Circulating IGF1 and IGFBP3 in relation to the development of β -cell autoimmunity in young children

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Abstract

Objective: This study aimed at investigating the role of IGF1 and IGF binding protein 3 (IGFBP3) in the development of β -cell autoimmunity.

Methods: Five hundred and sixty-three subjects with HLA-conferred susceptibility to type 1 diabetes (T1D) were monitored for signs of seroconversion to positivity for insulin and/or GAD, IA2, and zinc transporter 8 autoantibodies by the age of 3 years. In 40 subjects who developed at least one autoantibody, IGF1 and IGFBP3 plasma concentrations were measured and compared with 80 control subjects who remained negative for autoantibodies, and were matched for age, sex, country of origin, and HLA genotype. The increments of IGF1, IGFBP3, and IGF1/IGFBP3 molar ratio before and after seroconversion were compared with corresponding time intervals in controls.

Results: The IGF1 concentrations at the age of 12 months and the IGF1/IGFBP3 ratio at the age of 24 months were lower in the autoantibody-positive children ($P < 0.05$). The increase in circulating IGFBP3 was significantly higher in the autoantibody-positive children before seroconversion than in the corresponding time intervals in controls (0.43 mg/l; 95% CI 0.29–0.56 vs 0.22 mg/l; 95% CI 0.10–0.34 mg/l; $P < 0.01$). Children carrying the high-risk HLA genotype had lower plasma IGF1 and IGFBP3 concentrations at the age of 24 months than those with low-risk genotypes ($P < 0.05$ and < 0.01 respectively).

Conclusions: Circulating IGF1 and IGFBP3 appear to have a role in early development of β -cell autoimmunity. The decreased IGF1 concentrations in children with the high-risk HLA genotype may contribute to the reduced growth previously described in such children.

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Introduction

A growing body of evidence indicates an important role of insulin-like growth factor 1 (IGF1) in the regulation of islet β -cell growth, survival, and metabolism, with effects suggested to provide protection against type 1 diabetes (T1D) (1, 2, 3, 4, 5). The ability of the pancreas to generate new β -cells in response to an increased metabolic demand

or after injury has been described in experimental diabetes models and, importantly, in infants with T1D (6). The possible role of IGF1 in the regeneration of β -cells is based on studies demonstrating an increased IGF1 expression in focal areas of regeneration in rats and dogs with partial pancreatectomy (1, 2). Further studies showed that IGF1

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increases the replication and proliferation of pre-existing β -cells after damage, (3) and protects β -cells from apoptosis (4). Moreover, several studies have demonstrated that IGF1 is also involved in the regulation of immune tolerance. For example, the expression of IGF1 in the pancreatic islets of transgenic mice protects islets from lymphocytic infiltration, counteracts cytotoxicity and insulinitis induced by damage with multiple low doses of streptozotocin (5). In another mouse model, the IGF1 expression in the liver suppresses the progression of autoimmune diabetes and is associated with decreased islet inflammation (7).

The liver is the principal source of IGF1, producing up to 80% of circulating IGF1 (8), while the other 20–25% is synthesized locally by various tissues and acts mostly in an autocrine or paracrine manner (9). The role of circulating IGF1 in paracrine and autocrine regulation has not been fully understood. However, it has been shown that treatment with IGF1 protects islets against cytokine-mediated inhibition of insulin secretion and cell death by apoptosis (10), implying an important role of circulating IGF1 in the survival of pancreatic islets. There have also been studies demonstrating that the administration of IGF1 into the systemic circulation reduces the severity of insulinitis and delays the onset of diabetes in non-obese diabetic mice (11, 12).

The vast majority of IGF1 is bound to a family of six structurally and evolutionally related IGF-binding proteins (labelled as IGFBP 1–6), mostly to IGFBP3 (13, 14). The majority of IGF1 circulates in a large (150 kDa) ternary complex with IGFBP3 and an acid-labile subunit (ALS) (14). The major role of IGFBP3 is the transport of IGF1, prolonging its half-life time, and controlling its access to the IGF1 receptor (15). However, IGF-binding proteins also have functions that are considered to be IGF1 independent (15, 16). The possible role of those IGF1 independent actions in the pathogenesis of diabetes is not clear, but some cross-sectional studies have observed that individuals with impaired glucose tolerance or T2D have lower IGFBP1 and higher IGFBP3 levels when compared to controls (17, 18). A large, prospective case–control study has demonstrated a strong positive association between circulating IGFBP3 and T2D (19).

As the growth hormone (GH)-IGF1 axis is the major determinant of childhood growth, the increased linear growth observed in children with T1D before the manifestation of the disease (20, 21) implies a possible role of IGF1 in the pathogenesis of T1D in humans. Some previous studies have reported that the childhood growth is related, at least partly, to different HLA genotypes

conferring risk or protection against T1D (22, 23, 34). In particular, our previous work has demonstrated that subjects with the high-risk HLA genotype had significantly slower linear growth and weight gain compared to those with low-risk HLA genotypes (22). To our knowledge, the possible interaction between the IGF1/IGFBP3 system and HLA genotypes has not been studied before. Moreover, despite a large body of evidence implicating a role for the IGF1/IGFBP3 system in the development of T1D (1, 2, 3, 4, 5, 7, 11, 12), there is only one study on circulating IGF1 and IGFBP3 concentrations in prediabetic children with early signs of β -cell autoimmunity, i.e. positivity for diabetes-associated autoantibodies (24). That study did not find any difference between the level of IGF1 or IGFBP3 in subjects developing autoantibodies compared to those without autoantibodies (24). Therefore, because of the paucity of relevant studies, we set up a study to investigate the possible role of circulating IGF1 and IGFBP3 in the development of diabetes-associated autoantibodies in children with HLA-conferred susceptibility to T1D during the first 3 years of life.

Subjects and methods

Study population

The study subjects were derived from 258 Estonian and 305 Finnish children with HLA-conferred susceptibility to T1D, monitored for the development of diabetes-associated autoantibodies from birth up to the age of 36 months as a part of the DIABIMMUNE study. We included all 17 autoantibody-positive cases from Estonia (four with multiple autoantibodies, including one case that developed T1D by the age of 2.4 years) and all 23 autoantibody-positive cases from Finland (eight with multiple antibodies, including three cases that developed T1D by the age of 3.2, 3.7, and 2.6 years). In addition, for each case we selected two controls from the same DIABIMMUNE cohort with no diabetes-associated autoantibodies matched for sex, country of origin, and HLA-genotype and date of birth that were as close as possible. In this way 80 controls were selected for 40 autoantibody-positive subjects. All children were born between September 2008 and August 2010. The Estonian and Finnish study subjects were monitored according to the same protocol. The local Ethics Committees approved the study and the parents of all children gave written informed consent to participation in the study.

HLA genotyping

HLA genotyping was performed with a PCR-based lanthanide-labeled hybridization method using time-resolved fluorometry for detection as previously described (25). Initially, the samples were analyzed for the presence of HLA *DQB1**02, *DQB1**03:01, *DQB1**03:02, and *DQB1**06:02/3 alleles, as described by Kiviniemi *et al.* (26). Depending on the result of the initial analysis, the further analyses involved a low-resolution *DQB1* full-house typing, *DQA1* typing with three allele-specific probes, and subtyping of *DR4* (25). Samples positive for the *DQA1**05-*DQB1**02 combination (i.e. the DR3-DQ2 haplotype) and/or those with *DQB1**0302/4 without the presence of *DRB1**0403/6 (DR4-DQ8) were included into the study if no protective haplotype (i.e. *DQB1**06:02/3, *DQB1**05:03, or the combinations of *DQA1**05-*DQB1**03:01 or *DQA1**02:01-*DQB1**03:03) was found.

According to Hekkala *et al.* (27), all subjects were divided into three groups, depending on their HLA alleles. The subjects with the combination of two susceptibility-associated haplotypes, i.e. the DR3-DQ2/DR4-DQ8 genotype, were categorized as the high-risk group ($n=18$), those carrying the DR4-DQ8/X genotype (X=a non-protective allele, but not DR3-DQ2) as a group with moderate risk ($n=57$), and those with the DR3-DQ2/Y genotype (Y=a non-protective allele, but not DR4-DQ8) as a group with low, although increased, risk for T1D ($n=45$).

Diabetes-associated autoantibodies

Insulin autoantibodies (IAAs), and antibodies to glutamic acid decarboxylase (GADA), insulinoma-associated 2 (IA2A), and zinc transporter 8 antibody (ZnT8A) were analyzed with specific radiobinding assays, as described by Knip *et al.* (28). The cut-off level was 2.80 relative units

(RU) for IAA, 5.36 RU for GADA, 0.78 RU for IA2A, and 0.61 RU for ZnT8A. All samples obtained from cord blood (0 months) and at the follow-up visits at the ages of 3, 6, 12, 18, 24, and 36 months were analyzed for those four biochemical autoantibodies. Islet cell antibodies were analyzed with indirect immunofluorescence on the human blood group 0 donor pancreas from all samples from any child testing positive for one or more of the biochemical autoantibodies. The detection limit was 2.5 Juvenile Diabetes Foundation units.

IGF1 and IGFBP3 measurements

Plasma concentrations of IGF1 and IGFBP3 were measured with a solid-phase, enzyme-labeled chemiluminescent immunometric assay with the IMMULITE 2000 analyzer (Diagnostic Products, Los Angeles, CA, USA), according to the manufacturer's protocols. The detection limit for IGF1 was 25 μ g/l. According to Elmlinger *et al.* (29), the intra-assay and inter-assay coefficients of variability for that method are <4 and 8%, respectively, for IGF1 and <4.5 and 7% respectively for IGFBP3. In autoantibody-positive subjects, plasma IGF1 and IGFBP3 concentrations were measured from samples obtained at the age when seroconversion to positivity for at least one autoantibody was detected (T1) and at the closest time points before (T0) and after seroconversion (T2). For children with multiple autoantibodies, the IGF1 and IGFBP3 concentrations were measured from all available time points. In controls (autoantibody-negative children) IGF1 and IGFBP3 were analyzed from samples taken at the same time points as in the corresponding case children. The number of subjects studied at different time points is presented in Table 1.

Table 1 Numbers of IGF1 and IGFBP3 measurements at different ages in subjects with different HLA-related risk for T1D.

Age in months	HLA risk-groups						Number of subjects with seroconversion
	Low risk (n=45)		Moderate risk (n=57)		High risk (n=18)		
	Aab + (n=15)	Aab – (n=30)	Aab + (n=19)	Aab – (n=38)	Aab + (n=6)	Aab – (n=12)	
0 (n=5)	1	3	0	1	0	0	0
3 (n=19)	3	7	2	6	1	0	1
6 (n=39)	6	11	5	11	2	4	4
12 (n=67)	9	16	12	21	3	6	8
18 (n=98)	12	18	16	34	6	12	12
24 (n=79)	9	15	15	28	4	8	3
36 (n=54)	5	7	10	23	3	6	11

Aab-, Aab+ - autoantibody negative and autoantibody positive respectively.

Statistical analysis

The data were analyzed with the R-program (R 2.15.2, GraphPad, San Diego, CA, USA) and SAS (SAS 9.2, SAS Institute, Inc., Cary, NC, USA). The molar ratio of IGF1/IGFBP3, the possible marker of free bioactive IGF1 in plasma (30), was calculated on the basis of molecular weights of 7.6 kDa for IGF1 (31) and 42 kDa for IGFBP3 (32). The normality of data distribution was controlled using the Kolmogorov-Smirnov test. In cases when the concentration of IGF1 was below the detection limit, a level of 25 μ g/l was used for the statistical analysis.

To compare the possible differences in plasma IGF1 and IGFBP3 concentrations and IGF1/IGFBP3 ratios between subjects with different HLA-risk groups for T1D, the ANOVA test with *post-hoc* Tukey HSD analysis was used. If the data were not normally distributed, the Kruskal-Wallis test with the *post-hoc* Bonferroni (Dunn) *t*-test was applied. For comparison of IGF1, IGFBP3, and IGF1/IGFBP3 molar ratio between autoantibody-positive and negative subjects, an unpaired two-tailed Student's *t*-test or, in the case of data with a skewed distribution, the Mann-Whitney's *U* test were applied.

The changes in plasma IGF1 and IGFBP3 concentrations and in IGF1/IGFBP3 ratio were calculated between the time point of seroconversion (T1) and the closest time points before seroconversion (T0) to positivity for at least one autoantibody (T1–T0), as well as the closest time points after seroconversion (T2–T1). In matched control subjects, the changes in IGF1 and IGFBP3 concentrations as well as in the IGF1/IGFBP3 ratio were calculated between the same time points corresponding to those used in the cases. The paired *t*-test was applied to compare the changes in IGF1, IGFBP3, and IGF1/IGFBP3 ratio between cases and controls.

For the subgroup of autoantibody-positive children with multiple autoantibodies ($n=12$), a paired *t*-test analysis of possible changes in IGF1, IGFBP3, and IGF1/IGFBP3 ratio between the time points before and at the time of seroconversion to positivity for multiple antibodies was performed as well. In addition, the four subjects who progressed to clinical T1D were compared to their controls applying the paired *t*-test. A *P* value <0.05 was considered statistically significant.

Results

IGF1 and IGFBP3 age-related changes in relation to HLA risk genotypes for T1D

In general, there is a rapid decline in IGF1 concentrations after birth up to the age of 6 months, followed by a gradual

recovery until the age of 18 months, when the levels are comparable with those at birth. After that, the IGF1 concentrations continue to increase slowly. Similar age-related changes were also seen in the IGF1/IGFBP3 ratio. The IGFBP3 concentrations are, in contrast, lowest in cord blood and increase continuously throughout the study period. At the age of 24 months, the mean IGF1 concentration was significantly lower in the children carrying the high-risk HLA genotype for T1D in comparison with the low-risk group (57.1 μ g/l, 95% CI 45.1–69.1 μ g/l vs 84.0 μ g/l, 95% CI 68.1–99.8 μ g/l, $P=0.02$; Fig. 1). Simultaneously, the mean IGFBP3 concentration in the high-risk group (2.3 mg/l, 95% CI 2.1–2.6 mg/l; $P<0.01$) was also significantly lower when compared with both the moderate-risk (2.5 mg/l, 95% CI 2.4–2.7 mg/l; $P<0.01$) and low-risk groups (2.9 mg/l, 95% CI 2.7–3.2 mg/l) (Fig. 2). There were no differences in the IGF1/IGFBP3 ratio at any time between the three HLA risk groups (Fig. 3).

Comparison between autoantibody-positive and negative subjects

There were no significant differences in plasma concentrations of IGF1 and IGFBP3 concentrations or

Figure 1

Box plot of plasma IGF1 concentrations over the first 3 years of life in the study participants in relation to their HLA risk genotype for type 1 diabetes. The ends of the whiskers show the lower and the upper quartile (Q1 and Q4 respectively). The horizontal bar at 0 months represents the value measured for a single subject from the moderate-risk group, and that at the age of 3 months is for a single subject from the high-risk group. The asterisk marks $P=0.02$ for the high risk vs the low-risk group.

Figure 2

Box plot of plasma IGFBP3 concentrations over the first 3 years of life in the study participants in relation to their HLA risk genotype for type 1 diabetes. The ends of the whiskers show the lower and the upper quartile (Q1 and Q4 respectively).

The horizontal bar at 0 months represents the value measured for a single subject from the moderate-risk group, and that at the age of 3 months is for a single subject from the high-risk group. The two asterisks mark $P < 0.01$ for the high risk vs both the moderate-risk and low risk groups.

IGF1/IGFBP3 ratio between the autoantibody-positive and negative children at any time point from birth up to the age of 36 months, except a slightly lower mean IGF1 concentration in autoantibody-positive subjects ($39 \mu\text{g/l}$, 95% CI $30\text{--}48 \mu\text{g/l}$) compared to the autoantibody-negative infants ($48 \mu\text{g/l}$, 95% CI $42\text{--}54 \mu\text{g/l}$) at the age of 12 months ($P < 0.05$), and a slightly lower IGF1/IGFBP3 ratio level in autoantibody-positive children at the age of 24 months (0.136 , 95% CI $0.117\text{--}0.156$ vs 0.151 , 95% CI $0.140\text{--}0.161$; $P < 0.05$). In the four cases that progressed to clinical T1D, the concentrations of IGF1 and IGFBP3 and the IGF1/IGFBP3 ratio were not different from other cases or controls.

Changes in IGF1, IGFBP3, and IGF1/IGFBP3 ratio before and after seroconversion

When comparing the changes in IGF1 and IGFBP3 concentrations, as well as in the IGF1/IGFBP3 molar ratio between different time points, the mean increment in IGFBP3 concentration preceding the seroconversion ($T1\text{--}T0$) was significantly higher in autoantibody-positive children compared to the increase during the

corresponding time interval in the autoantibody-negative subjects (0.43 mg/l 95% CI $0.29\text{--}0.56 \text{ mg/l}$ vs 0.22 mg/l , 95% CI $0.1\text{--}0.34 \text{ mg/l}$; $P = 0.005$; Fig. 4). In addition, there was a trend towards a decrease in mean IGF1 and IGFBP3 concentrations after seroconversion among the autoantibody-positive children ($T2\text{--}T1$, $P = 0.06$ for both) compared to the autoantibody-negative subjects. A sub-cohort analysis of the autoantibody-positive children with multiple autoantibodies and their autoantibody-negative controls did not show any significant differences in the increase in mean IGF1 and IGFBP3 concentrations or in the IGF1/IGFBP3 molar ratio during the interval before and after seroconversion to multiple antibodies. The children who developed T1D showed similar trends to the other cases.

Discussion

In this study we observed several significant associations between the IGF1/IGFBP3 system on one hand and the development of diabetes-associated autoantibodies and HLA genotypes conferring risk for T1D on the other. First of all, we found that at the age of 24 months the mean IGF1 and IGFBP3 concentrations in subjects with the high-risk HLA genotype for T1D (DR3-DQ2/DR2-DQ8) were significantly lower compared to the subjects with

Figure 3

Box plot of plasma IGF1/IGFBP3 molar ratio over the first 3 years of life in the study participants in relation to their HLA risk genotype. The ends of the whiskers show the lower and the upper quartile (Q1 and Q4 respectively). The horizontal bar at 0 months represents the value measured for a single subject from the moderate-risk group, and that at the age of 3 months is for a single subject from the high-risk group.

Figure 4

Mean plasma IGFBP3 concentrations in autoantibody-positive (Aab+) children compared to the controls (Aab-) before (T0), at (T1) and after (T2) seroconversion for at least one diabetes-associated autoantibody. The statistical standard error of the mean (S.E.M.) is shown by error bars. The increase preceding the seroconversion was significantly greater (** $P=0.005$) in the Aab+ children than during the corresponding time interval in the Aab- children.

low-risk HLA genotypes. The results are in line with our previous study in the entire DIABIMMUNE study cohort, where we found that by the age of 18–24 months the subjects with high HLA-risk genotypes for T1D demonstrated a pronounced slowdown in linear growth as well as in weight (22). The current results suggest that the decreased circulating IGF1 and IGFBP3 may be involved in the slower growth in children carrying the high-risk genotype. In the study by Beyerlein *et al.* (24), the association of IGF1 and IGFBP3 in relation to HLA status was not analysed.

The GH-IGF1 axis starts to play a major role in the regulation of human growth around the age of 2 years (33). This may explain why the difference in IGF1 and IGFBP3 concentrations between subjects with different HLA genotypes was seen only at the age of 24 months and not before. Accordingly, it would be interesting to follow these children to see whether a slower growth rate and lower IGF1 and IGFBP3 concentrations will continue to persist or whether this is just a temporary finding followed by some catch-up both in growth as well as in hormone concentrations. Studies in slightly older children (23, 34) and in adults (35), in whom increased HLA-conferred risk for T1D was observed to be associated with lower BMI, indicate that the former scenario is more likely to occur.

We suggest that the slower linear growth and weight gain in DIABIMMUNE subjects (22) and lower BMI in other children and adults with high HLA-conferred risk for T1D may be (23, 34, 35) associated with lower circulating concentrations of IGF1 and IGFBP3. Since the mechanisms behind the HLA-conferred genetic susceptibility to T1D are poorly defined (36), we could speculate that the reduced IGF1 concentrations in subjects with the high-risk HLA genotype could explain, at least partially, the HLA-related risk for T1D, given that high IGF1 concentrations protect against the development of T1D (4, 5, 7, 10, 11, 12). However, Fourlanos *et al.* (35) recently proposed that an increased BMI simply promotes the development of T1D in subjects carrying lower-risk HLA risk genotypes by increasing the penetrance of lower-risk HLA genes, thus increasing the proportion of lower-risk HLA genes among subjects with higher BMI. The presence of HLA genotype-related differences in BMI in children without any signs of T1D, as was reported by Yang *et al.* (34), or as shown in the present study in children with early signs of β -cell autoimmunity, speaks against that suggestion.

The most important result of our study is probably the demonstration of a significantly higher increment in IGFBP3 before seroconversion to autoantibody positivity when compared to the corresponding time interval in autoantibody-negative subjects. In only one relevant study (24), the IGF1 and IGFBP3 concentrations were compared at certain time points irrespectively of the time of seroconversion, whereas our study looked at the rate of IGF1 and IGFBP3 increments before the time of seroconversion. This approach evaluates more directly the role of IGF1 and IGFBP3 in the process of autoimmunity development.

Our initial hypothesis was that there should be a significant difference in the increment of IGF1/IGFBP3 molar ratio as one of the proposed markers of bioavailable IGF1 (30). To a lesser degree, we expected to see differences in the changes in IGF1 or IGFBP3 concentrations. In our study, only the latter was observed. The interpretation of an isolated higher increment of IGFBP3 concentrations in autoantibody-positive subjects compared to autoantibody-negative ones represents a challenge, as the serum IGFBP3 concentration is predominantly regulated post-translationally by stabilization through its GH-dependent binding partners IGF1 and ALS. Thus, at the serum level, IGFBP3 is GH-dependent (37), but at the transcriptional level is independent of GH (38). In some diseases, such as T1D and obesity, the serum IGF1 concentration is often within the normal range, whereas the GH level is high,

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reflecting some degree of disintegration of the GH/IGF1 axis (39). We suggest that this type of disintegration of the GH/IGF1 axis may exist to some extent also in subjects seroconverting to positivity for diabetes-associated autoantibodies. This suggestion is supported by the absence of any significant difference in the increment in the IGF1/IGFBP3 molar ratio when autoantibody-positive and negative children were compared in the period preceding the seroconversion (Fig. 4). Such a change in the IGF1/IGFBP3 molar ratio should be expected if the IGFBP3 increase in the two groups is not accompanied by a similar increase in the IGF1 concentration. As a matter of fact, a small difference in the mean IGF1/IGFBP3 molar ratio was actually seen between autoantibody-positive and autoantibody-negative subjects when the entire groups were compared at the age of 24 months. However, the observations regarding the IGF1/IGFBP3 molar ratio should be interpreted cautiously in terms of its biological role. In fact, it has recently been questioned whether the IGF1/IGFBP3 molar ratio is a reliable marker of free bioactive IGF1, as this has not been validated and proven experimentally (40). The molar ratio changes in the same direction as bioavailable IGF1 in some, but not all situations (39).

It is not clear whether IGFBP3 protects β -cells against autoimmune damage or accelerates the death of β -cells. Increasing IGFBP3 concentrations right before seroconversion to autoantibody positivity could be a compensatory reaction that increases the half-life of circulating IGF1, thus providing β -cell protection. An independent role of IGFBP3 in the development of autoimmune β -cell damage cannot be excluded. The IGF-independent apoptotic and growth-inhibitory effects of IGFBP3 have been seen in many different cell types (16); furthermore, in several large studies an association between IGFBP3 and the development of T2D has been demonstrated (17, 18, 19). Accordingly, the role of the fast increase in plasma IGFBP3 concentration right before seroconversion will remain a subject for further studies.

In addition to the lower IGF1/IGFBP3 ratio at the age of 24 months in autoantibody-positive children compared to autoantibody-negative peers, we also found a similar difference in the IGF1 concentrations at the age of 12 months. Both findings are different from those reported by Beyerlein *et al.* (24), according to whom the IGF1 and IGFBP3 concentrations at 9 and 24 months of age did not differ between seropositive and seronegative subjects.

Our study has several limitations. First, the number of children who developed multiple autoantibodies or T1D

was small. It is known that only a minority of subjects with one autoantibody will progress to clinical T1D (41). A second limitation is the modest sensitivity of the IGF1 assay with a lower detection limit of 25 μ g/l, as quite a large number of subjects (13 cases and 14 controls) had such results at the time point before seroconversion. The interpretation of the results provides also some challenges because of the relatively small number of IGF1 and IGFBP3 samples in the particular subgroups based on HLA-risk genotypes and the autoantibody status. This drawback was due to the limited availability of plasma samples from the study participants. We should also take into consideration possible confounding factors, such as the duration of breastfeeding, which is known to influence the serum IGF1 concentrations (42). However, the differences in IGF1 and IGFBP3 plasma concentrations were seen around 24 months of age, when the potential effect of breastfeeding should be marginal.

In conclusion, our study shows that circulating IGF1 and IGFBP3 may play a role in the development of β -cell autoimmunity in young children. The current results suggest a possible link between the previously reported association between HLA genotypes and linear growth rate, and weight gain in children with HLA-conferred susceptibility for T1D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

A Peet was responsible for the follow-up of the Estonian study cohort, and A-M Hämäläinen for the Finnish study cohort. A Peet was also responsible for the IGF1 and IGFBP3 analyses, conceived the study along with V Tillmann and M Knip, participated in its design, and drafted the manuscript. P Kool designed and performed all statistical analyses. J Ilonen was responsible for HLA testing, defined the HLA subgroups and helped to draft the manuscript. M Knip was responsible for autoantibody assays. All co-authors read and approved the final manuscript.

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