

Effect of maternal diet during lactation on development of bovine insulin-binding antibodies in children at risk for allergy

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Background: The role of exposure to dietary antigens through maternal milk is intriguing, because it may result either in immunization or in tolerance. Exposure to cow's milk proteins results in antibody formation against bovine insulin in infants at risk for type 1 diabetes.

Objective: To study the appearance of IgG antibodies to bovine and human insulin in infants with an atopic family history whose mothers followed a cow's milk-free diet during the first 3 months of lactation.

Methods: In a prospective cohort study on prevention of food allergies, 123 infants were exclusively breast-fed or received supplementation with a hydrolyzed casein-based formula (Nutramigen) until the age of 6 months. The mothers either avoided cow's milk during the first 3 months of lactation (diet group) or had an unrestricted diet (nondiet group). The levels of IgG antibodies to bovine and human insulin were determined by enzyme immunoassay at 3, 6, 12, and 18 months and at 4 years. In addition, cord blood was obtained at birth and a maternal sample at delivery.

Results: At 3 months, IgG antibodies to bovine insulin were low in both dietary groups (median levels 0.150 and 0.114 optical density units in the diet and nondiet groups). After exposure to dietary insulin, IgG antibodies to bovine insulin increased in both groups, reaching a peak at 12 months in the nondiet group and at 18 months in the diet group. At 18 months, IgG antibodies to bovine insulin were lower in infants in the nondiet group than in infants in the diet group (0.287 vs 0.500, $P < .0001$). At 4 years, the antibodies no longer differed between the groups.

Conclusion: The exposure to cow's milk proteins through breast milk during the first 3 months of life resulted in decreased levels of antibodies to dietary bovine insulin at 18 months of age, suggesting a role for breast milk antigens in early tolerance induction. (*J Allergy Clin Immunol* 2000;106:302-6.)

Key words: Allergy, cow's milk-free diet, lactation, insulin-binding antibodies

Tolerance to dietary antigens normally develops early in life, but sometimes this process is disturbed, resulting in food allergy.¹ Although the concentrations of dietary antigens transferred to maternal milk are in the range of picograms to nanograms per milliliter of milk,² allergic sensitization can occur even during exclusive breast-feeding.^{3,4} The avoidance of dietary allergens such as cow's milk (CM), egg, and fish in the diet of a nursing mother only moderately reduces the incidence of allergic manifestations in the offspring.⁵ Although a maternal elimination diet for the first 3 months of lactation was associated with lower levels of IgE antibodies to CM and egg white in the infants during the period of allergen avoidance, there was no discernible effect at 9 months of age.⁶

The development of high-affinity autoantibodies to insulin is associated with type 1 diabetes.⁷ Early exposure to CM proteins and/or short breast-feeding has been associated with an increased risk for type 1 diabetes in epidemiologic studies.^{8,9} We have previously shown that early CM exposure results in antibody formation against bovine insulin (BI) in infants at risk for type 1 diabetes.^{10,11} The aim of the present study therefore was to address the effect of early CM exposure through breast milk on the appearance of IgG antibodies binding to BI and human insulin (HI) in infants whose mothers followed a CM-free diet or an unrestricted diet during lactation. This was studied in a group of infants with a family history of allergy who participated in a Swedish prospective study on prevention of food allergies.⁵

METHODS

Subjects

Infants from families with atopic disease in at least 2 first-degree relatives (parents, siblings) or in 1 first-degree relative and an elevated cord blood IgE level were recruited to a Swedish prospective study on prevention of food allergies. Patient selection and diet criteria for the 2 study groups have been previously reported in detail.⁵ During the first 6 months, the infants received only breast milk, or supplementation with a hydrolyzed casein-based formula (Nutramigen, Bristol-Meyers, Stockholm, Sweden). CM was introduced into the diet after 6 months, and egg-containing products after 9 months. The nursing mothers from the diet group avoided CM, egg white, and fish products during the first 3 months of lactation. The diet peri-

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Abbreviations used

- BI: Bovine insulin
- BLG: β -Lactoglobulin
- CM: Cow's milk
- EIA: Enzyme immunoassay
- HI: Human insulin

od was limited to 3 months to minimize dietary mistakes and to attain maximal compliance. The mothers from the nondiet group consumed an unrestricted diet during their entire lactation. The 2 groups were comparable with regard to heredity for atopic disease, pattern of breast-feeding, Nutramigen supplementation, and number of dietary errors.⁵ Serum samples from 123 infants ($n = 78$ in the diet group and $n = 45$ in the nondiet group) were available for insulin-binding antibody analyses from the original cohort of 140 infants. There were no dropouts in the original study cohort. Samples were available from 69 and 42 infants at birth, from 71 and 40 infants at 3 months, from 74 and 36 infants at 6 months, from 66 and 28 infants at 12 months, from 62 and 28 children at 18 months, and from 73 and 36 children at 4 years from the diet and nondiet groups, respectively. Serum samples taken at delivery were studied from 71 mothers in the diet group and from 34 mothers in the nondiet group. All parents of the participating infants gave their informed consent. The study was approved by the Human Research Ethics Committee of the Medical Faculty, University of Linköping, Sweden.

Enzyme immunoassay for antibodies to bovine insulin and human insulin

Polystyrene plates (Combiplate Enhanced binding, Labsystems, Helsinki, Finland) were coated with BI or HI 1 $\mu\text{g}/\text{well}$ (Sigma, St Louis, Mo). One-percent human serum albumin in PBS was used for residual coating and 0.05% polyoxyethylene sorbitanmonolaurate (Tween 20) in PBS as a washing buffer. The samples were diluted 1:20 in PBS containing 0.2% human serum albumin and 0.05% Tween. Alkaline phosphatase-conjugated rabbit anti-human IgG antibodies (Jackson ImmunoResearch, West Grove, Pa) were used as the secondary antibody and *p*-nitrophenyl phosphate (Sigma) as a substrate. The absorbance was measured by optical reader at 405 nm, and the results were expressed as optical density units. The samples were run blindly. A pool of 5 known positive and negative samples were run on each plate as quality controls. The mean intra-assay variation of the method was 12% and interassay variation was 20%. For inhibition assays, 1000 $\mu\text{g}/\text{mL}$ BI was incubated with the serum sample for 2 hours at room temperature before analyzing the sample in the enzyme immunoassay for antibodies to BI. The same serum sample without the inhibitor, but treated otherwise in a similar manner, was always run on the same plate. Samples with high IgG antibodies to BI were selected for inhibition analyses. The age of 6 months was chosen for this assay; samples were available from 16 infants in both groups.

Statistical analysis

The Mann-Whitney *U* test was used to compare the 2 groups. Wilcoxon signed ranks test was used to compare the levels of antibodies at different time points in a particular group. Correlation between different parameters was calculated by the Spearman correlation test. Significance was tested as 2-sided and a level of $P < .05$ was considered as significant.

RESULTS

Levels of IgG antibodies to BI and HI were low in both groups at birth and at 3 months of age (Table I and Fig. 1).

Because these values were at the background level of the assay, the differences between the groups were not considered biologically significant. The levels increased after 3 months of age in both groups. No difference in the median levels of insulin-binding antibodies was seen between the 2 groups at 6 months of age (ie, during the time when all the mothers consumed CM products) or at 12 months of age (ie, after exposure to CM products in the diet of the infants). At 18 months of age, IgG antibodies to both BI and HI were lower in infants whose mothers were on an unrestricted diet than in infants whose mothers followed the CM-free diet during the first 3 months of lactation (median levels 0.287 vs 0.500, $P < .0001$ for BI-IgG, and 0.249 vs 0.414, $P < .003$ for HI-IgG, respectively). At 4 years of age, the antibody levels no longer differed between the groups (Table I). An increase in the levels of IgG antibodies to BI was observed from 3 to 6 months of age in infants in the diet group (0.152 vs 0.202, $P < .0001$, Wilcoxon signed ranks test), after the cessation of mothers' dietary manipulation. No significant increase was seen in infants in the nondiet group from 3 to 6 months (0.123 vs 0.160, $P = .11$). A peak in the levels of IgG antibodies to BI and HI was reached at 12 months of age in the nondiet group and at 18 months of age in the diet group. The IgG antibodies to BI and HI correlated at all time points ($r = 0.53$, $P < .0001$ at birth; $r = 0.62$, $P < .0001$ at 3 months; $r = 0.75$, $P < .0001$ at 6 months; $r = 0.56$, $P < .0001$ at 12 months; $r = 0.69$, $P < .0001$ at 18 months; and $r = 0.58$, $P < .0001$ at 4 years). The binding of IgG-antibodies to solid-phase BI at a concentration of 1000 $\mu\text{g}/\text{mL}$ liquid-phase BI was inhibited to a similar degree in both groups. The median percentage of inhibition at 6 months of age was 47% and 40.5% (difference not significant) in the diet and nondiet groups, respectively.

DISCUSSION

The IgG antibody levels to BI were lower at 18 months of age in children whose mothers were on an unrestricted diet during the first 3 months of lactation than in infants whose mothers had followed a diet free of CM products during this time. An increase in the IgG antibody level to insulin was observed between the ages of 3 and 6 months in infants whose mothers started to use CM products after the elimination diet. This indicates that exposure to tiny amounts of CM proteins through breast milk during the first 3 months of life is associated with less antibody production later in life when CM proteins are introduced into the diet. Our findings may reflect early development of tolerance to small amounts of dietary BI present in breast milk. This effect may be dose dependent, because exposure to CM proteins present in infant formulas during the first 3 months of life results in increased levels of IgG antibodies to β -lactoglobulin (BLG), for example.¹² Alternatively, our finding may reflect differences in antigen processing when exposed to dietary antigens in breast milk or in intact CM formula.

Our finding is in accordance with animal studies showing that maternal antigen-containing diet during pregnan-

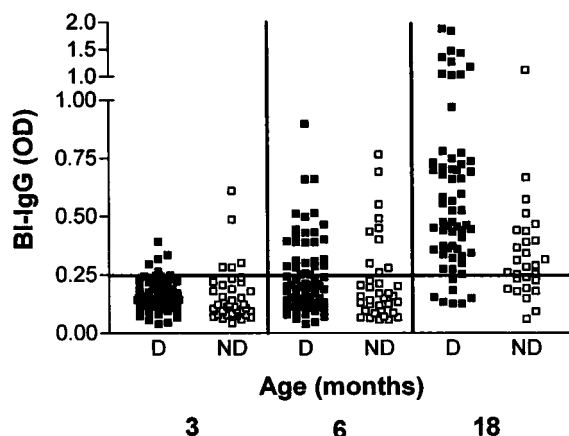


FIG 1. IgG antibodies to BI expressed as optical density units at 3 months ($P < .05$), 6 months ($P = .25$), and 18 months ($P < .0001$) in infants whose mothers avoided CM during the first 3 months of lactation (D), and in infants of mothers on an unrestricted diet during lactation (ND). The lower level of detection is shown by the horizontal line.

TABLE I. Median levels of BI-IgG and HI-IgG in infants whose mothers avoided CM during the first 3 months of lactation and in infants of mothers on an unrestricted diet during lactation

Sampling time		BI-IgG			HI-IgG		
		Diet	Nondiet	P value	Diet	Nondiet	P value
	0 mo	0.219	0.213	NS	0.195	0.163	<.05
Stop of maternal dietary elimination→	3 mo	0.150	0.114	<.05	0.169	0.176	NS
Start of CM formulas→	6 mo	0.202	0.159	NS	0.246	0.191	NS
	12 mo	0.318	0.303	NS	0.351	0.304	NS
	18 mo	0.500	0.287	<.0001	0.414	0.249	<.003
	4 y	0.226	0.221	NS	0.226	0.225	NS
	Mother	0.174	0.174	NS	0.212	0.208	NS

NS, Not significant.

Median levels of antibodies expressed as optical density units.

cy/lactation resulted in lower levels of dietary antibodies in the offspring when exposed to the same antigen after weaning.¹³ The effect of maternal diet on immune tolerance to parenteral antigen challenge was demonstrated in young animals that had been exposed to the antigen during gestation, lactation, or both.¹⁴ Telemo et al¹⁵ also demonstrated that fetal exposure of guinea pigs to dietary CM proteins through maternal CM consumption resulted in lower levels of IgG antibodies to BLG in offspring on immunization with milk whey-protein concentrate than in offspring of mothers following a milk-free diet. In this experiment the offspring were not nursed. The importance of early life for oral tolerance induction is also strengthened by a study showing that in piglets oral antigen exposure at birth resulted in a decreased antibody response to the same oral antigen fed from 3 weeks and after.¹⁶ The effect of prenatal and postnatal period in the infant immune system is reviewed by Björkstén.¹⁷ In human beings, a maternal hypoallergic diet during pregnancy did not alter the incidence of atopy or the levels of total serum IgE in the offspring.¹⁸ In the same cohort as we studied here, a maternal hypoallergic diet during lactation had no effect on the emergence of positive skin prick test results

to allergens or allergen-specific IgE antibodies later in life,^{5,6,19} but a decrease in the incidence of atopic eczema was observed.^{5,19} Our results on the appearance of IgG antibodies to BI indicate that elimination of food antigens from the diet of a nursing mother may modulate the development of food antigen-specific IgG antibodies in the child. The previous results on IgA antibody responses to BLG in the same cohort are in accordance with our findings. The number of children with a positive IgA response to BLG at 18 months of age was lower in children whose mothers had an unrestricted diet during the first 3 months of lactation than in children whose mothers avoided CM during this period. However, the levels of IgG antibodies to BLG and ovalbumin did not differ between the groups.⁶ The amount of these latter antigens in the diet is much higher than that of BI, and this may have had an effect on the results.

The newborn infant is normally first exposed to dietary antigens through maternal milk. Breast milk has major advantages over CM formulas—it enhances the maturation of the gut and protects the newborn from infections through maternal secretory antibodies.^{20,21} Secretory IgA in breast milk also affects antigen uptake

in the gut of the newborn infant, favoring the induction of tolerance.^{22,23} Furthermore, breast milk affects the gut flora, which likewise may be important for gastrointestinal host defense system and tolerance induction.²⁴ It is thus conceivable that exposure to dietary antigens through breast milk is a natural and safe route to encounter new antigens.

Despite this, some infants become sensitized to dietary antigens present in breast milk and develop allergic manifestations.^{3,4,25} As early as 1921, Shannon²⁶ demonstrated the adverse effects of food proteins in human breast milk by anaphylactic experiments on guinea pigs, even though the amounts of dietary antigens (eg, BLG and gluten) in breast milk are only in the range of picograms to nanograms per milliliter.^{2,27} These observations indicate that food antigens transferred to breast milk are immunologically active. The appearance of IgG antibodies to BI, even in exclusively breast-fed infants at 6 months of age, is an indicator of the immunogenicity of food antigens in human milk as also suggested by previous studies.⁶ A brief exposure to CM formula at birth has been shown to increase the risk of CM allergy compared with hydrolysate exposure, whereas exclusive breast-feeding at birth did not protect from allergy.²⁸ Contradictory findings on the role of breast-feeding in the outcome of immune responses in the infant might be explained by the combined effect of infant feeding, maternal food antigen-specific antibodies, maternal diet, and genetic factors.²⁹ In addition, nonantigen specific factors in breast milk may have importance in tolerance induction such as the levels of transforming growth factor- β .^{30,31}

However, the induction of systemic IgG antibody response to BI by exposure through breast milk was infrequent in the study (Fig. 1). It is also possible that some infants who received Nutramigen in our study might have been immunized to the insulin peptides present in this formula in the same way casein immunization occurs to casein peptides in some infants receiving Nutramigen.³² Because the groups did not differ in the exposure to Nutramigen, this should not have an effect on our results. No difference was observed between the groups at 3 months—during the period of maternal dietary manipulation—in the present study. Although the systemic immune response was not detectable at 3 months of age, the early antigen exposure through breast milk seemed to have had an effect on the kinetics of the IgG antibody responses to insulin later in life. The peak in the levels of insulin-binding antibodies appeared earlier in children of mothers consuming a normal diet during the first 3 months of lactation than in children whose mothers followed the CM-free diet during this period (ie, 12 vs 18 months of age; Table I). The difference in the levels of insulin-binding antibodies had disappeared by 4 years of age. It is possible that the observed difference between the groups reflects only a difference in timing of tolerance development. However, qualitative differences in the immune responses to dietary BI may exist despite the fact that no difference in the levels of the antibodies was observed later. The timing of development of tolerance may influ-

ence the characteristics of an immune response reactivated later in life (eg, by parenteral immunization).

The role of the gut immune system and oral tolerance in the pathogenesis of type 1 diabetes recently has been discussed.³³ Early exposure to CM formula, before 2 to 3 months of age, and/or have short breast-feeding, has been associated with type 1 diabetes in epidemiologic studies.^{8,9,34} Primary immunization to insulin occurs in early infancy by exposure to CM formula.^{10,11} Our results showing that exposure to BI through breast milk modifies the later development of immune responses to dietary insulin may have implications for the pathogenesis of type 1 diabetes. Exposure to dietary insulin through breast milk may account for a significant component of tolerance to this diabetes-associated autoantigen.

In conclusion, maternal avoidance of CM proteins during the first 3 months of lactation was associated with elevated levels of insulin-binding antibodies in infants later in life after introduction of CM products to the diet. This finding supports the notion that exposure to dietary antigens through breast milk during the first months of life may modify the induction of tolerance. This finding altogether emphasizes the importance of the first months of life in the development of tolerance.

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REFERENCES

1. Holt PG. Primary allergic sensitization to environmental antigens: perinatal T cell priming as a determinant of responder phenotype in adulthood. *J Exp Med* 1996;183:1297-301.
2. Kilshaw PJ, Cant JA. The passage of maternal dietary proteins into human breast milk. *Int Arch Allergy Appl Immunol* 1984;75:8-15.
3. Gerrard JW. Allergy in breast fed babies to ingredients in breast milk. *Ann Allergy* 1979;42:69-72.
4. de Boissieu D, Dupont C, Badoual J. Allergy to nondairy proteins in mother's milk as assessed by intestinal permeability tests. *Allergy* 1994;49:882-4.
5. Hattevig G, Kjellman B, Sigurs N, Björkstén B, Kjellman NIM. Effect of maternal avoidance of eggs, cow's milk and fish during lactation upon allergic manifestations in infants. *Clin Exp Allergy* 1989;19:27-32.
6. Hattevig G, Kjellman B, Sigurs N, Grodzinsky E, Hed J, Björkstén B. The effect of maternal avoidance of eggs, cow's milk, and fish during lactation on the development of IgE, IgG, and IgA antibodies in infants. *J Allergy Clin Immunol* 1990;85:108-15.
7. Greenbaum CJ, Palmer JP, Kuglin B, Kolb H. Insulin autoantibodies measured by radioimmunoassay methodology are more related to insulin-dependent diabetes mellitus than those measured by enzyme-linked immunosorbent assay: results of the Fourth International Workshop on the Standardization on Insulin Autoantibody Measurement. *J Clin Endocrinol Metab* 1992;74:1040-4.
8. Gerstein HC. Cow's milk exposure and type 1 diabetes. A critical overview of the literature. *Diabetes Care* 1994;17:13-9.
9. Åkerblom HK, Knip M. Putative environmental factors in type 1 diabetes. *Diabetes Metab Rev* 1998;14:31-67.
10. Vaarala O, Paronen J, Otonkoski T, Åkerblom HK. Cow milk feeding induces antibodies to insulin in children—a link between cow milk and insulin-dependent diabetes mellitus? *Scand J Immunol* 1998;47:131-5.
11. Vaarala O, Knip M, Paronen J, Hämäläinen A-M, Muona P, Väättäinen M, et al. Cow milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes* 1999;48:1389-94.
12. Jenmalm MC, Björkstén B. Exposure to cow's milk during the first 3 months of life is associated with increased levels of IgG subclass antibodies to β -lactoglobulin to 8 years. *J Allergy Clin Immunol* 1998;102:671-8.

13. Pathirana C, Goulding NJ, Gibney MJ, Pitts JM, Gallagher PJ, Taylor TG. Immune tolerance produced by pre- and postnatal exposure to dietary antigens. *Int Arch Allergy Appl Immunol* 1981;66:114-8.
14. Nicklin S, Miller K. Naturally acquired tolerance to dietary antigen: effect of in utero and perinatal exposure on subsequent humoral immune competence in the rat. *J Reprod Immunol* 1987;10:167-76.
15. Telemo E, Jakobsson I, Weström BR, Folkesson H. Maternal dietary antigens and the immune response in the offspring of the guinea pigs. *Immunology* 1987;62:35-8.
16. Bailey M, Miller BG, Telemo E, Stokes CR, Bourne FJ. Altered immune response to proteins fed after neonatal exposure of pigs to the antigen. *Int Arch Allergy Immunol* 1994;103:183-7.
17. Björkstén B. The intrauterine and postnatal environments. *J Allergy Clin Immunol* 1999;104:1119-27.
18. Fälth-Magnusson K, Kjellman N-I M. Development of atopic disease in babies whose mothers were receiving exclusion diet during pregnancy—a randomized study. *J Allergy Clin Immunol* 1987;80:869-75.
19. Sigurs N, Hattevig G, Kjellman B. Maternal avoidance of eggs, cow's milk and fish during lactation: effect on allergic manifestations, skin-prick tests, and specific IgE antibodies in children at age 4 years. *Pediatrics* 1992;89:735-9.
20. Sheard NF, Walker WA. The role of breast milk in the development of the gastrointestinal tract. *Nutr Rev* 1988;46:1-8.
21. Duchén K, Björkstén B. Sensitization via the breast milk. In: Mestecky J, editor. *Immunology of milk and the neonate*. New York: Plenum; 1991. p. 427-36.
22. Hansson LÅ, Ahlstedt S, Andersson B, Carlsson B, Fällström SP, Melander L, et al. Protective factors in milk and the development of the immune system. *Pediatrics* 1985;75:172-6.
23. Machtlinger S, Moss R. Cow's milk allergy in breast fed infants: the role of allergen and maternal secretory IgA antibody. *J Allergy Clin Immunol* 1986;77:341-7.
24. Gyorgy P, Norris RF, Rose CS. Bifidus factor. I. A variant lactobacillus bifidus requiring a special growth factor. *Arch Biochem Biophys* 1954;48:193-201.
25. Jakobsson I, Lindberg T. Cow's milk as a cause of infantile colic in breast-fed infants. *Lancet* 1978;2:437-9.
26. Shannon WR. Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea pigs. *Am J Dis Child* 1921;22:223-31.
27. Troncone R, Scarcella A, Donatiello A, Canataro P, Tarabuso A, Auricchio S. Passage of gliadin into human milk. *Acta Paediatr Scand* 1987; 76:453-6.
28. Saarinen KM, Juntunen-Backman K, Järvenpää A-L, Kuitunen P, Lope L, Renlund M, et al. Supplementary feeding in maternity hospitals and the risk of cows' milk allergy: a prospective study of 6209 infants. *J Allergy Clin Immunol* 1999;104:457-61.
29. Wright AL, Sherrill D, Holberg CJ, Halonen M, Martinez FD. Breast feeding, maternal IgE, and total serum IgE in childhood. *J Allergy Clin Immunol* 1999;104:589-94.
30. Saarinen KM, Vaarala O, Klemetti P, Savilahti E. Transforming growth factor- β_1 in mothers' colostrum and immune responses to cows' milk proteins in infants with cows' milk allergy. *J Allergy Clin Immunol* 1999; 104:1093-8.
31. Kalliomäki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor- β in breast milk: a potential regulator of atopic disease at an early age. *J Allergy Clin Immunol* 1999;104:1251-7.
32. Vaarala O, Saukkonen T, Savilahti E, Klemola T, Åkerblom HK. Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula. *J Allergy Clin Immunol* 1995;96:917-23.
33. Vaarala O. Gut and the induction of immune tolerance in type 1 diabetes. *Diabetes Metab Res Rev* 1999;15:353-61.
34. Virtanen SM, Räsänen L, Ylönen K, Aro A, Clayton D, Langholz B, et al. Early introduction of dairy products associated with increased risk of IDDM in Finnish children. *Diabetes* 1993;42:1786-90.