Persistent Alterations in Plasma Lipid Profiles Before Introduction of Gluten in the Diet Associated With Progression to Celiac Disease

Partho Sen, PhD1, Cecilia Carlsson, MSc2, Suvi M. Virtanen, MD, PhD3,4,5,6, Satu Simell, MD, PhD7, Heikki Hyöty, MD, PhD8,9, Jorma Ilonen, MD, PhD10,11, Jorma Toppari, MD, PhD7,12, Riitta Veijola, MD, PhD13,14,15, Tuulia Hyötyläinen, PhD6, Mikael Knip, MD, PhD16,17,18 and Matej Orešič, PhD1,19

OBJECTIVES: Celiac disease (CD) is a chronic enteropathy characterized by an autoimmune reaction in the small intestine of genetically susceptible individuals. The underlying causes of autoimmune reaction and its effect on host metabolism remain largely unknown. Herein, we apply lipidomics to elucidate the early events preceding clinical CD in a cohort of Finnish children, followed up in the Type 1 Diabetes Prediction and Prevention study.

METHODS: Mass spectrometry–based lipidomics profiling was applied to a longitudinal/prospective series of 233 plasma samples obtained from CD progressors (n = 23) and healthy controls (n = 23), matched for human leukocyte antigen (HLA) risk, sex, and age. The children were followed from birth until diagnosis of clinical CD and subsequent introduction of a gluten-free diet.

RESULTS: Twenty-three children progressed to CD at a mean age of 4.8 years. They showed increased amounts of triacylglycerols (TGs) of low carbon number and double bond count and a decreased level of phosphatidylcholines by age 3 months as compared to controls. These differences were exacerbated with age but were not observed at birth (cord blood). No significant differences were observed in the essential TGs.

DISCUSSION: Our preliminary findings suggest that abnormal lipid metabolism associates with the development of clinical CD and occurs already before the first introduction of gluten to the diet. Moreover, our data suggest that the specific TGs found elevated in CD progressors may be due to a host response to compromised intake of essential lipids in the small intestine, requiring de novo lipogenesis.

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A44

INTRODUCTION

Celiac disease (CD) is a chronic, systemic, autoimmune enteropathy triggered by dietary gluten and related prolamins from rye and barley in genetically susceptible individuals (1). Approximately 90%–95% of patients with CD express human leukocyte antigen (HLA)-DQ2 protein, whereas the remaining (5%–10%) express HLA-DQ8 (2). CD is characterized by a wide range of gastrointestinal and extraintestinal symptoms that include diarrhea, weight loss, abdominal distention, malabsorption, and iron deficiency anemia (1). Serologic tests such as measurement of serum immunoglobulin A (IgA) and/or immunoglobulin G (IgG) tissue transglutaminase antibodies, IgA

1Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland; 2Department of Chemistry, Örebro University, Örebro, Sweden; 3Public Health Promotion Unit, National Institute for Health and Welfare, Helsinki, Helsinki, Finland; 4Faculty of Social Sciences/Health, University of Tampere, Tampere, Finland; 5Center for Child Health Research, University of Tampere and Tampere University Hospital, Tampere, Finland; 6Science Centre, Tampere University Hospital, Tampere, Finland; 7Department of Paediatrics and Adolescent Medicine, Turku University Hospital, Turku, Finland; 8Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland; 9Fimlab Laboratories, Pirkanmaa Hospital District, Tampere, Finland; 10Institute of Biomedicine, University of Turku, Turku, Finland; 11Clinical Microbiology, Turku University Hospital, Turku, Finland; 12Institute of Biomedicine, Centre for Integrative Physiology and Pharmacology, University of Turku, Turku, Finland; 13Department of Paediatrics, PEDEGO Research Unit, Medical Research Centre, University of Oulu, Oulu, Finland; 14Department of Children and Adolescents, Oulu University Hospital, Oulu, Finland; 15Department of Women’s and Children’s Health, Karolinska Institutet, Stockholm, Sweden; 16Children’s Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; 17Research Programs Unit, Diabetes and Obesity, University of Helsinki, Helsinki, Finland; 18Tampere Centre for Child Health Research, Tampere University Hospital, Tampere, Finland; and 19School of Medical Sciences, Örebro University, Örebro, Sweden. Correspondence: Matej Orešič, PhD. E-mail: matej.oresic@utu.fi. Mikael Knip, MD, PhD. E-mail: mikael.knip@helsinki.fi.

Received December 5, 2018; accepted March 12, 2019; published online May 10, 2019
© 2019 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The American College of Gastroenterology
endomysial antibodies, and deamidated gliadin peptide antibodies (IgG class) are performed for screening and diagnosis of CD. In addition, a biopsy of small intestine is still required in many countries to confirm the diagnosis (3).

The incidence of CD and other autoimmune diseases such as type 1 diabetes (T1D) has been increasing in children and adults over the past decades (4,5). The occurrence of these autoimmune diseases is higher in the Nordic countries (6) than elsewhere, with the highest prevalence of CD occurring in Sweden (29/1,000 by age 12 years) and the highest incidence rate of T1D occurring in Finland (64/100,000/year for children younger than 15 years) (7,8). Approximately 10% of patients with T1D develop overt CD (9). On the other hand, people with CD are at risk of T1D before age 20 years (10). These autoimmune diseases share common, predisposing alleles in the class II HLA-region as the DR3-DQ2 and DR4-DQ8 haplotypes (11).

Recent studies show that in addition to genetic predisposition and exposure to dietary gluten, other factors such as the composition of the intestinal microbiota, birth delivery mode, infant feeding, and the use of antibiotics may also affect the onset of CD (12). Thus, the early pathogenesis of CD is still poorly understood, and the identification of molecular signatures associated with progression to overt CD remains an unmet medical need (13). The health burden of CD in terms of quality of life, complications, mortality, and cost of treatment is considerable, meaning that its prevention has become, in the last decade, an important area of research.

Metabolomics is the study of small (<1,500 Da) molecules and their functions in cells, tissues, and body fluids (14). Metabolic studies in adults diagnosed with active CD (15) identified marked changes in serum and urine metabolic profiles, along with altered intestinal microbiota (16–18). Interestingly, a recent, small prospective study suggests an altered early trajectory of the gut microbiome (ages 4 and 6 months) in children who later progressed to CD (19).

Lipids are a structurally and functionally highly diverse group of metabolites with many important biological functions including as components of cell membranes, intermediates in signaling pathways, and as energy sources. The emergence of lipidomics, a discipline closely related to metabolomics, has enabled researchers to study lipidomes and lipid metabolism at the systems level (20,21). Because of their diverse roles in biological systems, it is not surprising that lipid-related disturbances occur in many common diseases. In our previous lipidomics studies, for example, we found that dysregulated lipid metabolism precedes islet autoimmunity and overt disease in children who later progressed to T1D (22,23).

Here, we applied a lipidomics approach, in a longitudinal study setting, with the aim of elucidating the early events preceding onset of overt CD.

**METHODS**

**Study design and protocol**

The children included in the present study are from the Type 1 Diabetes Prediction and Prevention (DIPP) cohort, which is an ongoing prospective study initiated in 1994. In the DIPP study, parents of newborn infants at the University Hospitals of Turku, Tampere, and Oulu in Finland are asked for permission to screen the child for HLA alleles conferring the risk of T1D, using umbilical cord blood. Families of children identified as having an increased HLA-conferred risk of T1D are invited to join the study. Our analysis included children born at Tampere University Hospital between August 1999 and September 2005. During that period, 23,839 children were screened at birth for increased risk of T1D, and 2,642 eligible children were enrolled in the follow-up and had at least 2 visits to the study clinic. These children carry the high-risk HLA DQB1*02/*03:02 genotype or the moderate-risk HLA-DQB1*03:02/x genotype (x ≠ DQB1*02, 03:01, or 06:02). More than 1,200 of these children took part in the DIPP-CD study. The DIPP study in Tampere followed the children at regular intervals at the ages of 3, 6, 12, 18, and 24 months and subsequently at intervals of 12 months for children without T1D-related autoantibodies. At each visit, the families were interviewed for diet, infections, growth, and important family-related issues, and the children gave a nonfasting venous blood sample. The children were followed for 4 CD-related antibodies, anti-tissue transglutaminase (anti-tTG), anti-endomysium, anti-gliadin (AgA-IgG and AgA-IgA), and anti-reticulin antibodies, and for T1D-associated autoantibodies, islet cell antibody. If a sample was positive for islet cell antibody, insulin autoantibody, antibodies against tyrosine phosphatase-like protein (IA-2A), and glutamate decarboxylase were measured from all samples taken from that child. From the beginning of 2003, all samples were measured for the 4 T1D-associated autoantibodies.

All children participating in this study were of white origin. They were initially identified based on positivity for IgG class tissue transglutaminase antibodies. As a consequence, IgA-deficient subjects were excluded. One of the mothers had CD. Furthermore, the children in the CD follow-up cohort were annually screened for anti-tTG antibodies (tTGAs) using a commercial kit (Celikay Pharmacia Diagnostics, Freiburg, Germany). If any given child’s sample was found to be positive for tTGA, all of that child’s previous and following samples were analyzed for the entire set of CD-related antibodies. A duodenal biopsy was recommended for all tTGA-positive children. If the biopsy was consistent with the ESPGHAN criteria of 1990, a gluten-free diet (GFD) was recommended. Three of the CD cases were also diagnosed with T1D, one just before the diagnosis of CD and the other ones after the diagnoses of CD (62 and 76 months later).

We randomly selected 23 children (12 males and 11 females) with biopsy-proven CD (progressors) and a control for each progressor matched for age, sex, and with the same risk HLA alleles, and living in the Tampere region throughout the whole follow-up period. None of the controls were diagnosed with T1D. The original HLA screening for patients with CD and suitable controls was also completed to a “full-house” DQ typing to verify the presence of DQA1*05 in selected DQB1*02-positive children (24). These clinical and genetic annotations of the participants are given in Table 1. Furthermore, the children were exclusively breastfed until 1.6 months (median), and some were exclusively breastfed up to age 8.0 months. The first exposure to gluten in this study was at a median age of 6.0 months. The children (case and control) had comparable (nominal and nonsignificant difference) energy (kJ), fat (g), carbohydrate (oat, rye, and wheat), and gluten intakes (see Figures S1 and S2, Supplementary Digital Content 1, http://links.lww.com/CTG/A44).

Maternal total diet during pregnancy and lactation was ascertained by a validated food frequency questionnaire (25). Infant feeding was studied by structured questionnaires, which families filled in at home, and which were checked during the clinic visits. The child’s diet was assessed with 3-day food records at 3, 6, and 12 months, followed by 2, 3, 4, and 6 years of age visits. The food records were posted before the study visit or given at the previous visit with detailed, written instructions. The families and
daycare personnel were instructed to write down all the foods, drinks, and dietary supplements the child consumed, with the amounts and brand names during 1 weekend day and 2 weekdays. The study nurses checked the records at the study visit and helped to estimate any incomplete portion sizes with a picture booklet. The collection, processing, and calculation of food consumption to estimate any incomplete portion sizes with a picture booklet. The study nurses checked the records at the study visit and helped to estimate any incomplete portion sizes with a picture booklet.

Table 1. Demographic and clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Celiac disease</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>HLA DR-DQ genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DR3)-DQA1<em>05-DQB1</em>02/</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>DRB1<em>04-DQA1</em>03-DQB1*03/2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Duration of exclusive breastfeeding (median)</td>
<td>1.4 mo</td>
<td>1.6 mo</td>
</tr>
<tr>
<td>Duration of breastfeeding (median)</td>
<td>8.0 mo</td>
<td>8.0 mo</td>
</tr>
<tr>
<td>Age at first gluten intake (median)</td>
<td>6.0 mo</td>
<td>5.5 mo</td>
</tr>
<tr>
<td>Age at first tTGA positivity (median) (seroconversion)</td>
<td>58 mo</td>
<td>—</td>
</tr>
<tr>
<td>First tTGA level in plasma (median endotoxininduced uveitis (EIU))</td>
<td>116.8</td>
<td>—</td>
</tr>
<tr>
<td>Age at endoscopy (median)</td>
<td>58.5 mo</td>
<td>—</td>
</tr>
<tr>
<td>IFI level after GFD (median endotoxininduced uveitis (EIU))</td>
<td>7.1</td>
<td>—</td>
</tr>
<tr>
<td>Total follow-up age from birth (median)</td>
<td>6.1 yr</td>
<td>6.2 yr</td>
</tr>
<tr>
<td>Plasma samples analyzed by lipidomics</td>
<td>121</td>
<td>112</td>
</tr>
<tr>
<td>EIU, endotoxininduced uveitis; GFD, gluten-free diet; tTGA, tissue transglutaminase antibody.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Persistent Alterations in Plasma Lipid Profiles

The statistical analysis was performed using the “stats” package. The effect of different factors such as age, sex, and condition (healthy or CD) on the lipidomics data set was evaluated. The data were centered to zero mean and unit variance. The relative contribution of each factor to the total variance in the data set was estimated by fitting a linear regression model, where the normalized intensities of metabolites were regressed to the factor of interest, and thereby, median marginal coefficients ($R^2$) were estimated. This analysis was performed using the “stats” package (see Table S1, Supplementary Digital Content 1, http://links.lww.com/CTG/A44). Partial least squares discriminant analysis (PLS-DA) (29) and variable importance in projection (VIP) scores (30) were estimated by an array of functions coded in R package. Moreover, the PLS-DA models were cross-validated (31) by 7-fold cross-validation as implemented in “ropls” package. Furthermore, the PLS-DA models were cross-validated (31) by 7-fold cross-validation as implemented in “ropls” package, and $Q^2$ (an estimate of model’s predictability) and $R^2$ were obtained.

The longitudinal profiles of the lipids in the samples obtained from CD progressors and controls were compared using linear mixed-effects (LMEs) models (32) as implemented in the “nlme” function of “nlme” package. The intensity of a metabolite (i) in a sample (j) is a function of multiple factors such as follow-up age, sex, case/control, and subject-wise variability. The clinical and genetic factors of the children were matched and standardized in this study (Table 1); other factors such as subject-wise variability can be modeled and derived using LME models. The LME models were restricted to constant terms with the fixed effect being CD progression/nonprogression (control), age, sex, and the random effect being subject-wise variation in comparison to the group-specific mean level. Fitted LME models showed that sex difference has nominal effect on the metabolite intensities. The fully parametrized model was compared with a null model using analysis of variance (ANOVA) (33) (“aov” as deployed in the “stats” package). The lipid profiles that changed significantly ($P < 0.05$) were subjected to false discovery rate (FDR) adjustment using p-adjust(). Lipid profiles with FDR <0.05 were listed.

Next, post hoc analysis using the Tukey test for honest significant difference (HSD) was performed on these selected lipids to see, if at all, they are changing between progressors and non-progressors at a particular age. A list of differentially altered lipids that also showed HSD ($P < 0.05$) between CD progressors and healthy controls at a particular age was marked. These lipids were considered for further analysis.
correlation coefficient was calculated using “rcorr()” function implemented in “Hmisc” package. “Heatmap.2()” and “boxplot()” were used for data visualization.

RESULTS
Global plasma lipidome in progression to CD
The complete lipidomics data set was first explored using multivariate analysis. Among other factors affecting the lipidome, age was found to be a major confounding factor (see Figure S8, Supplementary Digital Content 1, http://links.lww.com/CTG/A44). PLS-DA (29) of lipidomics data from all 233 longitudinal samples suggested that children who later progressed to CD (progressors) may have different lipid profiles in comparison to their matched healthy controls (Figure 1a). Different classes of lipids such as cholesterol esters (CEs), phosphatidylcholines (PCs), lysophosphatidylcholines, phosphatidylethanolamines, phosphatidylinositol, sphingomyelins (SMs), and triacylglycerols (TGs) were affected (regression coefficient [≥0.05] and VIP scores (30) >1) (Figure 1b).

Differences between cases and controls were observed already by age 3 months, for example, before the introduction of gluten to the diet (Figure 1c). TGs, PCs, and CEs were mostly affected (regression coefficient [≥0.05] and VIP (30) scores >1) at this age (Figure 1d). The cord plasma lipidome clustered distinctly from other age groups (see Figure S9, Supplementary Digital Content 1, http://links.lww.com/CTG/A44). However, no significant differences (HSD, P values > 0.05) in the cord plasma lipids were observed between CD progressors and their matched healthy controls (Figure 2a).

Molecular lipids in progression to overt CD
Longitudinal analysis of lipidomic profiles at the individual lipid level identified 80 molecular lipids (from a total of 239) occurring at significantly different levels over time between CD progressors and healthy controls (FDR-adjusted P values < 0.05) (Figure 2a).

Figure 1. Classification of CD and healthy controls, based on their lipidome. (a) PLS-DA score plot showing difference in the lipidome between CD progressors (cyan) and healthy controls (magenta). (b) A regression plot of the lipids and their VIP scores obtained from the PLS-DA model. The lipids are grouped and color coded by their classes. (c) PLS-DA score plot showing difference in the lipidome in CD progressors along the age. (d) A regression plot of the lipids and their VIP scores at age 3 months. The lipids are grouped and color coded by their classes. CD, celiac disease; PLS-DA, partial least squares discriminant analysis; VIP, variable importance in projection.
The level and direction of regulation of TGs depend on their chemical structure. There was an association between the fold change in TGs (CD progressors vs healthy controls) and the TG double bond count as well as the TG carbon number (Figure 2b–e). At age 3 months, TGs with lower double bond and carbon numbers were found to have increased in CD progressors as compared to matched, healthy controls, with coefficients ($R = 0.13; P = 0.19$) and ($R = 0.54; P = 0.0001$), respectively, for double bond count and carbon number (Figure 2b, d). Interestingly, at a later age, these TGs with lower double bond and carbon numbers were downregulated with ($R = 0.79; P = 0.08$) and ($R = 0.44; P = 0.0002$), respectively (Figure 2c, e). Notably, 4 such TGs were elevated in progressors at age 3 months (HSD, $P < 0.05$). No significant changes were detected for dietary TGs, such as those containing polyunsaturated fatty acids. Representative longitudinal profiles for selected significantly altered lipids between CD progressors and healthy controls are shown in Figure 3.

Impact of tissue transglutaminase antibodies on lipidomic profiles

Tissue transglutaminase (tTG) is a multifunctional, calcium-dependent enzyme (Enzyme Commission number [EC] 2.3.2.13) that plays an important role in the pathogenesis of CD. Antibodies produced against tTG, anti-tissue transglutaminase antibodies (tTGA), are used as serological markers with high sensitivity (99%) and reasonable specificity (>90%) for the diagnosis of CD (34–36).

We examined tTGA titers in 23 CD progressors at 2 different stages: (i) immediately after the seroconversion for tTGA and (ii) 6–12 months after both seroconversion and introduction of GFD. First, we identified at least 7 “essential” TGs, i.e., TGs of dietary origin, in our lipidomics data set. The identities of these TGs were validated using external sources including both the Human Metabolome Database (37) and FooDB.ca (http://foodb.ca/). Spearman correlation analysis was then performed between the levels of selected TGs (34 significantly changed nonessential and 7 nonsignificantly changed essential TGs) and PCs (18 PCs $\pm$ 2 lysophosphatidylcholines) with measured tTGA levels at these stages. The aim was to determine whether the systemic levels of TGs and PCs are associated with antibody titers during CD progression. A positive correlation (Spearman rank coefficient, $\delta_{avg} = 0.174$; minimum $P$ value = 0.01) was found between the TG and tTGA levels after seroconversion (Figure 4a). However, a negative correlation was found at a later age, where the levels of tTGA decreased considerably ($\delta_{avg} = -0.163$; minimum $P$ value = 0.05). In addition, at least 16 PCs were negatively correlated at these 2 different time points ($\delta_{avg} = -0.02$ and $-0.13$; minimum $P$ values $= 0.01$ and 0.02, respectively) (Figure 4b). It is known that GFD decreases...
tTGA levels in patients with CD (38), while it also improves the total cholesterol levels and high density lipoprotein profiles without any significant increase in low density lipoproteins (39).

**Impact of gluten on molecular lipids**

We then divided the cohort into 3 subgroups: (i) before the introduction of gluten in the diet (3 months), (ii) after the introduction of gluten (12–36 months), and (iii) after the introduction of GFD (72 months, CD progressors only).

We observed a decrease in the total essential TG level in the plasma of the CD progressors after gluten intake (Figure 5a; see Figure S10A, Supplementary Digital Content 1, http://links.lww.com/CTG/A44). Introduction of GFD reversed this trend, but the changes were not significant (ANOVA, P values > 0.05) (Figure 5a, e). On the other hand, levels of nonessential endogenous TGs were decreased (ANOVA, P values < 0.004) in the plasma of the CD progressors after commencement of gluten intake and even after the diagnosis of clinical CD and introduction of GFD (P values < 0.001). The trend in the change of nonessential TGs was also observed in healthy controls (P values = 0.007 and 0.01, respectively) (Figure 5b, e; see Figure S10B, Supplementary Digital Content 1, http://links.lww.com/CTG/A44). Moreover, there was a difference (P < 0.05) in nonessential TGs levels between CD progressors and healthy controls as predicted by the Tukey test for HSD (Figure 2a).

These findings suggest that dysregulation of lipid metabolism might occur at an early stage of CD progression, even before the introduction of gluten in the diet.

PCs were elevated both in the progressors (P < 0.004) and controls (P < 0.007) after the commencement of gluten intake (Figure 5c, e; see Figure S10C, Supplementary Digital Content 1, http://links.lww.com/CTG/A44). PCs are the major class of phospholipids that form the cellular constituents required for the assembly of biological membranes. In contrast to healthy controls, no significant differences in the SM level were observed in the CD progressors after commencement of gluten intake. A difference in the plasma SM level was observed only at a later age after the introduction of GFD (Figure 5d, e; see Figure S10D, Supplementary Digital Content 1, http://links.lww.com/CTG/A44).

**DISCUSSION**

We identified systematic differences in plasma lipidomes between children who progressed to clinical CD during the follow-up as compared to children who remained healthy. These differences were observed before the first exposure to gluten in the diet and before the first signs of CD-associated autoimmunity. The dysregulation of plasma lipidome in CD progressors was dominated by complex lipids such as PCs, TGS, and CEs. Because there is no evidence of gut damage in any studies performed so far in individuals who are autoantibody negative for tTGA, it is unlikely that these systematic differences are caused by gluten from breast milk or other sources in the infants’ diet causing damage to the gut immediately after birth. An earlier lipidomics study in the PreventCD cohort did not find significant differences between CD progressors and matched controls at age 4 months (40). However,
that study used a targeted analysis focusing on a subset of phospholipids and acyl-carnitines and did not measure TGs and CEs, where the major changes were found to have occurred in our study.

In this study, several nonessential TGs were upregulated in CD progressors as early as age 3 months, i.e., before the first introduction of gluten to the diet. However, no significant changes in dietary TGs were found at this age. Lipid malabsorption is believed to be a side effect of flattened villi in the small intestine (15,17). However, our data suggest that lipid-related abnormalities are not caused by CD-related villous atrophy in the gut. One plausible explanation that arises is that lipid malabsorption can lead to a reduction in cholesterol-transporting lipoproteins and secretion of apolipoprotein-A1 (41,42). A decrease in the level of CEs in CD progressors during the first 3 months after birth supports this explanation. Moreover, CEs were upregulated at a later age after the introduction of GFD (Figures 2a and 3a). Lipid malabsorption may thus occur at a very early age in CD progressors and, therefore, de novo lipogenesis (43), as reflected by increased levels of TGs with low carbon number and double bond content, may be necessarily increased to compensate for said compromised lipid uptake. Interestingly, this phenomenon was not observed at birth (i.e., in the cord blood data), thus suggesting that the observed lipid dysregulation is not an inborn phenomenon.

Furthermore, an inverse relationship between endogenous TGs and tTGA titer after seroconversion and introduction of GFD in the CD progressors also reaffirms the involvement of TGs and other phospholipids (PCs) in CD progression. Indeed, the first 3 months after birth supports this explanation.
there was an increase in cholesterol levels after the introduction of GFD, and there was a decrease in tTGA titers, in agreement with previous studies (38,39). Presumably, these lipids may play a protective role against seroconversion to tTGA positivity and CD progression.

We found, in earlier studies, that T1D is preceded by dysregulation of lipid metabolism (22,44,45). The key findings from these studies were that islet autoimmunity and overt T1D are preceded by diminished phospholipid and TG levels. Although there are some similarities at an early age in phospholipid profiles in T1D and, as shown in the current study, CD progressors, no such similarities exist for TGs. The increase of TGs with low carbon number and double bond count does seem to be specific to CD progression. These TGs are associated, in adults, with elevated liver fat in nonalcoholic fatty liver disease (46,47), reflecting increased de novo lipogenesis and adipose tissue lipolysis. In fact, patients with CD have, interestingly, been found to be at an increased risk of nonalcoholic fatty liver disease (48). Our findings may thus offer an explanation for this association.

The main limitation of this study is the relatively small number of children included, and for that reason, our findings should be considered preliminary and hypothesis generating. However, the sample size is mitigated by the longitudinal study setting, with 5 prospective samples, from birth until after the introduction of GFD, on average, from each child. Moreover, the subjects of our study were well matched with their controls for genetic and environmental factors. In the current study, the patients were initially screened for HLA-conferred susceptibility to T1D. There was an overrepresentation of DR3/DR4-heterozygous (n = 17) and DR4-positive subjects (n = 6) in the study series. In a general population, most CD cases are DR3 positive. In our study, there were no significant differences in the lipidomic profiles between these 2 HLA groups. Nevertheless, because of this selection bias, our findings may not be translatable to other populations and thus need to be confirmed in a setting more representative of a general population.

In summary, our study suggests that lipid-related abnormalities in CD progressors demonstrably occur before the first introduction of gluten to the diet. These changes may be related to impaired lipid absorption and de novo lipogenesis, with both of these being part of the host response. Our preliminary findings may have important clinical implications for the detection of subjects at risk of CD and for the understanding of its early pathogenesis.

Data accessibility
The lipidomics data sets and the clinical metadata generated in this study have been submitted to MetaboLights (49) and can be located using accession number (MTBLS729).
Clinical metadata were linked to the lipidomics data set using the ISA-creator package from Metabolights.

Ethical approval and informed consent
The Ethics Committee of Tampere University Hospital approved the study. Written informed consent was obtained from the parents for HLA screening, autoantibody analysis, and intestinal biopsies.

CONFLICTS OF INTEREST
Article guarantor: Dr. Matej Orešič, PhD.
Specific author contributions: M.O. and M.K. designed and supervised the study. C.C. performed lipidomics experiments, which were supervised by T.H. P.S. analyzed the data. S.V., S.S., H.H., J.L., J.T., and R.V. contributed to the design of the clinical study. P.S. and M.O. wrote the manuscript. All authors critically reviewed and approved the final manuscript.

Financial support: This study was supported by the Academy of Finland (Centre of Excellence in Molecular Systems Immunology and Physiology Research—SyMMYS, Decision No. 250114, to M.O. and M.K.; and Personalised Health 2014 programme project, Decision No. 292568).

Potential competing interests: None declared.

ACKNOWLEDGEMENTS
We thank the families participating in the DIPP study for making this study possible; the expert staff of the DIPP study for their excellent work with the participating research families and sample collection; Professor Olli Simell for his important scientific contribution to the DIPP study; Dawei Geng for technical assistance in lipidomic analysis; and Aidan McGlinchey for editing the manuscript.

Study Highlights

WHAT IS KNOWN
✓ Previous studies have shown that individuals with clinical CD have altered lipid profiles as compared to nongenetically and non–age-matched control groups.
✓ Introduction of gluten to one’s diet has always been considered to be the main trigger for this disease, yet it has been unknown what are the other contributing factors needed for disease initiation.

WHAT IS NEW HERE
✓ Our study suggests that there are alterations in lipid metabolism before first signs of autoimmunity or disease symptoms in subjects who later progress to clinical CD as compared to healthy controls with the same HLA-associated risk. The dysregulated lipid profiles were observed already at age 3 months between CD progressors and healthy controls, i.e., before the first introduction of gluten in the diet. Furthermore, these differences exacerbated with age but were not observed at birth.

TRANSLATIONAL IMPACT
✓ The plasma lipid markers identified in this study may help to predict and isolate early changes in the lipidome of the children, who later progresses to CD.

REFERENCES


43. Sen et al.