British Journal of Nutrition (2006), **95**, 603–608
DOI: 10.1079/BJN20051676

Dietary risk factors for the emergence of type 1 diabetes-related autoantibodies in $2\frac{1}{2}$-year-old Swedish children

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(Received 21 April 2005 – Revised 11 November 2005 – Accepted 21 November 2005)

We studied dietary risk factors by analysing a questionnaire administered at birth, 1 year and $2\frac{1}{2}$ years of age, as well as the level of glutamic acid decarboxylase autoantibodies (GADA) and tyrosine phosphatase autoantibodies (IA-2A), in 7208 $2\frac{1}{2}$-year-old children from the All Babies in Southeast Sweden cohort, using the 95th percentile cut-off for autoantibodies to identify children at risk of type 1 diabetes. A total of 657 children had either IA-2A (*n* 360) or GADA (*n* 335), and thirty-eight children had both GADA and IA-2A. In univariate analysis, male gender and maternal coeliac disease implied a risk of possessing IA-2A. Maternal type 2 diabetes, a high consumption of fresh cows milk at the age of 1 year and a late introduction of gluten were associated with a risk of GADA. Early cessation of breast-feeding ($\leq$2 months of age) was associated with a risk of the simultaneous occurrence of both IA-2A and GADA. In logistic regression analysis, a high consumption of milk at the age of 1 year (odds ratio 2·6) represented a risk for GADA, and maternal coeliac disease (odds ratio 2·9) represented a risk for IA-2A. The combination of an early introduction of cows milk formula and a late introduction of gluten-containing food gave an odds ratio of 6·0 for positivity for at least one autoantibody at 1 and $2\frac{1}{2}$ years of age. The induction of autoantibodies by the age of $2\frac{1}{2}$ years has a male preponderance and is more common in children with maternal type 2 diabetes or maternal coeliac disease. Dietary risk factors for the induction of β-cell autoantibodies in $2\frac{1}{2}$-year-old children are a short duration of breast-feeding, an early introduction of cows milk formula and a late introduction of gluten, as well as a high consumption of milk at the age of 1 year.

Type 1 diabetes: GAD: IA-2: Cows milk: Breast-feeding: Gluten

The possible link between type 1 diabetes (T1D) and cows milk is controversial, at least partly because of methodological pitfalls when measuring exposure to cows milk in infants and children from different populations. Some retrospective studies have reported an inverse association between the length of exclusive breast-feeding, i.e. the avoidance of early cows milk or other foreign protein exposure in early infancy, and the occurrence of T1D (Borch-Johnsen *et al.* 1984; Mayer *et al.* 1988). An increased risk of T1D in individuals who have not been breast-fed or whose breast-feeding has been stopped within the first 3–4 months after birth has been shown in several studies (Virtanen *et al.* 1991; Kostraba *et al.* 1992, 1993; Gerstein, 1994; Norris & Scott, 1996; Perez-Bravo *et al.* 1996; Gimeno & de Souza, 1997). As most of the studies are retrospective, relying on long-time maternal dietary recall, inaccuracies have to be considered, especially when the data have been collected many years after the diagnosis of T1D. Prospective studies are therefore needed to clarify the issue.

Several prospective studies from Australia, Germany, North America and Finland have investigated the relationship between cows milk exposure and β-cell autoimmunity in children with an increased genetic risk of T1D (Couper *et al.* 1999; Kimpimaki *et al.* 2001; Norris *et al.* 2003; Ziegler *et al.* 2003). In the Finnish DIPP study, cows milk exposure before 4 months of age, as well as a short duration of breast-feeding, was associated with an increased risk of the development of tyrosine phosphatase autoantibodies (IA-2A) or multiple (i.e. two or more) autoantibodies (Kimpimaki *et al.* 2001). In the other studies in which the development of the β-cell autoantibodies was determined, multiple autoantibodies were not associated with duration of breast-feeding or age at introduction of cows milk (Couper *et al.* 1999; Norris *et al.* 2003; Ziegler *et al.* 2003). The discrepancy between the studies raises the question of whether cows milk exposure could be a risk factor linked with an unidentified 'true' risk factor of T1D in Finland, or whether other environmental factors could modify the aetiological importance of cows milk exposure as risk factor for T1D, leading to an increase in the latter in Finland and a decrease in the other countries mentioned.

Early exposure to wheat gluten was also found to carry a risk of β-cell autoimmunity in German and North American studies (Norris *et al.* 2003; Ziegler *et al.* 2003), which may

Abbreviations: ABIS, All Babies in Southeast Sweden; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, tyrosine phosphatase autoantibodies; OR, odds ratio; T1D, type 1 diabetes.

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simply indicate that early stimulation of the gut immune system with foreign proteins is associated with a risk of β-cell autoimmunity in children (Vaarala, 2002).

Here, we report dietary risk factors for the emergence of glutamic acid decarboxylase autoantibodies (GADA) and IA-2A at $2\frac{1}{2}$ years of age in an unselected population-based cohort of Swedish children born between October 1997 and October 1999. In these children, a short duration of breast-feeding was associated with an increased risk of double-positivity for GADA and IA-2A at $2\frac{1}{2}$ years of age. In logistic regression analysis, a high consumption of cows milk at the age of 1 year was a risk factor for the appearance of GADA at $2\frac{1}{2}$ years of age. An early cessation of breast-feeding and the introduction of cows milk were associated with positivity for at least one autoantibody in consecutive samples measured at 1 and $2\frac{1}{2}$ years of age. In these children, the combination of early cows milk exposure and the late introduction of porridge was associated with an odds ratio (OR) of 6·0 for permanent autoantibody positivity.

Material and methods

All Babies in Southeast Sweden (ABIS) is a population-based cohort study in which newborns have been followed prospectively with regular biological samples and questionnaires.

The infants were born between October 1997 and October 1999 in the south-east of Sweden. A total of 21 700 newborns and their families were recruited, 17 055 newborns (78·6 %) participating in the ABIS study. In total, 10 861 questionnaires were obtained when the infants were 1 year old and 8715 questionnaires when they were $2\frac{1}{2}$ years. Informed consent was obtained from the parents before enrolment. The ABIS population is representative of the general Swedish population (Statistics Sweden, 1999). The Research Ethics Committees of the Faculty of Health Sciences, Linköping University, and the Medical Faculty, Lund University, Sweden, approved the project.

In addition to a cord blood sample, other biological samples – blood, urine, stool, hair – were obtained from the children, and a questionnaire about environmental factors was administered at follow-up visits at the ages of 1, $2\frac{1}{2}$ and 5 years. Lysed whole blood samples taken at $2\frac{1}{2}$ years of age were stored at 20°C until analysed. Randomly non-selected children (n 7208) from the ABIS population who had given a blood sample at the age of $2\frac{1}{2}$ years were included for the present study on dietary factors and the occurrence of autoantibodies.

GADA and IA-2A were determined by an immune precipitation method using methionine-labelled antigens. The method is described in detail elsewhere (Wahlberg *et al.* 2005). Positivity for IA-2A and GADA was determined as an antibody level above the 95th percentile for healthy $2\frac{1}{2}$-year-old children, which corresponds to 26 WHO units for IA-2A and 82·2 for GADA. Using the Second International Workshop of Diabetes Autoantibody Standardisation program (2003), the specificity of the assay was 100 % for IA-2A and 98 % for GADA, the corresponding sensitivities being 50 % and 78 %. The intra-assay CV was 5·2 %, and the interassay variation 13–18 %.

Questionnaires were completed at birth and when the children were 1 and $2\frac{1}{2}$ years of age. In the questionnaires, mothers were asked for duration of exclusive breast-feeding and total duration of breast-feeding (number of months). Families were asked to keep food records, that is forms on which the parents noted when certain foods were introduced, specifically cows milk and gluten-containing foods. Age (by month) at introduction of the food items was asked for. Parents and other first-degree relatives with diabetes and coeliac disease were self-reported. The physician reported any instance of diabetes in the children.

Statistical analysis

The data from all the questionnaires were optically scanned and manually checked for errors. The register data in relation to autoantibody status were analysed statistically using the two-tailed Fisher exact test after classifying the children into groups with positive or negative islet autoimmunity results at the age of 2·5 years. All variables were categorized into two categories. We used univariate analysis to identify variables individually predictive of the development of autoantibodies (stage 1). The OR was estimated by entering the significant variables in a logistic regression. Those found to be significant at the 5 % level were then included in the multivariate model (stage 2). Variables were analysed simultaneously and included in the multivariate model by stepwise forward selection of the most significant. Multivariate logistic regression was used to estimate the OR, with a 95 % CI, for the significance of the explanatory variable. Correlations were measured using Spearman's correlation coefficient. All P-values are two-tailed. Statistics were calculated on a PC using the Statistical Package for Social Science (SPSS 11.5 software; SPSS Inc, Chicago, IL, USA).

Results

We found positivity above the 95th percentile at the age of $2\frac{1}{2}$ years in 360 children for IA-2A, in 335 for GADA and in 38 for both GADA and IA-2A.

Family history of autoimmune disease

The risk of developing GADA was increased in the offspring of mothers with type 2 diabetes (OR 3·3, CI 1·3, 8·6, $P<0·05$), as was the risk of developing IA-2A in children whose mother had coeliac disease (OR 2·8, CI 1·1, 7·2, $P<0·05$; Table 1). Male gender of the child was associated with a risk of IA-2A (OR 1·3, CI 1·0, 1·6, $P<0·05$; Table 1). Using the stepwise logistic regression model, maternal coeliac disease was associated with the induction of IA-2A (OR 2·9, CI 2·9, 7·5, $P<0·05$; Table 2). Maternal or paternal educational level did not affect the risk of developing diabetes-related autoantibodies at the age of $2\frac{1}{2}$ years. Parental age did not affect autoantibody status.

Nutrition history

Mother's diet during pregnancy and breast-feeding. The maternal intake of fresh cows milk or cows-milk-containing products, such as yoghurt, sour milk or bread, during pregnancy did not correlate with a higher risk of the child developing autoantibodies at the age of $2\frac{1}{2}$ years.

Table 1. Results of univariate analysis of risk factors related to increased levels of β-cell autoantibodies (exceeding the 95th percentile level) in an unselected population of Swedish children from the All Babies in Southeast Sweden study

	IA-2A		GADA		IA-2A and GADA		At least one autoantibody	
Independent risk factors ≥95 %	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI
Gender (boys/girls)	1·3	1·0, 1·6, $P=0·027$						
Type 2 diabetes (maternal)			3·3	1·3, 8·6, $P=0·014$				
Coeliac disease (maternal)	2·8	1·1, 7·2, $P=0·035$						
Cessation of breast-feeding (1 year old; month 0–2 *v.* later)*					2·9	1·1, 7·8, $P=0·036$		
Consumption of milk at the age of 1 year (four times per d *v.* no consumption)*			2·4	1·1, 5·2, $P=0·028$				
Introduction of gluten (porridge; ≥7 *v.* other months)*			1·6	1·2, 2·3, $P=0·006$			1·4	1·1, 1·8, $P=0·015$

IA-2A, tyrosine phosphatase autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; OR, odds ratio.
*The questionnaire asked: 'For how long was the child exclusively breast-fed (exclusively breast milk?)'; 'How often does your child drink cows milk nowadays?' and 'At what age did your child get porridge for the first time?'.
For details of subjects and procedures, see p. 604.

Out of the whole ABIS cohort at the age of 1 year, 1494 mothers out of 10 205 (15 %) reported that their babies had been breast-fed totally for 2 months, and 820 of 10 309 (8 %) had stopped breast-feeding before their baby was 3 months old. This is in agreement with figures in the cohort of 7208 children analysed for autoantibodies: according to the questionnaire when the child was 1 year old, 850 of 5435 (16 %) children had been breast-fed totally for 3 months, and 466 of 5531 (8 %) mothers had stopped breast-feeding before their baby was 3 months of age.

Early weaning of the child before 3 months of age, that is, a total duration of breast-feeding of less than 3 months, was associated with a risk of developing multiple autoantibody positivity above the 95th percentile against both GADA and IA-2A (OR 2·9, CI 1·1, 7·8, $P=0·036$; Table 1). From the questionnaire at 1 year, the age at introduction of formulas containing cows milk and gluten-containing foods (porridge) correlated with both the duration of exclusive breast-feeding (R 0·6, $P<0·001$ and R 0·366, $P<0·001$ respectively) and the total duration of breast-feeding (R 0·575, $P<0·001$ and R 0·249, $P<0·001$ respectively).

Cows milk. Children consuming cows milk four or more times per d had an increased risk of developing GADA (OR 2·4, CI 1·1, 5·2, $P<0·05$; Table 1). In a stepwise logistic regression model, only a high consumption of cows milk at the age of 1 year was associated with a risk of developing GADA (OR 2·6, CI 1·2, 5·7, $P<0·05$).

A high consumption of cows-milk-containing formula or yoghurt at the age of 1 year was not associated with the presence of GADA and/or IA-2A at $2\frac{1}{2}$ years of age. Similarly, a high consumption of cows milk, cows-milk-containing formula or yoghurt at the age of $2\frac{1}{2}$ years was not associated with the GADA and/or IA-2A at $2\frac{1}{2}$ years.

Porridge containing gluten. The late introduction of porridge (later than 6 months *v.* any other age in months) was related to a risk of the emergence of GADA (OR 1·6, CI 1·2, 2·3, $P<0·01$) and GADA and/or IA-2A (OR 1·4, CI 1·0, 1·8, $P<0·05$). A high consumption of porridge at the age of 1 or $2\frac{1}{2}$ years was not significantly related to the induction of autoantibodies against GADA and/or IA-2A at $2\frac{1}{2}$ years. The early introduction of porridge (before 3 months of age) was not related to an increased risk of developing autoantibodies at the age of $2\frac{1}{2}$ years, but only three children were exposed to porridge before 3 months of age in the ABIS study cohort.

The consumption of other gluten-containing foods (pasta, macaroni, spaghetti) was not associated with GADA and/or IA-2A at $2\frac{1}{2}$ years.

High-risk individuals. Children who remained positive for both GADA and/or IA-2A (n 37) during the follow-up (consecutive samples taken at 1 and $2\frac{1}{2}$ years of age) were considered to be high-risk individuals (Table 3). The mean duration of exclusive breast-feeding was equal in the affected children and the unaffected children (4·47 *v.* 4·47 months). A total of six of thirty (20 %) affected, compared with 519 of 4935 (11 %, $P<0·05$, OR 2·12) unaffected, children had stopped exclusive breast-feeding during the first 2 months of life.

The mean duration of total breast-feeding was less in the affected children than the unaffected children (6·61 *v.* 7·09 months; data from the questionnaire at the age of 1 year).

Table 2. The results of stepwise logistic regression analysis of risk factors related to increased levels of β-cell autoantibodies (exceeding the 95th percentile level) in an unselected population of Swedish children from the All Babies in Southeast Sweden study

	IA-2A		GADA	
Independent risk factors ≥95 %	OR	95 % CI	OR	95 % CI
Consumption of milk at the age of 1 year (four times per d *v.* no consumption			2·6	1·2, 5·7, $P = 0·020$
Coeliac disease (maternal)	2·9	2·9, 7·5, $P = 0·028$		

IA-2A, tyrosine phosphatase autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; OR, odds ratio.
For details of subjects and procedures, see p. 604.

In the cohort, five of thirty-one (16 %) affected, compared with 239 of 5026 (5 %, $P<0·001$, OR 3·85) unaffected, children had ceased breast-feeding in the first 2 months of life. Likewise, eleven of thirty-one (35·5 %) affected and 937 of 4730 (14 %, $P=0·01$, OR 2·23) unaffected children had started formula milk within the first 2 months of life.

An early introduction of cows milk formula (before 2 months of age) correlated to an increased risk of β-cell autoantibodies (OR 2·3, CI 1·1, 4·7, $P<0·01$). The late introduction of gluten-containing food (porridge; later than 6 months) was not significantly associated with β-cell autoantibodies (OR 1·3, CI 0·4, 3·6, $P=0·7$)).

The introduction of cows milk formula (before 2 months of age) and gluten-containing food (porridge; after 6 months) increased the risk of developing GADA and/or IA-2A in consecutive samples at the age of 1 year and $2\frac{1}{2}$ years (OR 6·0, CI 1·4, 26, $P<0·05$). In a logistic regression model, the early introduction of cows milk formula and the late introduction of gluten-containing food increased the risk of GADA and/or IA-2A (OR 5·8, CI 1·3, 25·5, $P<0·05$).

None of the high-risk individuals has yet developed T1D.

Discussion

In our prospective, population-based follow-up study of Swedish infants, we showed that early weaning of the child before 3 months of age was associated with a risk of β-cell autoimmunity determined as positivity for both GADA and IA-2A. A short duration of breast-feeding rather than a short duration of exclusive breast-feeding was a risk factor for β-cell autoimmunity, which suggests that breast-feeding for longer than 3 months protects against β-cell autoimmunity at 2 years of age.

Table 3. The results of frequency analysis of infant diet characteristics related to increased levels of β-cell autoantibodies (exceeding the 95th percentile level) in an unselected population of Swedish children from the All Babies in Southeast Sweden study

Variables	GADA and/or IA-2A at 1 and 2 years of age (*n* 37)	Unaffected (*n* 6551)
Duration of exclusive breast-feeding (mean, months)†	4·47 (*n* 30)	4·48 (*n* 4935)
Cessation of exclusive breast-feeding†	*n* 30	*n* 4935
Month 0–1	6 (20 %)	519 (11 %)*
Month 2–4	7 (23 %)	2057 (42 %)
Month 5 +	17 (57 %)	2359 (48 %)
Breast-feeding duration†	6·61 (*n* 31)	7·09 (*n* 5026)
Mean (months)	7·91 (*n* 32)	8·73 (*n* 5209)
Cessation of total breast-feeding*	*n* 31	*n* 5026
Month 0–1	5 (16 %)	239 (5 %)***
Month 2–4	1 (3 %)	614 (12 %)
Month 5 +	25 (81 %)	4173 (83 %)
Age at cows milk formula exposure†	*n* 31	*n* 4730
Month 0–1	11 (35·5 %)	937 (14·3 %)**
Month 2–4	7 (23 %)	1114 (24 %)
Month 5 +	13 (42 %)	2679 (57 %)
Age at gluten-containing food exposure†	*n* 30	*n* 5134
Month 1–3		71 (1 %)
Month 4–6	26 (87 %)	4496 (88 %)
Month 7 +	4 (10·8 %)	567 (11 %)
High consumption of cows milk†		
At the age of 1 year	2/37 (5·4 %)	124/607 (20·4 %)
At the age of $2\frac{1}{2}$ years	0/37 (0 %)	190/448 (42·4 %)
High consumption of gluten-containing food (porridge)†		
At the age of 1 year (*n* 18)	15 (83·3 %)	2714/3604 (75·3 %)
At the age of $2\frac{1}{2}$ years (*n* 19)	1 (5·3 %)	320/3067 (10·4 %)

IA-2A, tyrosine phosphatase autoantibodies; GADA, glutamic acid decarboxylase autoantibodies; OR, odds ratio.
*$P<0·05$, **$P<0·01$, ***$P<0·001$.
†The questionnaire stated: 'For how long was the child exclusively breast-fed (exclusively breast milk?)', 'At what age did the child stop being breast-fed?', 'When did the child get cow's milk formula for the first time?', 'At what age did your child get porridge for the first time?', 'How often does your child drink cows milk nowadays?' and 'How often does your child eat porridge nowadays?'.
For details of subjects and procedures, see p. 604.

As most Swedish children receive cows milk formula as their first foreign protein source, the early introduction of cows milk formula correlated strongly with both the length of exclusive breast-feeding and the duration of breast-feeding, which is a confounding factor in this kind of dietary study (Virtanen *et al.* 1993; Akerblom & Knip, 1998). The consumption of cows milk formulas is higher among the children who are no longer breast-fed compared with children who receive cows milk formula during breast-feeding, and the risk of β-cell autoimmunity may be related to the dose of cows milk and not only to the age at exposure. It should, however, be noted that a higher weight gain may be more rapid and greater in infants fed formula compared with breast-milk-fed infants, and this might be a confounding factor (Johansson et al. 1994; Akerblom & Knip, 1998). Supporting the importance of cows milk consumption for developing a risk of β-cell autoimmunity, we found that a high consumption of cows milk at 1 year of age was associated with a risk of GADA. In some previous reports, a high consumption of cows milk has been associated with a risk of T1D, both at a population level (Dahl-Jorgensen et al. 1991; Fava et al. 1994) and in case-control studies (Verge *et al.* 1994; Virtanen *et al.* 1998). Our finding of cows milk consumption as a risk factor was restricted to consumption at 1 year of age, and a high consumption of cows milk at $2\frac{1}{2}$ years of age did not imply a risk of β-cell autoimmunity. We did not find any association between maternal intake of cows milk or cows-milk-containing products during pregnancy and diabetes-related autoantibodies in the child at the age of $2\frac{1}{2}$ years.

Exposure to wheat gluten has also been associated with the emergence of β-cell autoimmunity in children (Norris *et al.* 2003; Ziegler *et al.* 2003). It is thus possible that the introduction of foreign proteins in early infancy, when the maturation of the gut immune system is not complete, is somehow harmful and predisposes to β-cell autoimmunity (Vaarala, 2002). Inflammatory activity of the gut immune system has been associated with T1D in children (Westerholm-Ormio *et al.* 2003), and in animal models dietary factors have been shown to modify the functional profile of islet-infiltrating lymphocytes (Scott *et al.* 1997). Accordingly, stimulation of the gut immune system by foreign proteins in early infancy may non-specifically activate islet-cell immunity by so-called bystander mechanisms.

In Sweden, and also in Finland, cows milk proteins represent the first foreign proteins introduced to infants. Wheat-gluten-containing foods are introduced later, after the age of 4 months according to national recommendations, which are followed by the families who receive dietary advice at regular visits to health-care centres. In the present study, only three children received wheat gluten-containing foods before 3 months of age, and thus it was impossible to evaluate early gluten exposure as risk factor for β-cell autoimmunity. The rare exposure to wheat-gluten-containing foods before 3 months of age in Nordic countries does not support the view that an early exposure to wheat gluten could be responsible for a considerable aetiological fraction of T1D in Nordic countries.

Instead, we found that the late introduction of wheat-gluten-containing porridge carried a risk of development of GADA, which supports findings from a recent North American study (Norris *et al.* 2003). The mechanisms of oral tolerance are poorly known in man, but the dose of the antigen and the age of the host seem to be important determinants of the development of oral tolerance. Changes in dietary recommendations that favoured the later introduction of gluten-containing foods to infants in Sweden resulted in a rapid increase in the incidence of coeliac disease in the 1990s (Ivarsson *et al.* 2000). When the Swedish Paediatric Association reintroduced the old recommendations, which suggested an earlier introduction and lower doses of gluten during breast-feeding, the incidence of coeliac disease fell back to the earlier levels. This shows that later introduction with higher doses may be harmful and break oral tolerance in some children.

Furthermore, the Swedish experience suggests that the introduction of foreign proteins during breast-feeding favours the development of oral tolerance. Breast milk supports the development of tolerance as it contains, for example, transforming growth factor-β and other regulatory cytokines. In addition, the introduction of new food items during breast-feeding usually means small increasing doses, whereas the late introduction of wheat is more often associated with larger doses, which may explain the poor development of tolerance. The consumption of wheat-gluten-containing foods at the age of 1 or $2\frac{1}{2}$ years (a high consumption of breakfast cereal such as cornflakes and/or muesli) was not associated with the occurrence of β-cell autoimmunity at $2\frac{1}{2}$ years of age in the present study.

Coeliac disease in the mother implied a risk of IA-2A, probably owing to the shared human leukocyte antigen-risk genotype of coeliac disease and T1D.

The question of whether cows milk or wheat gluten contains a diabetogenic factor and could be thus involved in the pathogenesis of T1D needs further clarification; it is not possible to evaluate risk mechanisms in this kind of epidemiological study. Cows milk insulin is a diabetogenic candidate, and a later-activated insulin-specific immune response primed by bovine insulin could be the link between cows milk exposure and T1D (Vaarala *et al.* 1999; Paronen *et al.* 2000). Antibodies to the wheat-gluten-derived protein Glb1 have been associated with T1D in human subjects and in BB-rats (MacFarlane *et al.* 2003), so Glb1 could be an antigen linking β-cell autoimmunity and gluten stimulation. Gluten may also trigger the immune system of individuals who have the shared human leukocyte antigen genotype of T1D and coeliac disease, as suggested by Italian studies (Auricchio *et al.* 2004).

There are discrepant findings relating to whether or not cows milk and/or cereal may be diabetogenic. One explanation could be that dietary exposure may be correlated with actual diabetogenic exposure in some populations but not others. Factors (foods or behaviours) in the infant diet differ across populations, and patterns of exposure, recommendations and feeding habits differ from country to country.

Autoantibodies to insulin are the first detectable antibodies and appear most frequently in children with early islet autoimmunity (Savola *et al.* 1998). Insulin autoantibodies have not been analysed as the radiobinding assay is not reliable for the lysed whole blood that we have received from children at $2\frac{1}{2}$ years of age. Background levels are unfortunately too high, and the sensitivity of the assay is poor. Analyses of insulin autoantibodies are not recommended owing to a high level of unspecific binding. Because of this limitation, the conclusion of our results is that the risk factors we have found

might be involved in the induction, or progression, of autoimmunity.

We conclude that a short duration of breast-feeding is associated with a risk of β-cell autoimmunity in an unselected population of Swedish children. Our results further suggest that the late introduction of wheat could contribute synergistically with the early introduction of cows milk to the induction of β-cell autoimmunity.

Acknowledgements

We are very grateful to all the children and their parents participating in ABIS, as well as to the health centres where all questionnaires were collected and the biological samples taken. We would also like to thank Lena Berglert, Gosia Smolinska and Cecilia Rundquist, who were of great help in the autoantibody determinations. This study, as a part of the ABIS project, was generously supported by JDRF-Wallenberg foundations (K 98-99D-12 813-01A), the Swedish Medical Research Council (MFR; Vetenskapsrådet; K99-72X-11 242-05A), the Swedish Child Diabetes Foundation (Barndiabetesfonden), the Swedish Diabetes Association, the Söderberg Foundation, and the Novo Nordisk Foundation.

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