*Rapid communication***Short-term exclusive breastfeeding predisposes young children with increased genetic risk of Type I diabetes to progressive beta-cell autoimmunity****T. Kimpimäki¹, M. Erkkola³, S. Korhonen³, A. Kupila⁴, S.M. Virtanen², J. Ilonen⁵, O. Simell⁶, M. Knip⁷**¹ Department of Pediatrics, Tampere University Hospital, Tampere, Finland² School of Public Health, University of Tampere, Tampere, Finland³ Department of Pediatrics, University of Oulu, Oulu, Finland⁴ Department of Pediatrics, University of Turku, Turku, Finland⁵ Turku Immunology Center and Department of Virology, Turku, Finland⁶ The Juvenile Diabetes Foundation Center for Prevention of Type I Diabetes in Finland⁷ Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland**Abstract**

Aims/hypothesis. This study aimed to establish the relation between early infant nutrition and signs of beta-cell autoimmunity in young children.

Methods. We identified and observed from birth 2949 infants with increased genetic risk of Type I (insulin-dependent) diabetes mellitus (HLA DQB1*02/*0302 or DQB1*0302/x, x = other than *02, *0301 or *0602) and monitored them for islet cell antibodies at 3 to 6 month intervals. If an infant seroconverted to islet cell antibody positivity, all of his or her samples were also analysed for autoantibodies to insulin, GAD65 (GADA) and to the protein tyrosine phosphatase related IA-2 molecule (IA-2A). Our case-control study comprises the first 65 children who seroconverted to islet cell antibody positivity before the age of 4 years and 390 control children who were islet cell antibody-negative (six control children/case). We monitored the duration of exclusive and total breastfeeding and the age at which cows' milk was introduced.

Results. Infants who had been breastfed exclusively for at least 4 months had lower risk of seroconversion to positivity for IA-2A or all four autoantibodies [odds ratio (OR) 0.24; 95 % CI 0.06–0.94 and OR 0.17; 95 % CI 0.03–0.86, respectively] than those infants who had been breastfed exclusively for less than 2 months. The risk of seroconversion to positivity for IA-2A or all four autoantibodies was higher in those younger than 2 months (OR 4.37; 95 % CI 1.33–14.42 and OR 5.02; 95 % CI 1.27–19.89) or aged 2 to 3.9 months (OR 5.50; 95 % CI 1.21–25.04 and 6.19; 95 % CI 1.10–34.84) when they first received cows' milk than in those aged 4 months or older.

Conclusions/interpretation. These observations suggest that short-term breastfeeding and the early introduction of cows' milk-based infant formula predispose young children who are genetically susceptible to Type I diabetes to progressive signs of beta-cell autoimmunity. [Diabetologia (2001) 44: 63–69]

Keywords Type I diabetes, autoantibodies, HLA risk markers, breastfeeding, cows' milk

Received: 10 July 2000 and in revised form: 13 September 2000

Corresponding author: Mikael Knip, MD, Hospital for Children and Adolescents, University of Helsinki, P.O. Box 281, 00029 HUCH, Finland

Abbreviations: CI, 95 % confidence interval; DIPPP, Type I Diabetes Prediction and Prevention Project; GADA, antibodies to GAD65; GAD65, 65 kilodalton isoform of glutamic acid decarboxylase; HLA, human leukocyte antigen; IAA, insulin autoantibodies; IA-2A, antibodies of the protein tyrosine phosphatase related IA-2 molecule; ICA, islet cell antibodies; IQR, interquartile range; OR, odds ratio.

It has been suggested that various environmental factors initiate the autoimmune process of Type I (insulin-dependent) diabetes mellitus [1] but the causal role of breastfeeding or of early exposure to cows' milk in the destruction of beta cells remains controversial. Several experimental models of autoimmune diabetes indicate that dietary interventions aiming to modify the exposure to cows' milk proteins have a preventive effect [2,3] and a positive correlation has been found between the consumption of cows' milk and the incidence of Type I diabetes [4–6]. An inverse

relation between the duration of breastfeeding and the development of Type I diabetes was first reported in 1984 [7] and when a meta-analysis of 13 studies was carried out in 1994, short-term breastfeeding was associated with a 1.4-fold risk of Type I diabetes, while an early introduction of cows' milk resulted in a relative risk of 1.6 [8]. Risks of similar magnitude were observed 2 years later but the authors concluded that the relatively weak association between early exposure of cows' milk and the development of Type I diabetes might result from biases due to the methodological limitations of previous studies [9]. A Finnish case-control study reported an association between early exposure to cows' milk and an increased risk of Type I diabetes [10], independently of the duration of breastfeeding [11]. In immunological studies increased titres of cows' milk antibodies have been observed in children with newly diagnosed diabetes. This supports an association between exposure to cows' milk and Type I diabetes [12,13], whereas the data on T-cell responses to proteins of cows' milk remains controversial [14]. Cows' milk was recently reported to be an environmental trigger of an immune response to insulin during infancy [15].

Our case-control study was based on a birth cohort in Finland the country with the highest annual incidence of Type I diabetes in the world. To evaluate the relation between early infant nutrition and the emergent signs of beta-cell autoimmunity, we identified from the general population, and observed, children with increased genetic susceptibility to Type I diabetes from birth.

Subjects and methods

Subjects. The Finnish Type I Diabetes Prediction and Prevention (DIPP) Study was established to predict Type I diabetes in the general population and to develop effective strategies to prevent of delay progression to clinical disease. Families with their infants born in one of the three participating university hospitals in Finland were invited to participate. The study was initiated at Turku University Hospital in November 1994, at Oulu University Hospital in September 1995 and at Tampere University Hospital in October 1997. We excluded families in which both parents were not Caucasian, had difficulties understanding Finnish, Swedish or English or whose newborn infant had a severe congenital malformation or disease. Umbilical cord blood samples were obtained from all the newborn infants and the families were offered the opportunity to have their infants screened for HLA-defined genetic risk of Type I diabetes after delivery. The parents were given both oral and written information on the study and more than 95 % of them gave their written informed consent to genetic screening.

Families whose infant had increased genetic susceptibility to Type I diabetes (high risk: DQB1*02/*0302; moderate risk: DQB1*0302/x, x = other than *02, *0301, or *0602) were invited to take part in the observational phase, after once again having given their written informed consent when the baby was approximately 3 months old. The first blood samples were taken at the age of 3 months, the next at the age of 6 months and sub-

sequently at intervals of 3 to 6 months during the initial 2 years and at an interval of 6 to 12 months thereafter up to the age of 4 years. Islet cell antibodies (ICA) were used as the primary screening test for beta-cell autoimmunity. If an infant tested positive for ICA at the age of 3 months, then, autoantibodies to insulin (IAA), the 65000 m/r isoform of glutamic acid decarboxylase (GADA) and the tyrosine phosphatase related IA-2 molecule (IA-2A) were also analysed in the cord blood sample. All 3-month-old infants that were ICA-positive also had ICA in their cord blood, suggesting that these antibodies had been transferred transplacentally. Such antibodies were excluded from the analysis, as they always disappeared by the age of 15 months at the latest. If an infant seroconverted to ICA positivity, he or she was observed at 3-month intervals and all of his or her blood samples including those obtained previously, were analysed for IAA, GADA and IA-2A.

By the end of September 1998, written informed parental consent for genetic screening had been obtained for 25 938 newborn infants, all of whom had been analysed for HLA-DQB1 alleles. Out of these 3596 (13.8 %) carried the high or moderate risk genotype and at least one ICA measurement was done on 2949 (82 %) of them. Of the children, 65 (32 boys), tested positive for ICA at least once during the follow-up, with a median observation period of 2.5 years (range 0.8–3.8 years). Of these 65 index subjects 26 (41.5 %) had the high risk genotype HLA-DQB1*02/*0302. Our analysis comprised the 65 children with diabetes-associated autoantibodies (cases) and 390 children (six control children/cases) without diabetes-associated autoantibodies (control children) matched for sex, HLA-DQB1 genotype, geographical region and birth date with the cases. Matching for birth date was done as closely as possible and the median difference in birth date was 7 days between the cases and the control children. The DIPP study protocol had been approved by the ethical committees of the three participating hospitals.

Genotyping. HLA-DQB1 alleles *02, *0301, *0302, *0602, *0603, and *0604, were analysed as described [16].

Autoantibody assays. Autoantibodies associated with diabetes were measured in the Research Laboratory of the Department of Pediatrics, University of Oulu. The ICA, GADA, and IA-2A were quantified as described previously [17]. Serum levels of IAA were quantified with a microassay as described [18].

Assessment of early infant nutrition. The children's height and weight were measured and data on breastfeeding and the introduction of cows' milk were recorded at every visit for as long as was appropriate. The duration of exclusive and overall breastfeeding and the age at which cows' milk was introduced were assessed for each individual. During exclusive breastfeeding a child did not receive any food other than breast milk and the introduction of cows' milk formula, cows' milk or other cows' milk products was defined as the age at which cows' milk feeding was initiated. The mothers' age and the duration of their general education was recorded at the time of their child's birth. The duration of exclusive breastfeeding was classified into less than 2 (short), 2 to 3.9 (intermediate) and 4 or more months (long). Overall breastfeeding was classified into less than 4, 4 to 5.9, and 6 or more months and the age when cows' milk was introduced into less than 2 (early), 2 to 3.9 (intermediate) and 4 months or more (late). The mothers' age, duration of maternal education and child's relative height and weight are known to be related to early infant nutrition, and accordingly we considered them possible confounding factors. The mothers' age and their child's height and weight were included in the model as continuous variables and their general education as a categorized variable (< 13 years and ≥ 13 years).

Statistical analysis. Spearman's coefficients of correlation were calculated between infant feeding variables and mothers' age and child's height and weight at the age of 12 months. The Mann-Whitney U -test was used to compare the association between infant feeding variables and the duration of the mothers' education. Conditional matched logistic regression analysis evaluated the relation between breastfeeding and positivity for autoantibodies with the STATA statistical software package, version 6.0 (Stata Corporation, College Station, Texas, USA). Odds ratios with confidence intervals (CI) of 95% were calculated to assess the relative risk of diabetes-associated autoantibodies with each exposure.

Results

Appearance of diabetes-associated autoantibodies. Among the 65 children who tested positive for ICA at least once during the follow-up visit, 22 had no other detected autoantibodies, eight tested positive for two autoantibodies, 16 for three, and 19 for all four. Nine out of the 65 children became antibody-negative during the observation and they all tested positive for ICA only. Altogether, 41 children tested positive for IAA at least once and 32 for GADA. Nineteen of the 24 children who tested positive for IA-2A had all four autoantibodies, three had three autoantibodies, and two had two autoantibodies.

Characteristics of early infant feeding. The characteristics of early infant feeding, the duration of the mothers' general education and relative height and weight at the age of 12 months are shown in Table 1. The median duration of exclusive breastfeeding was 0.2 months shorter in cases than in control children [1.8 (interquartile range, IQR, 0.5–3.9) months vs 2.0 (IQR 1.0–4.0) months]. The median duration of total breastfeeding was 6.0 (IQR 2.9–10.5) months in cases and 6.5 (IQR 3.0–10.0) months in control children. Median age at introduction of cows' milk was 2.0 months in cases (IQR 0.5–5.0) and control children (IQR 1.0–5.0).

Case and control mothers were similar in age (mean 29.6 vs 29.2 years). Mothers' age correlated with the duration of overall breastfeeding and the mothers with longer education had breastfed their children for a longer period and introduced cows' milk later than the other mothers. There was an inverse correlation between weight at the age of 12 months and the duration of overall and exclusive breastfeeding and the age when cows' milk was introduced.

Infant feeding and the appearance of beta-cell autoimmunity. There were differences in the distribution of the duration of exclusive breastfeeding and the age at which cows' milk was introduced between children who tested positive for IA-2A or for all four autoantibodies and their control children (Table 2). The risk

Table 1. Duration of mothers' general education, early infant feeding and height and weight at the age of 12 months of the study children

	Cases (<i>n</i> = 65)	Controls (<i>n</i> = 390)
Duration of mothers' general education, <i>n</i> (%)		
< 13 years	20 (31)	122 (31)
≥ 13 years	39 (60)	225 (58)
data missing	6 (9)	43 (11)
Duration of exclusive breastfeeding, <i>n</i> (%)		
< 2 months	32 (49)	185 (47)
2–3.9 months	16 (25)	85 (22)
≥ 4 months	16 (25)	110 (28)
data missing	1 (2)	10 (3)
Duration of total breastfeeding, <i>n</i> (%)		
< 4 months	18 (28)	99 (25)
4–5.9 months	12 (19)	62 (16)
≥ 6 months	34 (52)	214 (55)
data missing	1 (2)	14 (4)
Age at introduction of cows' milk feeding, <i>n</i> (%)		
< 2 months	30 (46)	177 (45)
2–3.9 months	10 (15)	50 (13)
≥ 4 months	24 (37)	152 (39)
data missing	1 (2)	11 (3)
Relative height at the age of 12 months, SDS ^a		
Means (SD)	0.1 (1.0)	0.1 (0.9)
Data missing	7 (11)	46 (12)
Relative weight at the age of 12 months, % ^a		
Means (SD)	96.8 (8.9)	96.8 (7.8)
Data missing	7 (11)	46 (12)

^a Relative height is expressed in SD scores and relative weight as a percentage in relation to mean weight for height in healthy Finnish boys and girls [32]

of seroconversion to positivity for IA-2A was lower in children who were exclusively breastfed long-term than in those who were exclusively breastfed short-term ($p = 0.04$, Table 3). Also the risk of seroconversion to positivity for IA-2A was higher in those who received cows' milk at an early ($p = 0.015$) or intermediate ($p = 0.027$) age (Table 3). These associations remained significant after adjustment for the mothers' age, duration of their education and the relative height and weight of the child at the age of 12 months ($p = 0.032$, $p = 0.015$, $p = 0.011$, respectively). Positivity for ICA, IAA, and GADA and duration of breastfeeding or the age when cows' milk was introduced showed no significant association (Table 3).

The crude ORs for the risk of seroconversion for one or more diabetes-associated autoantibodies in relation to the duration of exclusive breastfeeding (A) and the age at the introduction of cows' milk (B) are shown in Figure 1. Long-term exclusive breastfeeding protected against the development of all four autoantibodies also after adjustment for the confounding factors (adjusted OR 0.07; CI 0.01–0.86; $p = 0.031$). Intermediate duration of exclusive breastfeeding failed to influence the development of the combination of all four autoantibodies (adjusted OR 0.87; CI 0.20–3.79; $p = 0.848$). When infants first received

Table 2. Duration of exclusive breastfeeding and age at introduction of cows' milk among children who tested positive for IA-2A or all four autoantibodies and control children

	Four antibodies		IA-2A	
	Cases (<i>n</i> = 19)	Controls (<i>n</i> = 114)	Cases (<i>n</i> = 24)	Controls (<i>n</i> = 144)
Duration of exclusive breastfeeding, months				
< 2	13 (68 %)	54 (47 %)	16 (67 %)	70 (49 %)
2–3.9	4 (21 %)	18 (16 %)	5 (21 %)	25 (17 %)
≥ 4	2 (11 %)	40 (35 %)	3 (13 %)	47 (33 %)
Data not available	0	2 (2 %)	0	2 (1 %)
Age at introduction of cows' milk feeding, months				
< 2	13 (68 %)	51 (45 %)	16 (67 %)	65 (45 %)
2–3.9	3 (16 %)	8 (7 %)	4 (17 %)	12 (8 %)
≥ 4	3 (16 %)	53 (47 %)	4 (17 %)	65 (45 %)
Data not available	0	2 (2 %)	0	2 (1 %)

Table 3. ORs (CI) for the positivity for ICA, IAA, GADA, and IA-2A associated with early infant feeding variables

	ICA (<i>n</i> = 65) ^a OR (95 % CI)	IAA (<i>n</i> = 41) ^a OR (95 % CI)	GADA (<i>n</i> = 32) ^a OR (95 % CI)	IA-2A (<i>n</i> = 24) ^a OR (95 % CI)
Duration of exclusive breastfeeding				
Crude OR				
2–3.9 vs < 2 months	1.11 (0.58–2.13)	1.07 (0.47–2.43)	1.15 (0.45–2.95)	0.81 (0.27–2.41)
≥ 4 vs < 2 months	0.86 (0.45–1.66)	0.72 (0.30–1.72)	0.71 (0.26–1.94)	0.24 (0.06–0.94)
Adjusted OR ^b				
2–3.9 vs < 2 months	1.25 (0.60–2.57)	1.14 (0.44–2.95)	1.13 (0.40–3.21)	0.97 (0.30–3.18)
≥ 4 vs < 2 months	0.89 (0.43–1.83)	0.60 (0.22–1.61)	0.62 (0.21–1.82)	0.10 (0.01–0.82)
Age at introduction of cows' milk				
Crude OR				
< 2 vs ≥ 4 months	1.05 (0.59–1.89)	1.37 (0.63–2.95)	1.33 (0.56–3.14)	4.37 (1.33–14.42)
2–3.9 vs ≥ 4 months	1.29 (0.57–2.94)	2.02 (0.70–5.81)	1.45 (0.41–5.07)	5.50 (1.21–25.04)
Adjusted OR ^b				
< 2 vs ≥ 4 months	1.02 (0.53–1.97)	1.50 (0.62–3.60)	1.56 (0.61–3.97)	7.29 (1.48–36.04)
2–3.9 vs ≥ 4 months	1.61 (0.66–3.96)	2.77 (0.84–9.15)	2.02 (0.54–7.62)	11.98 (1.76–81.68)

^a Number of cases in parenthesis^b Adjusted for mothers' age, duration of general education and relative height and weight at the age of 12 month

cows' milk younger than 2 months or 2 to 3.9 months of age they had a higher risk of developing positivity for all four autoantibodies than those with late introduction of cows' milk (adjusted ORs 9.46; CI 1.50–59.8; *p* = 0.017 and 17.9; CI 1.87–172; *p* = 0.012, respectively).

Discussion

Our finding of an association between short-term exclusive breastfeeding and positivity for IA-2A or the combination of all four diabetes-associated autoantibodies suggests that long-term breastfeeding protects genetically susceptible children from progressive beta-cell autoimmunity during the first years of life. Also, cows' milk introduced into the infants' diet before the age of 4 months could increase the risk of beta-cell autoimmunity in genetically susceptible children. On the other hand, we found no association between the overall duration of breastfeeding and the

presence of any of the diabetes-associated autoantibodies or any of their combinations.

Results from three birth cohort studies on first-degree relatives of subjects with Type I diabetes did not show any relation between the age at which cows' milk was introduced or duration of total or exclusive breastfeeding and emergence of at least one [19,20], or one or at least two [21] of the three diabetes-associated autoantibodies (IAA, GADA, IA-2A) studied. Neither did we observe any association between infant feeding patterns and the emergence of one or two to three of the four antibodies (IAA, GADA, IA-2A, and ICA) that we analysed. In contrast to previous studies, however, we also looked for the four different antibody specificities and the development of all four antibodies separately and found that long-term exclusive breastfeeding was inversely related whereas an early age at introduction of cows' milk was positively related to the emergence of IA-2A or all four autoantibodies. In the present series the number of seroconverters was somewhat higher

Fig. 1 (A,B). Panel **A** shows crude ORs and CI of exclusive breastfeeding for 2 to 3.9 months (l-■-l) or for at least 4 months (l-■-l) vs for less than 2 months in relation to the number of positivity for any or any combination of the diabetes-associated autoantibodies (* $p = 0.032$). Panel **B** shows crude ORs and CI of early introduction of cows' milk at the age of less than 2 months (l-■-l) or at 2 to 3.9 months (l-■-l) vs. at 4 months or more in relation to positivity for diabetes-associated autoantibodies. Twenty-two children tested positive for ICA only, eight for two antibodies, 16 for three and 19 for all four antibodies (** $p = 0.021$; *** $p = 0.039$)

($n = 65$) than in the previous United States' (cross-sectional analysis with retrospective data, $n = 18$), Australian ($n = 52$) and German ($n = 31$) prospective studies. Our DIPP study has some virtues compared to the other reports. Firstly, it represents a population-based prospective cohort of children with an increased risk of Type I diabetes who are followed from birth. Secondly, we analysed all four diabetes-associated autoantibodies and their association with early infant nutrition separately, which has not been the case in earlier studies. Thirdly, we matched the controls for the HLA-DQB1 genotype, which has

not been done before. The vast majority of the children in the general population do not have an increased genetic susceptibility to Type I diabetes and accordingly they are presumably relatively insusceptible to environmental factors. Also, the different susceptible or protective HLA risk alleles, or both, could affect the influence of specific environmental factors in initiating and promoting the autoimmune process. It has been implied that an even stronger association between breastfeeding and diabetes might have been detected in previous case-control studies, if the cases and control subjects had been genetically matched [8] and this hypothesis has actually been confirmed by a Chilean study [22].

Those children who tested positive for all four diabetes-associated autoantibodies most probably have an ongoing beta-cell destructing process in their pancreatic islets. Positivity for all four antibody specificities is a late sign of the autoimmune process affecting the pancreatic beta cells and is highly predictive for the future development of diabetes in siblings of children with Type I diabetes [23,24]. Usually IA-2A emerge as the last, or one of the last, autoantibody specificities in preclinical Type I diabetes [25] and they have the highest positive predictive value for the disease in siblings of affected children [24,26]. In addition, IA-2A have been implicated as a specific marker of beta-cell destruction [17].

The effect of short-term breastfeeding in promoting the destructive process of the disease could be triggered by early introduction of cows' milk. Infants could be most susceptible to various environmental agents during the first months of life, when their intestine is relatively permeable [27]. Breast milk protects the infant against infections during the first months by maternally transmitted immunity and this could also increase the child's resistance to other potential triggers of diabetes-associated autoimmunity [28].

The possible role of nutrition in beta-cell autoimmunity and also the influence of infections and different combinations of putative environmental factors and their timing could vary individually. The possible deleterious effect of such factors might be cumulative over time [29] and might therefore also act as promoters of the destructive beta-cell process. Cows' milk proteins have been proposed as a trigger for the activation of the immune system in the destructive process leading to Type I diabetes early in childhood, possibly during the first years of life [13]. We found an association between positivity for all four autoantibodies and an early exposure to cows' milk. This suggests that an early introduction of cows' milk could act as a promoter of progressive beta-cell destruction rather than as a trigger of the process, at least in some children developing signs of autoimmunity during the first years of life. This association could also reflect a general effect of complex diets and not be

specific to cows' milk. Cows' milk-based formulas, however, happen to be the first complex diet of non-human origin, to which most infants are exposed in industrialized countries. The influence of early infant nutrition as well as that of the later childhood diet in the pathogenesis of Type I diabetes should be assessed in extensive prospective cohort studies starting from birth. Also, the possible role of other environmental factors, their interactions and their relations to genetic predisposition [30,31] remain to be defined.

From our observations we cannot determine whether long-term exclusive breastfeeding or late exposure to cows' milk protects people against severe beta-cell autoimmunity or merely delays the emergence of diabetes-associated autoantibodies. Similarly, it is not possible at this point to draw any conclusions on the possible protection from overt diabetes by long-term exclusive breastfeeding or late introduction of cows' milk, because few children have progressed to clinical diabetes in the present series so far.

In conclusion, short-term exclusive breastfeeding and early introduction of cows' milk could predispose genetically susceptible children to progressive beta-cell autoimmunity during the first years of life. These observations emphasize the need for an intervention study to examine whether weaning infants to a fully hydrolysed formula provides protection against destructive beta-cell autoimmunity and subsequent progression to clinical Type I diabetes, or merely delays the process. Long-term studies are needed to fully explore the mechanisms that trigger and regulate beta-cell destruction in humans.

Acknowledgements. This work was supported by the Foundation for Pediatric Research, Medical Research Funds, Tampere, Oulu and Turku University Hospitals, the Medical Research Council, Academy of Finland, the Juvenile Diabetes Foundation International (grants 197032 and 4-1998-274), the Novo Nordisk Foundation and EU Biomed 2 (BMH4-CT98-3314). We thank T. Simell, P. Muona, P. Arvilommi, R. Hakala, H. Savolainen, A.-M. Hämäläinen, M. Törmä, B. Nurmi, K. Rasimus, H. Pohjola, A. Stenius, K. Riikonen, U. Markkanen, P. Asunta, A. Suutari, R. Sihvo, and E. Mäntymäki for superb team-work in the DIPP-project. We are also grateful to S. Anttila, S. Heikkilä, P. Koramo, S. Pohjola, R. Pääkkilä, T. Lauren, R. Suominen, J. Hakalax, and M. Jaakkola for their skilful technical assistance.

References

- Åkerblom HK, Knip M (1998) Putative environmental factors in type 1 diabetes. *Diabetes Metab Rev* 14: 31-67
- Elliott RB, Reddy SN, Bibby NJ, Kida K (1988) Dietary prevention of diabetes in the non-obese diabetic mouse. *Diabetologia* 31: 62-64
- Coleman DL, Kuzava JE, Leiter EH (1990) Effect of diet on incidence of diabetes in nonobese diabetic mice. *Diabetes* 39: 432-436
- Scott FW (1990) Cow milk and insulin-dependent diabetes mellitus: is there a relationship? *Am J Clin Nutr* 51: 489-491
- Dahl-Jørgensen K, Joner G, Hanssen KF (1991) Relationship between cows' milk consumption and incidence of IDDM in childhood. *Diabetes Care* 14: 1081-1083
- Virtanen SM, Hyppönen E, Läärä E et al. (1998) Cows' milk consumption, disease-associated autoantibodies and type 1 diabetes mellitus: a follow-up study in siblings of diabetic children. *Diabetic Med* 15: 730-738
- Borch-Johnsen K, Joner G, Mandrup-Poulsen T et al. (1984) Relation between breastfeeding and incidence rates of insulin-dependent diabetes mellitus: a hypothesis. *Lancet* ii: 1083-1086
- Gerstein HC (1994) Cows' milk exposure and type 1 diabetes mellitus: a critical overview of the clinical literature. *Diabetes Care* 17: 13-19
- Norris JM, Scott FW (1996) A meta-analysis of infant diet and insulin-dependent diabetes mellitus: do biases play a role? *Epidemiology* 7: 87-92
- Virtanen SM, Räsänen L, Aro A et al. (1991) Infant feeding in Finnish children < 7 yr of age with newly diagnosed IDDM. *Diabetes Care* 14: 415-417
- Virtanen SM, Räsänen L, Ylönen K et al. (1993) Early introduction of dairy products associated with increased risk of IDDM in Finnish children. *Diabetes* 42: 1786-1790
- Savilahti E, Saukkonen TT, Virtala ET, Tuomilehto J, Åkerblom HK (1993) Increased levels of cows' milk and β -lactoglobulin antibodies in young children with newly diagnosed IDDM. The Childhood Diabetes in Finland Study Group. *Diabetes Care* 16: 984-989
- Saukkonen T, Virtanen SM, Karppinen M et al. (1998) Significance of cows' milk protein antibodies as risk factor for childhood IDDM: interactions with dietary cows' milk intake and HLA-DQB1 genotype. *Diabetologia* 41: 72-78
- Norris JM, Pietropaolo M (1999) Controversial topics series: Milk proteins and diabetes. *J Endocrinol Invest* 22: 568-580
- Vaarala O, Knip M, Paronen J et al. (1999) Cows' milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes* 48: 1389-1394
- Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T (1995) Triple-label hybridization assay for type-1 diabetes-related HLA alleles. *Biotechniques* 18: 870-877
- Savola K, Bonifacio E, Sabbah E et al. (1998) IA-2 antibodies - a sensitive marker of IDDM with clinical onset in childhood and adolescence. *Diabetologia* 41: 424-429
- Sabbah E, Savola K, Kulmala P et al. (1999) Diabetes-associated autoantibodies in relation to clinical characteristics and natural course in children with newly diagnosed type 1 diabetes. *J Clin Endocrinol Metab* 84: 1534-1539
- Norris JM, Beaty B, Klingensmith G et al. (1996) Lack of association between early exposure to cows' milk protein and β -cell autoimmunity. *Diabetes Autoimmunity Study in the Young (DAISY)*. *JAMA* 276: 609-614
- Hummel M, Füchtenbusch M, Schenker M, Ziegler A-G (2000) No major association of breastfeeding, vaccinations, and childhood viral disease with early islet autoimmunity in the German BABYDIAB study. *Diabetes Care* 23: 969-974
- Couper JJ, Steele C, Beresford S et al. (1999) Lack of association between duration of breastfeeding or introduction of cows' milk and development of islet autoimmunity. *Diabetes* 48: 2145-2149
- Pérez-Bravo F, Carrasco E, Gutierrez-López MD, Martínez MT, López G, García de los Ríos M (1996) Genetic predisposition and environmental factors leading to the development of insulin-dependent diabetes mellitus in Chilean children. *J Mol Med* 74: 105-109

23. Bingley PJ, Christie MR, Bonifacio E et al. (1994) Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 43: 1304–1310
24. Kulmala P, Savola K, Petersen JS et al. (1998) Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes – a population-based study. *J Clin Invest* 101: 327–336
25. Roll U, Christie MR, Fuchtenbusch M, Payton MA, Hawkes CJ, Ziegler A-G (1996) Perinatal autoimmunity in offspring of diabetic parents. The German multicenter BABY-DIAB study: detection of humoral immune responses to islet antigens in early childhood. *Diabetes* 45: 967–973
26. Leslie RD, Atkinson MA, Notkins AL (1999) Autoantigens IA-2 and GAD in Type I (insulin-dependent) diabetes. *Diabetologia* 42: 3–14
27. Jakobsson I, Lindberg T, Lothe L, Axelsson I, Benediktsson B (1986) Human α -lactalbumin as a marker of macromolecular absorption. *Gut* 27: 1029–1034
28. Boggetti E, Meschi F, Malavasi C et al. (1992) HLA antigens in Italian type 1 diabetic patients: role of DR3/DR4 antigens and breast feeding in the onset of the disease. *Acta Diabetol* 28: 229–232
29. Scott FW, Cloutier HE, Kleemann R et al. (1997) Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats. *Diabetes* 46: 589–598
30. Martin JM, Trink B, Daneman D, Dosch H-M, Robinson B (1991) Milk proteins in the etiology of insulin-dependent diabetes mellitus (IDDM). *Ann Med* 23: 447–452
31. Hawerkos HW (1997) Could the aetiology of IDDM be multifactorial? *Diabetologia* 40: 1235–1240
32. Sorva R, Perheentupa J, Tolppanen EM (1984) A novel format for a growth chart. *Acta Paediatr Scand* 73: 527–529