environment, including gestational, perinatal, and postnatal factors, cannot be excluded (8). It is known that gestational events might increase disease risk for the offspring (9), including type 1 diabetes (10,11) and celiac disease (12). It is hypothesized that the risk for type 1 diabetes at an early age might be increased by a pathogenic pregnancy and be reflected in altered cord-blood composition. It is therefore critical to identify biomarkers of type 1 diabetes risk as early as possible in life.

Global profiling of serum metabolites has been used to dissect clinical and pathogenic aspects of the progression to islet autoimmunity and type 1 diabetes (13–15). Metabolomics analysis of samples from children in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study diagnosed with type 1 diabetes at 0.5–13.5 years of age (14) showed that serum metabolites may mark progression to islet autoimmunity and from islet autoimmunity to diabetes. In that study (14), umbilical cord-blood levels of phosphatidylcholines (PCs) were reduced in children who developed diabetes compared with healthy autoantibody-negative children matched for HLA (14). This observation supports the notion that children exposed to gestational events may have increased risk for type 1 diabetes (16). More recently and independent of the present investigation, low cord-blood content of choline-containing phospholipids (PLs) was associated with progression to diabetes (17). In the Diabetes Prediction in Skåne (DiPiS) study, we found that gestational events affect birth weight and length (18) as well as the appearance of islet autoantibodies (19). We therefore analyzed umbilical cord serum by lipidomics (20,21) to test the hypothesis that a specific molecular lipid profile predicts type 1 diabetes.

The DiPiS study is a population-based study of a cohort of more than 35,000 children (17,22,23). We compared 76 DiPiS children who developed type 1 diabetes before 8 years of age with 76 control subjects matched for HLA, sex, date of birth, and the mother's age. In this group of 152 children, we also analyzed the cord-blood lipidomic profile in relation to gestational events reported by the parents in clinical questionnaires (18).

RESEARCH DESIGN AND METHODS

Population and study design. The DiPiS study was initiated in the Skåne region of Southern Sweden between September 2000 and August 2004. Umbilical cord-blood samples were collected from more than 35,000 newborns (24) and analyzed for HLA genotypes and islet autoantibodies (Fig. 1) (17,22). DiPiS children at increased risk for type 1 diabetes and islet autoantibody appearance have been analyzed every 4–12 months, depending on the number of islet autoantibodies. Gestational and perinatal events were reported by ~25,000 parents in a questionnaire administered at the second postnatal visit (18).

Until April 2010, when the first sample selection was initiated, 112 children had developed type 1 diabetes (Fig. 1). Twins/triplets, children born to diabetic (any type) mothers, and children positive for islet autoantibodies in the cord blood were not included in this study (Fig. 1). The suitable control children had not developed persistent islet autoantibodies or type 1 diabetes by April 2010. The case-control matching was based on date of birth (difference, 0–27 days), sample storage time, HLA, sex, the mother's age, and gestational age. The study population consisted of 76 index children (40 boys) diagnosed with type 1 diabetes and 76 control children. The median age at diagnosis was

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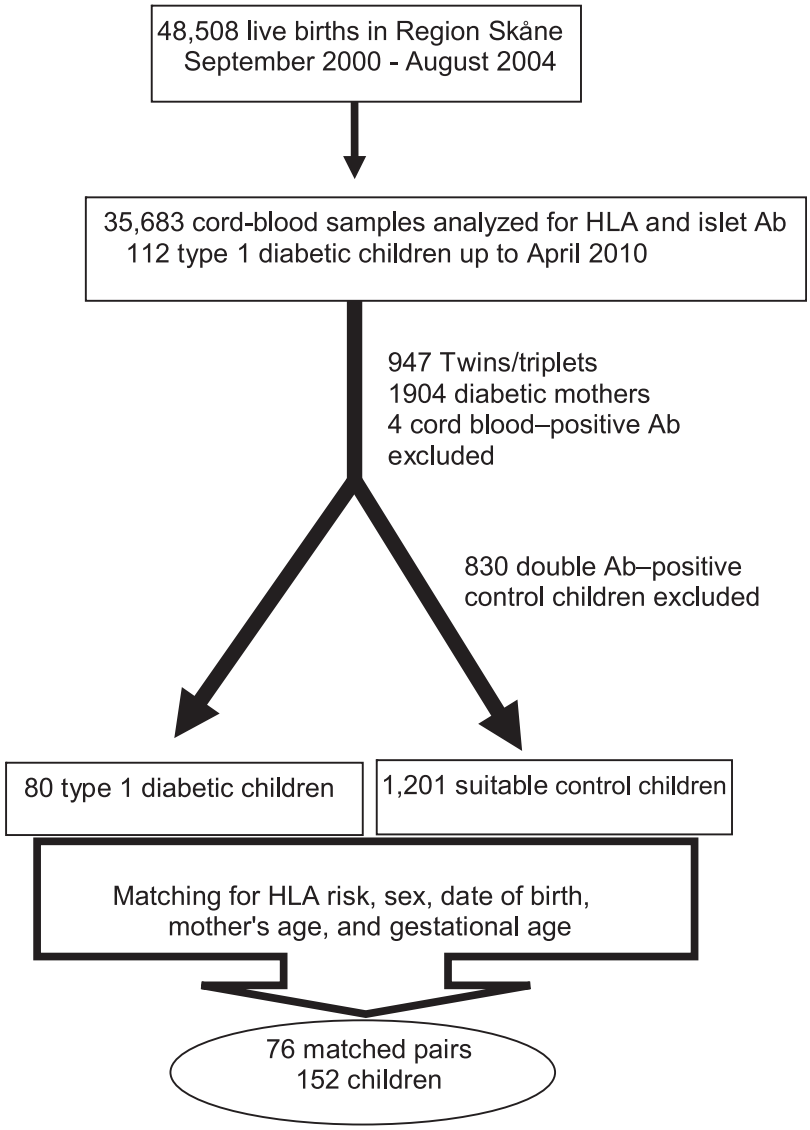

FIG. 1. Flowchart of study design. Index and control children were selected from a population-based newborn screening study (DiPiS study) between 2000 and 2004. Ab, autoantibody.

4.5 years (range 10 months–8 years). Index and control children did not differ in birth weight ($P = 0.88$) and length ($P = 0.76$), whereas index children (39.0 ± 1.7 weeks) showed slightly shorter gestational age than the control children (39.2 ± 1.3 weeks; $P = 0.046$) (Table 1). Retrospective questionnaires were available for 101 children, including 55 index and 46 control children, and reported information on the newborn (birth weight and length, jaundice) and gestational events (infections, vaccinations, diet, drugs, weight gain).

The study was approved by the Regional Ethics Board (2009/244), and parents gave informed consent when the child was 2 months of age.

Lipidomics analysis. The samples were blinded, prepared, and analyzed in random order in duplicates with ultra–high-performance liquid chromatography combined with mass spectrometry (MS) (20). MS analysis was performed with electrospray ionization in the positive ion mode.

Data processing, detection and alignment of peaks, and peak integration and normalization were performed as already described in detail (14). Lipid identification was carried out through an in-house reference compound library or the MS/MS spectrum (20). Only identified lipids were included in the final dataset. The established lipidomics platform applied in this study covers the major lipid classes found in serum, including PCs, sphingomyelins (SMs), phosphatidylethanolamines (PEs), ceramides, and triglycerides (TGs).

Metabolite stability. All samples were stored in the same place for 6.1–9.9 years at −20°C. The strict case-control matching for date of birth implies that each matched case-control pair has been stored for the same time and has undergone approximately the same number of freeze–thaw cycles. The concentration of the most abundant lysophospholipid (lysophosphatidylcholine [LPC] 16:0) was not affected by storage (R^2 linear 0.008, $P = 0.1$), whereas the PCs PC(40:5), PC(40:7), PC(38:4), PC(38:5), PC(38:5e), PC(36:4), and PC (36:5e)/PE(38:5e) decreased significantly with increasing sample storage time (Supplementary Fig. 1).

TABLE 1
Baseline characteristics of index and control children

	Index children (n = 76)	Control children (n = 76)	P
Male sex (n)	40	40	
Birth weight (g)	3,581 ± 536	3,592 ± 439	0.8
Birth length (cm)	50.2 ± 2.4	50.3 ± 2.0	0.7
Gestational age (weeks)	39.0 ± 1.7	39.2 ± 1.3	0.046
Age at diagnosis (years)	4.5 (10 months–8 years)	—	NA

NA, not applicable. Continuous data are shown as mean ± standard deviation or median (range).

Statistical analysis. The index child 117 was a strong outlier (Grubbs' procedure, $P < 0.005$, and the multivariate Hotelling's T^2 range plot) and excluded from further analysis along with the matched control.

Multivariate data analysis was performed on 150 samples by SIMCA-P+ 12.0 statistical software (Umetrics, Umeå, Sweden). Multivariate methods appear to be a valid tool for the analysis of complex and interdependent variables requiring multiple adjusting. The data were centered, Pareto-scaled to reduce the influence of noisy variables on the modeling results, and analyzed through projections methods as principal component analysis (PCA) and orthogonal partial least squares projections to latent structures discriminant analysis (OPLS-DA). PCA reduced the complexity of multidimensional datasets, creating low-dimensional coordinates from the principal determinants of the data variance. OPLS divided the data into related (predictive) and unrelated to a preset outcome and was applied to correlate the metabolite pattern with type 1 diabetes development, clinical features, and sample storage time. The data were evaluated through S-plots and shared and unique structure (SUS)-like plots for the identification of metabolic biomarkers (25). Additional statistical analysis was done with SPSS 18.0 software (PASW 18 Inc., Chicago, IL) with $P < 0.05$ considered significant. Case-control differences were calculated with parametric or nonparametric matched-pair tests, as appropriate. To account for false discovery rate in multiple comparisons (26), q values were calculated with the q-Value Calculator (statistical software R). The correlation between metabolite levels and storage duration or clinical features was evaluated through the Spearman ρ nonparametric correlation.

RESULTS

Metabolites detected in cord-blood samples. A total of 106 lipid metabolites were identified in the cord-blood samples of the 152 children, including PLs (PCs, PEs, SMs, and LPCs/lyso phosphatidylethanolamines [LPEs]) and TGs (Table 2). First, a PCA of the whole dataset was performed, yielding an estimated predictive ability of 60% ($Q2\times = 0.6$). The related loading plot showed that the lipid class was the strongest determinant of metabolite distribution (data not shown). Consistently, the levels of total PCs (PCtot), LPCs (LPCtot), PEs (PEtot), and SMs (SMtot) were strongly interrelated ($P < 0.001$ Spearman's ρ) in index and control children. LPCtot ($P = 0.02$) and SMtot ($P = 0.03$) were also related to total TGs (TGtot).

Multivariate analysis of cord-blood lipidome data. Second, models were calculated to compare case-control lipidomics pattern in relation to the age of type 1 diabetes diagnosis. The best predictive model for type 1 diabetes was achieved in children younger than 2 years of age at diagnosis, where PCs, PEs, and TGs explained 41% of the case-control separation ($n = 18$ observations; OPLS A 1 + 0 + 0; goodness of separation [*R2Ycum*], 32%; goodness of prediction [*Q2cum*], 16%). The groups of children older than 4 years at diagnosis could not be modeled.

Then, S-plots were used to identify metabolic markers of age at diagnosis. Cord-blood PCs were significantly lower in children developing type 1 diabetes before 4 years of age than in matched controls (85% negative correlation between PCtot and type 1 diabetes) (Supplementary Fig. 2*A*). Among children diagnosed before 2 years of age, low TGs were a better marker than PCs (0.95 negative correlation between TGtot and type 1 diabetes) (Supplementary Fig. 2*B*). The 3-year-olds did not differ from the 4-year-olds (data not shown). A SUS-like plot comparing children diagnosed before 2 and at 2–4 years of age (Supplementary Fig. 3) confirmed that low PLs (PCs, PEs, and SMs) marked type 1 diabetes in all children diagnosed when younger than 4 years of age, although reduced TGs showed a better predictive value in children diagnosed when younger than 2 years (Supplementary Fig. 3).

Additional analysis of cord-blood lipidome data. Pairwise case-control analysis showed that median cord-blood levels of 50 of 58 PLs (PCs, PEs, and SMs) but only of 7 of 36 TGs were reduced in children diagnosed before 4 years of age compared with their matched controls (significant PC[36:4], $P = 0.02$; and PE[36:0], $P = 0.02$). Stepwise analysis of the index children showed that median cord-blood levels of 39 of 44 PCs/PEs were reduced in children diagnosed before 4 years of age compared with index children diagnosed when older than 4 years, and of these, 18 different lipids reached statistical significance (Table 3 and Supplementary Fig. 4). Interestingly, the matched controls did not show the same trend (Table 3 and Supplementary Fig. 4). A test on all age classes (10 months–8 years) confirmed that several PCs and PEs were significantly decreased in the cord blood of index children with younger age at onset (Table 3). Finally, as suggested by the multivariate plots (Supplementary Figs. 2 and 3), children diagnosed before 4 years of age showed lower levels of 34 of 36 TGs than matched controls, and 7 of these lipids reached statistical significance. However, cord-blood TGs decreased significantly with decreasing gestational age ($P = 0.001$, Kruskal-Wallis test). Cord-blood TGs and gestational age appeared more strongly correlated in index children ($P = 0.007$) than in control children ($P = 0.049$; Supplementary Fig. 5*A*). Moreover, gestational age was significantly related to the

TABLE 2
Metabolites identified in cord-blood samples

LysoPCs/LysoPEs ($n = 12$)
LysoPC(14:0), LysoPC(16:0), LysoPC(16:1), LysoPC(18:0), LysoPC(18:1), LysoPC(18:2), LysoPC(20:3), LysoPC(20:4), LysoPC(20:5), LysoPC(22:6), LysoPE(18:2), LysoPE(20:1)
PCs ($n = 35$)
PC(31:1)/PE(34:1), PC(32:0), PC(32:1), PC(32:2), PC(33:1)/PE(36:1), PC(33:2)/PE(36:2), PC(34:1), PC(34:2), PC(34:2e), PC(34:3), PC(34:3e)/PE(37:3e), PC(35:2), PC(36:1), PC(36:1e), PC(36:2), PC(36:2e), PC(36:3), PC(36:3e), PC(36:4), PC(36:5), PC(36:5e)/PE(38:5e), PC(37:4)/PE(40:4), PC(38:2), PC(38:3), PC(38:3e), PC(38:4), PC(38:5), PC(38:5e), PC(38:6), PC(40:4), PC(40:5), PC(40:5e), PC(40:6), PC(40:7), PC(40:8)
PEs ($n = 9$)
PE(34:0), PE(34:3e), PE(36:0), PE(36:1), PE(38:1), PE(38:4), PE(38:4e), PE(40:4), PE(40:7e)
SM ($n = 14$)
SM(d18:1/14:0), SM(d18:1/16:0), SM(d18:1/16:1), SM(d18:1/18:0), SM(d18:1/18:2), SM(d18:1/18:3), SM(d18:1/20:0), SM(18:1/20:1), SM(d18:1/21:0), SM(d18:1/22:0), SM(d18:1/22:1), SM(d18:1/22:3), SM(18:1/23:1), SM(d18:1/24:1)
TGs ($n = 36$)
TG(18:1/16:1/18:2)/TG(18:2/18:2/16:0), TG(18:1/18:2/18:1), TG(46:0), TG(46:1), TG(46:2), TG(47:1), TG(48:0), TG(48:1), TG(48:2), TG(48:3), TG(49:1), TG(49:2), TG(50:0), TG(50:1), TG(50:2), TG(50:3), TG(51:1), TG(51:2), TG(51:3), TG(52:1), TG(52:2), TG(52:3), TG(52:4), TG(52:5), TG(53:3), TG(53:8), TG(54:1), TG(54:2), TG(54:3), TG(54:4), TG(54:5), TG(54:6), TG(54:9), TG(56:5), TG(56:6), TG(56:7)

TABLE 3
PLs differing between children who developed diabetes before and after 4 years of age

	Children developing type 1 diabetes (n = 75)						Control children (n = 75)	
	Median index						Median <4 to >4 years	
Metabolites	<4 years	>4 years	Ratio	P value*	q value	P value†	Ratio	P value§
PC(38:5)	3.01	4.21	0.71	*0.001*	0.1		0.92	0.3
PC(36:4)	79.11	96.45	0.82	*0.003*	0.1	*0.01*	1.02	0.6
PC(36:5+PE385:e)	2.56	3.21	0.78	*0.004*	0.1	*0.002*	0.93	0.3
PC(38:5e)	0.33	0.39	0.84	*0.004*	0.1	*0.02*	0.91	0.2
PC(40:4)	1.21	1.57	0.77	*0.007*	0.12		0.98	0.6
PE(38:4)	13.84	16.85	0.82	*0.007*	0.12	*0.03*	0.95	0.7
PC(37:4+PE40:4)	1.76	2.14	0.82	*0.009*	0.13	*0.046*	0.94	0.4
PC(38:6)	28.82	33.19	0.87	*0.01*	0.13		0.96	0.7
PE(40:4)	2.46	3.12	0.79	*0.01*	0.14		1.01	0.9
PC(40:5)	1.26	1.84	0.68	*0.02*	0.14		0.97	0.7
PC(40:5e)	0.26	0.31	0.85	*0.02*	0.14		0.3	0.3
PC(38:4)	2.54	3.14	0.81	*0.02*	0.14	*0.049*	0.88	0.2
PC(32:1)	5.77	8.02	0.72	*0.02*	0.15		1.14	0.7
PC(40:8)	0.39	0.32	1.21	*0.03*	0.16		1.02	0.8
PE(38:4e)	60.65	56.02	1.08	*0.03*	0.16		1.10	0.021
PC(31:1+PE34:1)	1.26	1.48	0.85	*0.03*	0.16		0.96	0.8
Pctot	447.65	502.83	0.89	*0.03*	0.17		0.8	0.8
PC(34:3e+PE37:3e)	0.56	0.69	0.81	*0.03*	0.17		0.6	0.6
PC(40:7)	0.45	0.51	0.88	*0.047*	0.23		1.08	0.7

The q value estimates the false discovery rate (FDR) for a given P value cutoff in multiple hypothesis testing (26). In these series, the estimated q value is reported for each test. Pi_0 is the maximum estimated q value among all P values < 0.05 and estimates the FDR when considering all P values <0.05 significant. In these series, the FDR (Pi_0) was 0.84 for Mann-Whitney and 0.5 for Kruskal-Wallis test. All PCs, except PC(40:8), and PEs, except PE(38:4e), were significantly reduced in children who developed diabetes when younger than 4 years of age. The same analysis performed on all control children matched to the two age classes of index children did not show the same trend. *Mann-Whitney test between the two classes of age at diagnosis (index children). Italics indicates statistically significant values. †Kruskal-Wallis nonparametric ANOVA test on all age classes (index children). §Mann-Whitney test between control children matched to the two classes of age at diagnosis.

age at onset (P = 0.02). The S-plot for biomarkers of gestational age (Supplementary Fig. 5*B*) confirmed that TGs strongly increased with increasing gestational age, whereas PLs (PCs, PEs, and SMs) showed a weak, negative correlation with gestational age (Supplementary Fig. 5*B*).

Gestational infections, gestational age, and sex differences. A total of 112 mothers reported gestational infections. Only 9 of 54 index children (16.6%) but 21 of 58 control children (36.2%) had a mother reporting gestational infection in the first trimester (P = 0.02). Among children diagnosed before 4 years of age, 5 of 19 (26%) had a mother reporting gestational infection during the first trimester, compared with 8 of 23 matched control children (34%) (P = 0.7). Among children diagnosed when older than 4 years of age, only 3 of 33 (9%) had a mother reporting a gestational infection during the first trimester, compared with 13 of 34 control children (38%) (P = 0.009). Lower levels of cord-blood LPCs were observed in index and control children (n = 112) born to mothers reporting a gestational infection (P = 0.04).

Male children (50.9 ± 2.3 cm) were taller at birth than female children (49.7 ± 1.8 cm; P = 0.03, controlling for gestational age). Index female children had shorter gestational age than female control children (P = 0.01) or index male children (P = 0.005). Index female children also showed significantly higher levels of 8 of 10 LPCs/PEs than female control children or index male children. Finally, we also observed that low TGs marked neonatal jaundice (90% positive correlation among 150 children, data not shown), as expected by the significantly shorter gestational age of children presenting with jaundice (index children, P = 0.003; controls, $P < 0.001$).

DISCUSSION

Our study of cord-blood lipidomics demonstrates that low levels of PCs and PEs increased the risk for type 1 diabetes diagnosed before 4 years of age. This finding may be of clinical relevance because epidemiological studies indicate that children diagnosed with type 1 diabetes at a young age are increasing. The importance of gestational events for disease risk in the offspring has been reviewed elsewhere (9,11,12,24). Altered cord-blood lipoproteins were reported in pathological conditions, such as intrauterine growth restriction and eclampsia, known to confer cardiovascular disease risk for the offspring (27). It would be of general importance to identify metabolite patterns that would distinguish the risk for different diseases in the offspring.

We tested the hypothesis that the lipidomic profile at birth might predict type 1 diabetes later in life. Fetal and neonatal serum lipids are markedly altered in women with maternal diabetes (28,29) but may be minimally affected by maternal lipids in normal pregnancies (28). Therefore, we excluded any type of maternal diabetes from the study (Fig. 1).

First, our study supports the recent observation of low cord-blood PC levels in 39 Finnish DIPP children who progressed to type 1 diabetes (14). We now extend this report to 75 Swedish children who developed type 1 diabetes before 8 years of age and 75 healthy and autoantibody-negative control children matched for HLA risk, sex, and date of birth (Fig. 1). Our major finding was that children developing type 1 diabetes before 4 years of age showed low levels of 50 different PLs, mainly PCs (Supplementary Figs. 2*A* and 4). Because PCs are the main source of choline, together with

diet intake (30), it is possible to postulate that DiPiS children who will develop type 1 diabetes might be choline-deficient at birth (14). Also, an imbalance in the maternal diet during pregnancy might possibly influence fetal/neonatal availability of choline (30). Low-level PCs may have a role in an imbalanced oxidative stress, because PCs are thought to have anti-inflammatory properties (31).

The second result was that cord-blood levels of TGs were low in children diagnosed before 2 years of age (Supplementary Fig. 2*B*). The finding that TGs were strongly related to gestational age complicated this observation, because the very young children showed a significantly shorter gestational age ($P = 0.02$) than children diagnosed when older than 6 years of age. Thus, low-level TGs may not represent a risk factor for type 1 diabetes. In normal pregnancies, the main source of fetal TGs is de novo synthesis by the liver (32). However, cord-blood TG levels may partly reflect maternal hypertriglyceridemia in late pregnancy (28). The observed direct correlation between cord-blood TG levels and gestational age (Supplementary Fig. 5), already reported in normal pregnancies (33), is probably physiological and unrelated to any gestational event conferring type 1 diabetes risk in the offspring but is of possible significance for any disorder related to short gestational age. Indeed, several clinical conditions are reported related to short gestational age, including type 1 (34) and type 2 diabetes (35). In the entire DiPiS study (18), index children have significantly shorter gestational age than control children ($P < 0.001$). Our observation that age at diagnosis was related to gestational age was surprising but may be because all children were diagnosed when younger than 8 years of age.

Taken together, short gestational age, perhaps in combination with reduced TG levels, may confer risk for type 1 diabetes at very early ages (<2 years). This subgroup of very young children may explain the significant clinical heterogeneity in relation to age at diagnosis reported in type 1 diabetes (36). Further metabolomics studies are needed (37) to disclose whether cord-blood TG levels are more important than gestational age per se. We note in this respect that PC levels, inversely related to gestational age (Supplementary Fig. 5*B*), were still reduced in the children diagnosed before 4 years of age (Supplementary Figs. 2*A* and 3). Low PC levels therefore remain the best marker for type 1 diabetes in children younger than 4 years of age because TG levels were influenced by gestational age, especially among the index children.

Because the samples were taken at birth, that no markers for type 1 diabetes risk were found for children with later onset may not be surprising. Future investigation will be needed to determine at regular follow-up visits to what extent low PCs combined with serum C-peptide measurements can be used in prevention clinical studies. In the DIPP study, as recently reported (17) after an analysis carried out independent of and in parallel to the current study, seven lipids were identified that predicted high risk for progression to type 1 diabetes. Reduction in choline-containing PLs in cord blood was specifically associated with progression to type 1 diabetes. In addition, our observation that LPC/PE levels were significantly increased in female index children, regardless of age at onset, may suggest the presence of an imbalance of oxidative environment (38). Finally, the low level of LPCs that was found to be related to gestational infections would be consistent with the hypothesis of a decreased LPC content in patients with infective conditions (39).

We could not reinvestigate the effect of maternal age at delivery, a reported risk factor for type 1 diabetes in the offspring (40), because our case-control pairs were matched for the mother's age to limit the influence of maternal metabolome.

A potential weakness of the study is the retrospective analysis of samples stored for a long time and of retrospective questionnaires collected by mothers after the delivery. The issue of metabolite stability is crucial for metabolomics evaluation because of the potential confounding effects of nonenzymatic lipid oxidation during long storage at suboptimal temperature. In this study, however, the stringent case-control matching controlled for metabolite stability and made it possible to conclude with a grade of confidence that observed metabolite variations were not due to storage. Our report on possible storage effects on metabolite concentration is of interest because no data have been published on samples stored more than a few weeks (41).

In conclusion, cord-blood metabolic patterns, rather than single metabolites, may be a valuable measure of type 1 diabetes risk. It may be important to include essentially all phospholipidic moieties in studies aimed at dissecting gestational events conferring disease risk in the offspring. Longitudinal studies of mothers from the first trimester until delivery will be important to disclose the character of gestational events that may lower cord-blood LPCs or affect TG levels in relation to the development of islet autoimmunity and diabetes in the offspring.

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D.L.T. designed the study, analyzed and researched data, and wrote the manuscript. T.S-L., H.E.L., T.H., S.A.I., and Å.L. designed the study, researched data, contributed to discussion, and reviewed and edited the manuscript. M.O. was responsible for the lipidomics analysis, analyzed data, contributed to discussion, and reviewed and edited the manuscript. D.L.T. and M.O. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. DIAMOND Project Group. Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. Diabet Med 2006;23:857–866
2. EURODIAB ACE Study Group. Variation and trends in incidence of childhood diabetes in Europe. Lancet 2000;355:873–876
3. Eisenbarth GS. Prediction of type 1 diabetes: the natural history of the prediabetic period. Adv Exp Med Biol 2004;552:268–290
4. La Torre D, Lernmark A. Immunology of beta-cell destruction. Adv Exp Med Biol 2010;654:537–583
5. Törn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ; Participating Laboratories. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia 2008;51:846–852
6. DPT-1 Study Group The Diabetes Prevention Trial-Type 1 diabetes (DPT-1): implementation of screening and staging of relatives. DPT-1 Study Group. Transplant Proc 1995;27:3377
7. Cucca F, Lampis R, Congia M, et al. A correlation between the relative predisposition of MHC class II alleles to type 1 diabetes and the structure of their proteins. Hum Mol Genet 2001;10:2025–2037
8. TEDDY Stydy Group. The Environmental Determinants of Diabetes in the Young (TEDDY) Study. Ann N Y Acad Sci 2008;1150:1–13
9. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 2008; 359:61–73
10. Dahlquist GG, Patterson C, Soltesz G. Perinatal risk factors for childhood type 1 diabetes in Europe. The EURODIAB Substudy 2 Study Group. Diabetes Care 1999;22:1698–1702
11. Elfving M, Svensson J, Oikarinen S, et al. Maternal enterovirus infection during pregnancy as a risk factor in offspring diagnosed with type 1 diabetes between 15 and 30 years of age. Exp Diabetes Res 2008;2008:271958
12. Lindehammer SR, Björck S, Lynch K, et al.; Diabetes and Celiac Disease Prediction in Scania Study Group. Early human pregnancy serum cytokine levels predict autoimmunity in offspring. Autoimmunity 2011;44:445–452
13. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB. Metabolomics applied to diabetes research: moving from information to knowledge. Diabetes 2009;58:2429–2443
14. Oresic M, Simell S, Sysi-Aho M, et al. Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes. J Exp Med 2008;205:2975–2984
15. Pflueger M, Seppänen-Laakso T, Suortti T, et al. Age- and islet autoimmunity-associated differences in amino acid and lipid metabolites in children at risk for type 1 diabetes. Diabetes 2011;60:2740–2747
16. Knip M, Veijola R, Virtanen SM, Hyöty H, Vaarala O, Akerblom HK. Environmental triggers and determinants of type 1 diabetes. Diabetes 2005;54 (Suppl. 2):S125–S136
17. Oresic M, Gopalacharyulu P, Mykkänen J, et al. Cord serum lipidome in prediction of islet autoimmunity and type 1 diabetes. Diabetes 29 April 2013 [Epub ahead of print]
18. Lynch KF, Lernmark B, Merlo J, Cilio CM, Ivarsson SA, Lernmark A; Diabetes Prediction in Skåne (DiPiS) Study Group. Cord blood islet autoantibodies and seasonal association with the type 1 diabetes high-risk genotype. J Perinatol 2008;28:211–217
19. Lindehammer SR, Hansson I, Midberg B, et al.; Diabetes Prediction in Skåne (DiPiS) Study Group. Seroconversion to islet autoantibodies between early pregnancy and delivery in non-diabetic mothers. J Reprod Immunol 2011;88:72–79
20. Nygren H, Seppänen-Laakso T, Castillo S, Hyötyläinen T, Orešič M. Liquid chromatography-mass spectrometry (LC-MS)-based lipidomics for studies of body fluids and tissues. Methods Mol Biol 2011;708:247–257
21. Quehenberger O, Dennis EA. The human plasma lipidome. N Engl J Med 2011;365:1812–1823
22. Larsson HE, Lynch K, Lernmark B, et al.; DiPiS Study Group. Diabetes-associated HLA genotypes affect birthweight in the general population. Diabetologia 2005;48:1484–1491
23. Larsson HE, Hansson G, Carlsson A, et al.; DiPiS Study Group. Children developing type 1 diabetes before 6 years of age have increased linear growth independent of HLA genotypes. Diabetologia 2008;51:1623–1630
24. Larsson K, Elding-Larsson H, Cederwall E, et al. Genetic and perinatal factors as risk for childhood type 1 diabetes. Diabetes Metab Res Rev 2004; 20:429–437
25. Wiklund S, Johansson E, Sjöström L, et al. Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. Anal Chem 2008;80:115–122
26. Käll L, Storey JD, MacCoss MJ, Noble WS. Posterior error probabilities and false discovery rates: two sides of the same coin. J Proteome Res 2008;7:40–44
27. Rodie VA, Caslake MJ, Stewart F, et al. Fetal cord plasma lipoprotein status in uncomplicated human pregnancies and in pregnancies complicated by pre-eclampsia and intrauterine growth restriction. Atherosclerosis 2004;176:181–187
28. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. Endocrine 2002;19:43–55
29. Kilby MD, Neary RH, Mackness MI, Durrington PN. Fetal and maternal lipoprotein metabolism in human pregnancy complicated by type I diabetes mellitus. J Clin Endocrinol Metab 1998;83:1736–1741
30. Li Z, Vance DE. Phosphatidylcholine and choline homeostasis. J Lipid Res 2008;49:1187–1194
31. Treede I, Braun A, Sparla R, et al. Anti-inflammatory effects of phosphatidylcholine. J Biol Chem 2007;282:27155–27164
32. Chow SN, Chen HY, Ouyang PC, Lee TY, Wei PY, Chen JS. Serum triglyceride levels in newborns and mothers at parturition. Int J Biol Res Pregnancy 1982;3:30–32
33. Christensen NC. Concentrations of triglycerides, free fatty acids and glycerol in cord blood of newborn infants with a birth weight of less than or equal to 2700 grams. Acta Paediatr Scand 1977;66:43–48
34. Svensson J, Carstensen B, Mortensen HB, Borch-Johnsen K; Danish Study Group of Childhood Diabetes. Early childhood risk factors associated with type 1 diabetes—is gender important? Eur J Epidemiol 2005;20:429–434
35. Kaijser M, Bonamy AK, Akre O, et al. Perinatal risk factors for diabetes in later life. Diabetes 2009;58:523–526
36. Andersson C, Larsson K, Vaziri-Sani F, et al. The three ZNT8 autoantibody variants together improve the diagnostic sensitivity of childhood and adolescent type 1 diabetes. Autoimmunity 2011;44:394–405
37. Varmus H. Ten years on—the human genome and medicine. N Engl J Med 2010;362:2028–2029
38. Fuchs B, Muller K, Paasch U, Schiller J. Lysophospholipids: potential markers of diseases and infertility? Mini Rev Med Chem 2012;12:74–86
39. Lissauer E, Johnson B, Shi S, Gentle T, Scalea M. Decreased lysophosphatidylcholine levels are associated with sepsis compared to uninfected inflammation prior to onset of sepsis. J Surg Res 2007;137:206
40. Cardwell CR, Stene LC, Joner G, et al. Maternal age at birth and childhood type 1 diabetes: a pooled analysis of 30 observational studies. Diabetes 2010;59:486–494
41. Zivkovic AM, Wiest MM, Nguyen UT, Davis R, Watkins SM, German JB. Effects of sample handling and storage on quantitative lipid analysis in human serum. Metabolomics 2009;5:507–516