# Early Exposure to Cow's Milk and Solid Foods in Infancy, Genetic Predisposition, and Risk of IDDM

JILL N. KOSTRABA, KAREN J. CRUICKSHANKS, JANET LAWLER-HEAVNER, LUIZ F. JOBIM, MARIAN J. REWERS, ELIZABETH C. GAY, H. PETER CHASE, GEORGEANNA KLINGENSMITH, AND RICHARD F. HAMMAN

**Using a case-control study design, we examined the hypothesis that early exposure to cow's milk and solid foods increased the risk of IDDM. An infant diet history was collected from 164 IDDM subjects from the Colorado IDDM Registry with a mean birth year of 1973, and 145 nondiabetic population control subjects who were frequency matched to diabetic subjects on age, sex, and ethnicity. Early exposure was defined as exposure occurring before 3 mo of age. After controlling for ethnicity, birth order, and family income, more diabetic subjects were exposed early to cow's milk (OR 4.5, 95% CI 0.9–21.4) and solid foods (OR 2.5, CI 1.4–4.3) than control subjects. To examine this association while accounting for the genetic susceptibility to IDDM, we defined individuals as high and low risk by an HLA-DQB1 molecular marker. Early exposure to cow's milk was not associated with elevated risk for IDDM in low-risk individuals. Relative to unexposed low-risk individuals, early exposure to cow's milk was strongly associated in individuals with a high risk marker (OR 11.3, CI 1.2–102.0). Similar findings were observed for early exposure to solid foods. These data indicate that early exposure to cow's milk and solid foods may be associated with increased risk of IDDM. The inclusion of HLA-encoded risk in the analyses demonstrates the combined effect of genetic and environmental factors. *Diabetes* 42:288–95, 1993**

From the Departments of Preventive Medicine and Biometrics and Pediatrics, University of Colorado School of Medicine, Denver; and The Children's Hospital, Denver, Colorado; and the Nuffield Department of Surgery, University of Oxford, John Radcliff Hospital, Headington, Oxford, United Kingdom.

Address correspondence and reprint requests to Jill N. Kostraba, PhD, Department of Preventive Medicine and Biometrics, University of Colorado School of Medicine, Box C-245, 4200 E. Ninth Avenue, Denver, CO 80262.

Received for publication 19 March 1992 and accepted in revised form 10 September 1992.

IDDM, insulin-dependent diabetes mellitus; OR, odds ratio; CI, confidence interval; SSO, sequence-specific oligonucleotides; NIH, National Institutes of Health.

Investigations of IDDM suggest that there is both a genetic (1–3) and an environmental component (4–7) in the etiology of the disease. Susceptibility to IDDM has been shown to be highly correlated with the absence of Asp at position 57 of the HLA-DQβ chain (1,2). Ecological and case-control studies have shown that risk of IDDM is lower in those who had been breast-fed (8–11). Moreover, some data suggest that infants breast-fed for longer periods of time have a lower risk of IDDM than those breast-fed for shorter periods (10,12). These observations suggest that the breast-feeding association is related to the protective effect of delaying exposures in the infant diet. For example, diabetic subjects were introduced to infant milks other than breast milk earlier than nondiabetic control subjects (13,14).

The relatively small increased risks associated with infant diet exposures that have been reported suggests that either the correct exposure has not been evaluated, or a better defined control group, i.e., a group that is susceptible to the disease, is needed to adequately evaluate the impact of the exposure. Without accounting for genetic susceptibility, investigations of potential environmental agents are likely to detect only weak associations, if any at all, by including individuals in their exposed category who are not genetically predisposed to the disease.

This retrospective study of infant diet and IDDM was conducted to determine whether early exposure to cow's milk and solid food in infancy was associated with IDDM risk. Moreover, our data allowed us to investigate whether an association was present in individuals at low and high risk for IDDM, as defined by the susceptibility marker on the HLA-DQβ chain.

## RESEARCH DESIGN AND METHODS

The Colorado IDDM Registry is a statewide, population-based incidence registry that identifies cases through a

physician surveillance network and periodic hospital record review (15). Criteria for inclusion in the registry were *1*) resident of Colorado at the time of diagnosis, *2*) <18 yr of age at diagnosis, *3*) placed on insulin within 2 wk of diagnosis, and *4*) diabetes not secondary to other conditions. The level of case ascertainment was estimated to be 93%. Diabetic individuals diagnosed between 1978 and 1988 were recruited from the Colorado IDDM Registry for a study of ethnic differences in IDDM. All living subjects in the registry who reported an Hispanic origin (16) or had a Spanish surname were eligible for the ethnicity study ($n$ = 118). A similar size random sample of 122 registry subjects who reported being non-Hispanic white was also selected. Seventy-five Hispanic subjects and 99 non-Hispanic white subjects participated in the ethnicity study. There were no significant differences in age, duration of IDDM, age at diagnosis of diabetes, or gender by participation status for either ethnic group (17).

Nondiabetic, healthy control subjects were recruited from a random sample of households generated from a tape of licensed motor vehicle drivers who were residents in 1987 of a 10-county region just east of the Rocky Mountains that includes ~82% of the population of the state, encompasses both rural and urban areas, and is easily accessible from Denver (19). Control subjects were frequency matched to diabetic subjects on sex, age-group, and ethnicity. One hundred thirty-two eligible people (50.4%) agreed to participate in the ethnicity study. Additional control subjects (5 Hispanics and 18 non-Hispanic whites) were recruited through announcements at the University of Colorado Health Sciences Center. There were no differences in age or years of maternal education between these control subjects and those obtained via the motor vehicle license tape.

Participants and their parents completed a questionnaire on early childhood, including questions concerning breast-feeding and at what age they started consuming infant formula, cow's milk, and any type of solid food. The survey instrument is available from the author (J.N.K.). Ten diabetic subjects and 10 control subjects were unable to provide an infant diet history. Birth order of the participant was evaluated because it could confound the analyses of infant diet. The variable, relative birth order, was calculated by dividing the child's actual birth order by the number of children in the family, such that the first born child had a value of 1/× in a sibship size of x, and the last born child had a value of ×/× or 1.

One hundred forty-four diabetic subjects and 131 control subjects provided a blood sample for genetic analysis. B-Cells were separated by nylon-wool columns (20), frozen in liquid nitrogen and shipped on dry ice for oligonucleotide analysis in the laboratory of Dr. John Todd. The identification of the HLA-DQA1 and DQB1 alleles by use of polymerase chain reaction (21) and dot-blot analysis with labeled oligonucleotides was conducted as previously described (1,2,21–23). Four DQA1 and seven DQB1 SSOs were used (1,2,24–26). Each oligonucleotide probe detected at least one allele. DQA1 alleles were grouped into four families (24,26): A1 (DQA1*0101, DQA1*0102, DQA1*0103), A2 (DQA1*0201), A3 (DQA1*0301), and A4 (DQA1*0401, DQA1*0501, DQA1*0601). The DQB1 SSOs were 1.1 (DQw5, DQB1*0501, DQB1*0502, DQB1*0604), 1.2 (DQw6, DQB1*0602, DQB1*0603), DQw2 (DQB1*0201), 3.1–57 (DQw7, DQw9, DQB1*0301, DQB1*0303), 3.1–26 (DQw7, DQB1*0301), 3.2 (DQw8, DQB1*0302), and DQw4 (DQB1*0402, DQB1*0401).

Diabetic and control subjects were divided into two susceptibility risk groups according to whether they had Asp at position 57 of the DQβ chain, which has been shown to be protective of IDDM in our population (K.J.C., unpublished observations). Those who had at least one Asp (i.e., Asp/Asp, Asp/non-Asp, or Asp/blank) were placed in the low susceptibility risk group, and those who were homozygous for amino acids other than Asp (i.e., non-Asp/non-Asp or non-Asp/blank) were placed in the high susceptibility risk group (1). Alleles encoding Asp in position 57 included DQw4, DQw6, DQw7, and DQw9.

**Analysis cohort.** There were two multiple case families in which both diabetic siblings participated in the ethnicity study. For the purposes of this study, one of the diabetic subjects was randomly chosen from each family for inclusion in the analyses. The analyses of infant diet exposures by IDDM status included the 164 diabetic subjects and 145 control subjects who had completed the infant diet history. Because some participants did not answer all of the questions in the infant diet history, the total number in the individual comparisons vary. In addition, due to technical problems including insufficient numbers of cells available for the SSO typing, HLA-DQB1 data were available on 115 diabetic subjects and 108 control subjects. Diet exposure comparisons stratifying by low and high genetic risk groups were limited to the participants on whom we had both genetic marker and infant diet data. Therefore, these analyses included 106 diabetic subjects and 99 control subjects.

**Statistical analyses.** The SAS system was used to compute ORs and 95% CI using Woolf's Method and Haldane's correction for small samples (27). The Student's $t$ test was used to compare normally distributed variables (e.g., birth yr) and Wilcoxon's rank test was used to compare non-normally distributed variables (e.g., relative birth order, breast-feeding duration, and age exposed to cow's milk and solid foods). Life table analysis was used to compare the cumulative percentage exposed to cow's milk or solid foods, and the log-rank statistic was used to test the difference between two curves. Multiple logistic regression analysis was used to control for confounding variables.

## RESULTS

We examined characteristics that may confound our analyses of infant diet, such as birth year, birth order, maternal age at birth, current maternal education, and current family income (Table 1). There were only small differences in gender and birth year between diabetic and control subjects. Significantly fewer case families reported an annual income of >$20,000 compared with control families. This difference was seen primarily in Hispanics. In Hispanics, diabetic subjects were more

TABLE 1
Demographic characteristics of study participants by diabetes status and ethnicity

| | Diabetic subjects | | Nondiabetic control subjects | |
|---|---|---|---|---|
| | % | *n* | % | *n* |
| All participants | | | | |
| Birth year | 1972.8 ± 5.4 | 164 | 1971.8 ± 6.6 | 145 |
| Sex* | 42.1 | 164 | 53.1 | 145 |
| Family income†‡ | 62.5 | 152 | 76.3 | 135 |
| Maternal education§ | 85.8 | 162 | 87.6 | 137 |
| Maternal age at birth‖ | 21.6 | 162 | 20.6 | 136 |
| Relative birth order | 0.78 ± 0.25 | 163 | 0.75 ± 0.27 | 143 |
| Hispanics | | | | |
| Birth year | 1972.5 ± 5.1 | 71 | 1972.0 ± 6.3 | 59 |
| Sex* | 42.2 | 71 | 49.1 | 59 |
| Family income†‡ | 43.1 | 65 | 65.4 | 55 |
| Maternal education§ | 70.4 | 71 | 75.4 | 57 |
| Maternal age at birth‖ | 22.5 | 71 | 16.4 | 55 |
| Relative birth order‡ | 0.77 ± 0.26 | 70 | 0.66 ± 0.26 | 59 |
| Non-Hispanic whites | | | | |
| Birth year | 1973.1 ± 5.6 | 93 | 1971.7 ± 6.8 | 86 |
| Sex* | 41.9 | 93 | 55.8 | 86 |
| Family income† | 77.0 | 87 | 83.7 | 80 |
| Maternal education§ | 97.8 | 91 | 96.3 | 80 |
| Maternal age at birth‖ | 20.9 | 91 | 23.5 | 81 |
| Relative birth order | 0.79 ± 0.24 | 93 | 0.79 ± 0.26 | 84 |

*Percent male.
†Current annual income ≥$20,000.
‡$P < 0.05$.
§Percent ≥12 yr of education (current).
‖Percent ≥30 yr of age at birth.

likely to be in the higher birth order of the family than control subjects.

The prevalence of breast-feeding was similar by diabetes status (Table 2). Overall and in non-Hispanic whites, diabetic subjects were younger when they were first exposed to solid foods compared with nondiabetic control subjects. Diabetic subjects were also exposed to cow's milk earlier than control subjects. Similar trends were seen in Hispanics but they were not statistically significant. Because ethnic-specific analyses showed similar trends in the infant diet comparisons between diabetic and control subjects (Table 2), the ethnic groups were pooled to maximize the sample size for the following analyses.

Figure 1 displays the cumulative percentage exposed to cow's milk (A) and solid foods (B) by age and diabetes status. The log-rank test of the difference between curves suggests that diabetic subjects were exposed to cow's

TABLE 2
Infant nutrition variables by diabetes status and ethnicity

| | Diabetic subjects | *n* | Nondiabetic control subjects | *n* |
|---|---|---|---|---|
| All participants | | | | |
| Breast-fed | 52.1% | 163 | 54.3% | 140 |
| Breast-feeding duration* | 24.8 ± 20.4 | 78 | 27.8 ± 25.0 | 62 |
| Exposure to cow's milk†‡ | 32.5 ± 17.8 | 110 | 37.2 ± 17.8 | 96 |
| Exposure to solid foods†§ | 13.4 ± 8.9 | 142 | 16.9 ± 11.5 | 117 |
| Hispanics | | | | |
| Breast-fed | 38% | 71 | 35.1% | 57 |
| Breast-feeding duration* | 24.3 ± 25.0 | 23 | 26.2 ± 18.3 | 16 |
| Exposure to cow's milk† | 35.4 ± 20.9 | 46 | 37.6 ± 15.5 | 40 |
| Exposure to solid foods† | 15.2 ± 8.6 | 55 | 18.0 ± 10.9 | 44 |
| Non-Hispanic whites | | | | |
| Breast-fed | 63% | 92 | 67.5% | 83 |
| Breast-feeding duration* | 25.0 ± 18.4 | 55 | 28.3 ± 27.1 | 46 |
| Exposure to cow's milk† | 30.5 ± 15.1 | 64 | 36.9 ± 19.5 | 56 |
| Exposure to solid foods†§ | 12.3 ± 9.0 | 87 | 16.2 ± 11.9 | 73 |

*Mean age in weeks when breast-feeding ceased of those who were breast-fed.
†Mean age in weeks when first exposed.
‡$0.05 < P < 0.10$.
§$P = 0.01$.

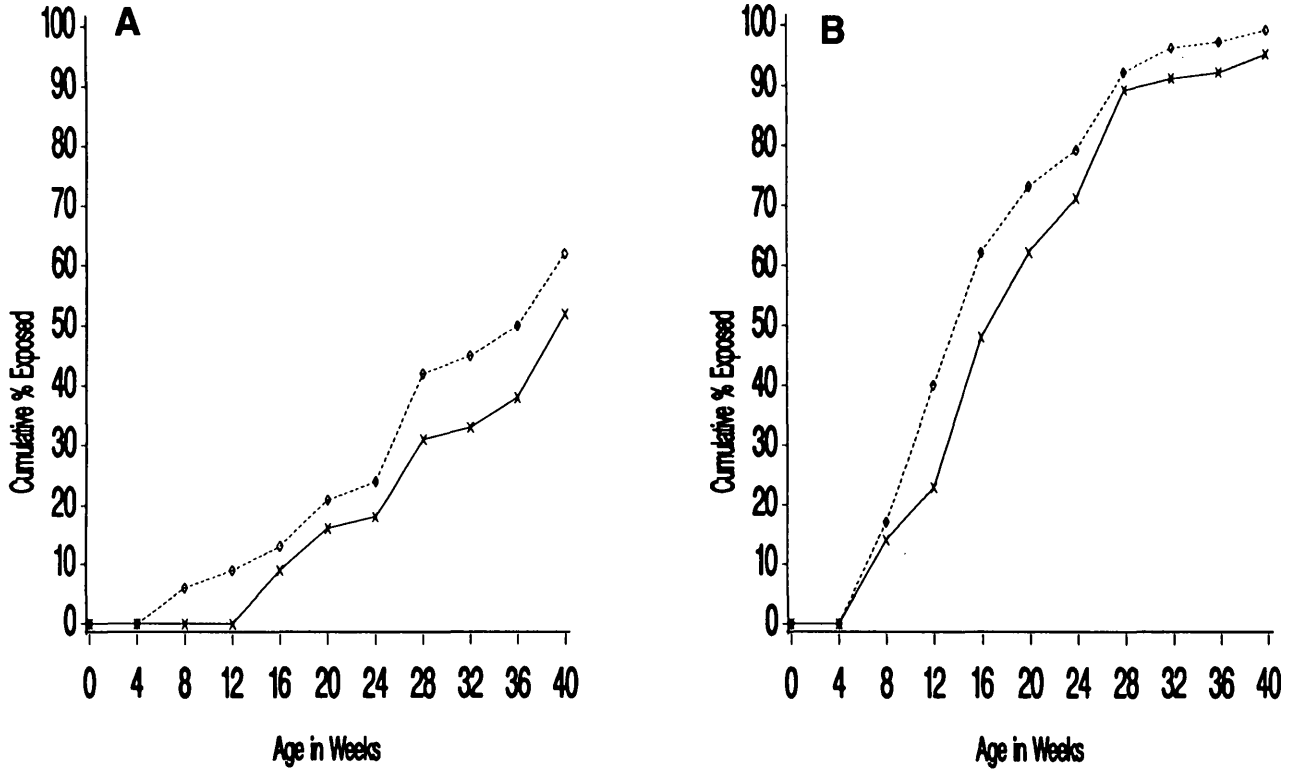


**FIG. 1. Age at exposure to cow's milk (*A*, 110 diabetic subjects and 96 control subjects) and solid foods (*B*, 142 diabetic subjects and 117 control subjects) in diabetic subjects (◇) and control subjects (×).**

milk (Fig. 1*A*, $P = 0.07$) and solid foods (Fig. 1*B*, $P = 0.008$) earlier than control subjects.

To investigate whether this association was present in individuals at low and high risk for IDDM as defined by HLA-DQB1 markers, the population was stratified on risk status and the infant nutrition comparisons were repeated. In high-risk individuals, diabetic subjects were introduced to cow's milk significantly earlier than nondiabetic control subjects (Fig. 2*A*, $P = 0.05$ [log-rank test]). In individuals who were low risk, there were no differences in age at exposure to cow's milk by diabetes status (Fig. 2*B*, $P = 0.48$). No differences were seen between the cumulative exposure to solid foods curves by diabetes status in either the high-risk (Fig. 3*A*, $P = 0.24$) or low-risk (Fig. 3*B*, $P = 0.52$) group.

Analysis of the diet data in their continuous form assumes a linear relationship between infant diet exposures and IDDM risk. This is unlikely, because everyone is exposed to these foods eventually. To determine whether the time relationship was linear or whether there was a threshold effect, one-half of the cohort was randomly selected for an exploratory analysis. The ORs for IDDM by age cutoffs for exposure were plotted. As shown in Fig. 4, the relationship between infant diet exposures and IDDM is not linear, and suggests a defined susceptibility window. The largest OR for exposure to cow's milk (OR 5.9, CI 0.9–40.7) or solid food (OR 2.6, CI 1.3–5.3) was found using the age cutoff of exposure before 3 mo of age. This suggests that exposures that occur before 3 mo may be particularly important in terms of diabetes risk. After adjusting for ethnicity, birth order, and family income in the entire study cohort, the ORs for exposures to cow's milk or solid foods before 3 mo were 4.5 (CI 0.9–21.4) and 2.5 (CI 1.4–4.3), respectively.

To measure the magnitude of the differences in risk by exposure status in high and low genetic risk individuals, ORs were calculated after adjusting for ethnicity, birth order, and income. Table 3 shows that in low-risk individuals, exposure to cow's milk before 3 mo of age did not have a significantly elevated OR for IDDM. However, relative to unexposed low-risk individuals, early exposure to cow's milk was associated with a significantly elevated odds ratio in individuals at high risk (OR 11.3, CI 1.2–102). Similar findings were observed for exposure to solid foods before 3 mo of age. When the unexposed high (genetic) risk group was used as the referent, the ORs related to exposure to cow's milk and solid foods in the high (genetic) risk group were elevated but not significant (Table 3).

## DISCUSSION

Our data suggest that by including HLA-encoded risk in the odds ratios for infant diet exposures, we draw a more coherent picture of the contributions of both host and environment in the etiology of IDDM. Although the sample sizes in some of the analyses are small and therefore should be interpreted cautiously, this study is one of the first to incorporate both genetic and environmental data in the investigation of IDDM etiology.

We found little difference in the percentage breast-fed or the duration of breast-feeding by diabetes status in our

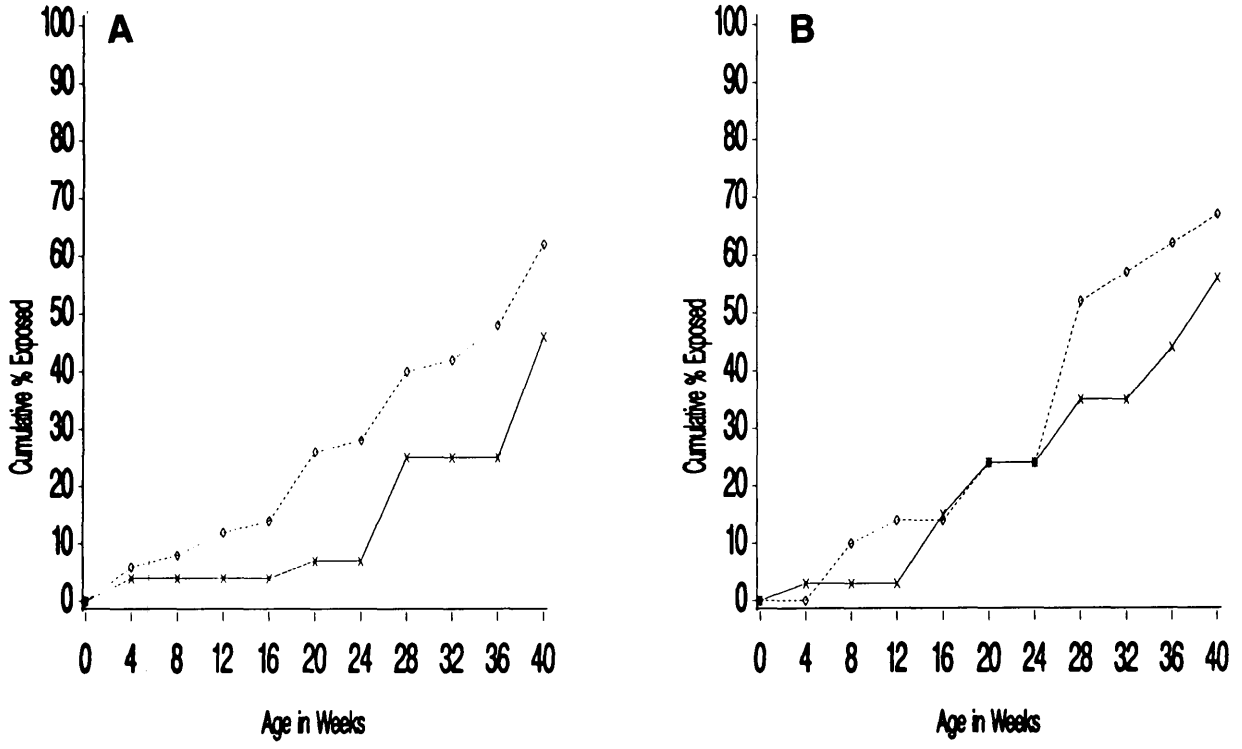


**FIG. 2. Age at exposure to cow's milk in diabetic subjects (◇) and control subjects (×) by genetic risk status as defined by the HLA-DQB1 marker (*A*, high risk, 50 diabetic subjects and 28 control subjects; *B*, low risk, 21 diabetic subjects and 34 control subjects).**

population, even in the high-risk groups (data not shown). This, coupled with the inconsistent findings in the literature (28–30), suggests that breast-feeding initiation and duration may be inadequate measures of IDDM risk. Moreover, animal models show that introduction of wheat and cow's milk protein sources at weaning may trigger diabetes (31–33). Therefore, in humans, duration of breast-feeding may not be as etiologically important as the age at introduction of alternative infant milks or foods.

Two ecological analyses showed that national consumption of milk proteins was directly related to the national IDDM incidence (11,34). In addition, two case-control studies demonstrated that early introduction of supplementary (non-breast) milks increased IDDM risk (13,14), whereas one study found no relationship by diabetes status (12). We did not have adequate data to examine cow's milk–based formulas separately as had been done in a previous study (13). The one study that examined age at introduction of solid foods in infancy found no difference by diabetes status (28). We observed a weaker association between IDDM risk and exposure to solid foods (as determined by the size of the overall OR) compared with the association found with cow's milk. The weaker relationship may be due to the wide variety of protein sources (e.g., cereal, fruit, vegetable) that were included in the loosely defined solid foods variable in our study.

A recently proposed hypothesis by Karjalainen et al. (35) suggests that sensitization and development of immune memory to cow's milk protein is the initial step in the etiology of IDDM. We propose that sensitization may occur in one of two ways—very early exposure to cow's milk before gut cellular tight junction closure, or exposure to cow's milk during an infection-caused gastrointestinal alteration when the intestinal barrier is compromised, allowing antigens to cross and initiate an immune reaction (36,37). In our study, the risk of IDDM was greatest when children were exposed to cow's milk before 3 mo of age, which may reflect the importance of the former route of exposure. The latter route of exposure would be impossible to observe without intensive follow-up in infancy. Children with newly diagnosed IDDM had higher levels of IgG anti-bovine serum albumin antibodies than age-matched control subjects (35), which may be a marker of immune sensitization by cow's milk. An observed amino acid similarity between a core sequence of bovine serum albumin and an HLA major histocompatibility complex II region encoding for HLA-DQB1 and a β-cell surface protein led to speculation (35) that after sensitization there is the potential for immune mimicry resulting in β-cell destruction. However, adequate experimental evidence for this hypothesis is not available.

The length of recall required to complete the infant diet history in our study varied by participant, but the mean was similar in the case and control populations. Accurate maternal recall of initiation of breast-feeding has been reported in the literature (38–40); however, recall of the duration of breast-feeding may be somewhat less reliable (41). As demonstrated by Vobecky et al. (42), it may be more difficult to detect a significant association with retrospective infant diet data because of its increased variability. Therefore, a significant finding using retrospective data may lead to a conservative rather than an

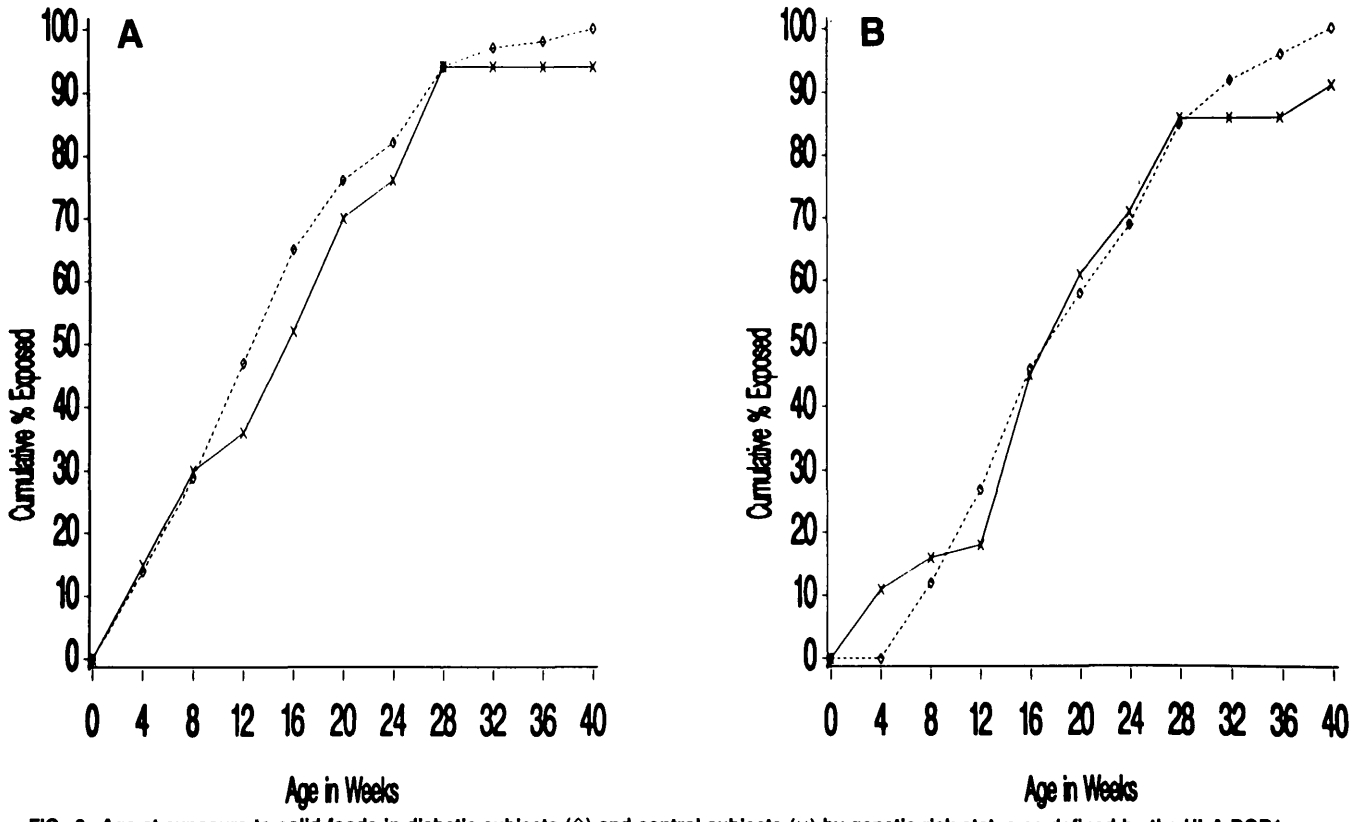


**FIG. 3. Age at exposure to solid foods in diabetic subjects (◇) and control subjects (×) by genetic risk status as defined by the HLA-DQB1 marker (*A*, high risk, 66 diabetic subjects and 33 control subjects; *B*, low risk, 26 diabetic subjects and 44 control subjects).**

invalid conclusion as long as recall bias was not present. Recall bias is unlikely in this study because of the length of time between infancy and onset of IDDM, and the lack of general knowledge of a potential relationship between infant diet and IDDM.

The potential misclassification of some of our study control subjects who possessed high-risk genetic markers and could thus develop IDDM in the future would have biased our findings toward the null. The only way to prevent this would be to select older control subjects who were no longer in the high-risk age-group; however, this would result in poorer recall of infant diet. It is also possible that we did not entirely control for the influence of birth order in these analyses because the relative birth order variable treated children from single child families as if they were a last born child, whereas their characteristics are more likely to resemble those of a first born child. Analysis of the data while excluding children from single child families (data not shown) resulted in findings that were similar to those presented herein, suggesting that the unique characteristics of these children did not bias our results.

The attributable risk was calculated to estimate the proportion of cases that could be prevented by the removal of a risk factor (i.e., early exposure to cow's milk or solid foods) from a population (43). The attributable risk for exposure before 3 mo of age to cow's milk and solid foods was 0.08 and 0.25, respectively. This means that if all parents in Colorado waited until after the age of 3 mo to introduce cow's milk or solid foods into the infant

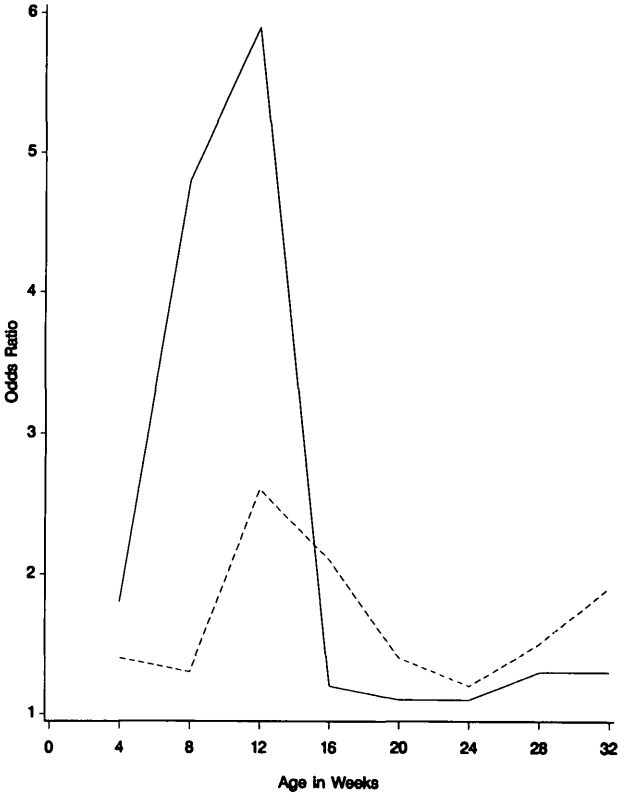


**FIG. 4. ORs for IDDM associated with different ages at exposure to cow's milk (*solid line*, 55 diabetic and 48 control subjects) and solid foods (*dashed line*, 71 diabetic and 59 control subjects) in a sample of the study cohort.**

TABLE 3
Adjusted and unadjusted ORs and 95% CIs for the risk of IDDM by exposures before 3 mo of age to cow's milk and solid foods in the infant diet

| Genetic Risk Groups | | Cow's milk | | | Solid foods | | |
|---|---|---|---|---|---|---|---|
| | | *n* | OR | CI | *n* | OR | CI |
| Low risk* | Unexposed | 50 | 1.0 | Referent | 55 | 1.0 | Referent |
| | Exposed† | 4 | 2.9 (5.3) | 0.2–36.5 (0.2–55.5) | 15 | 1.7 (1.7) | 0.5–5.8 (0.5–5.3) |
| High risk‡ | Unexposed† | 70 | 3.2 (3.0) | 1.5–6.8 (1.4–6.4) | 55 | 3.1 (3.1) | 1.4–7.2 (1.4–6.6) |
| | Exposed† | 7 | 11.3 (10.6) | 1.2–102.0 (1.8–71.4) | 44 | 6.3 (5.1) | 2.5–16.1 (2.1–15.0) |
| High risk‡ | Unexposed | 70 | 1.0 | Referent | 55 | 1.0 | Referent |
| | Exposed§ | 7 | 3.7 (3.5) | 0.4–33.6 (0.4–31.1) | 44 | 2.3 (1.6) | 0.9–5.9 (0.7–3.9) |

Values were adjusted for ethnicity, birth order, and current family income; unadjusted values are in parentheses.
*Low risk: at least one Asp in position 57 of the HLA-DQB1 chain.
†Referent group: low-risk individuals who were unexposed.
‡High risk: homozygous for amino acids other than Asp in position 57 of the HLA-DQB1 chain.
§Referent group: high-risk individuals who were unexposed.

diet, 8 and 25%, respectively, of the cases of IDDM in the state could be prevented. The attributable risks of exposure to cow's milk in low- and high-risk individuals (as defined by the HLA-DQB1 marker) were 0.11 and 0.09, respectively. Even though the magnitude of the OR is large for exposure to cow's milk, the attributable risk is relatively small because of the small percentage (3%) of the population that is exposed to cow's milk before 3 mo. As mentioned earlier, other IDDM cases may result from an immune sensitization occurring during a gastrointestinal illness; however, we were unable to account for this form of sensitization in our study.

The search for an environmental determinant of IDDM is driven, in part, by the possibility of preventing the disease through the removal (or addition) of a particular factor in the environment. Manipulation of the infant diet could be a relatively benign intervention that would have a strong public health impact. However, as shown by the low attributable risk associated with the exposures measured in our study, additional research into the timing and exact nature of the diabetogenic exposure in the infant diet is required before screening and/or interventions are implemented.

## ACKNOWLEDGMENTS

This research was funded by NIH Grant DK-32493; Grant BRSG-05357 awarded by the Biomedical Research Grant Program, Division of Research Resources, NIH; Grant RR-69 from the General Research Centers Program of the Division of Research Resources, NIH; PHS Research Grant 5-MO1-RR-00051 from the Division of Research Resources; and the Medical Research Council (United Kingdom).

We thank Phyllis Carosone-Link, Randall Deyle, Susan Gale, Susan Stengl, Mary Wilfawn, Terri Neville, and Suzanne Nash for efforts in identifying and recruiting participants. We also thank the participating hospitals and outpatient clinics in Colorado who provided facilities and phlebotomy support: San Luis Valley Diabetes Study, Alamosa; Memorial Hospital Pediatric Outpatient Clinics, Colorado Springs; Poudre Valley Hospital, Fort Collins; St. Mary's Hospital and Mesa County Health Department, Grand Junction; St. Mary Corwin Hospital, Pueblo; and the Barbara Davis Center for Childhood Diabetes and the Adult and Pediatric Clinical Research Centers at University Hospital, Denver. We thank Dr. John Todd for comments on this manuscript. We also thank members of Dr. Todd's laboratory for help with the nucleotide analyses.

## REFERENCES

1. Todd J, Bell J, McDevitt H: Amino acid 57 of the HLA-DQ beta chain is a major component in the genetic susceptibility to insulin-dependent diabetes mellitus. *Nature* (Lond) 329:599–604, 1987
2. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M: Aspartic acid at position 57 of the DQ chain protects against type I diabetes: a family study. *Proc Natl Acad Sci USA* 85:1–6, 1988
3. Svejgaard A, Platz P, Ryder LP: Insulin-dependent diabetes mellitus: joint results of the 8th workshop study. In *Histocompatibility Testing 1980.* Terasaki PI, Ed. Los Angeles, CA, UCLA Tissue Typing Laboratory, 1980, p. 638–56
4. Gamble DR: The epidemiology of insulin dependent diabetes, with particular reference to the relationship of virus infection to its etiology. *Epidemiol Rev* 2:49–70, 1980
5. Helgason T, Jonasson MR: Evidence for a food additive as a cause of ketosis-prone diabetes. *Lancet* 2:716–20, 1981
6. Robinson N, Fuller JH: Role of life events and difficulties in the onset of diabetes mellitus. *J Psychol Res* 29:583–91, 1985
7. Diabetes Epidemiology Research International: Preventing insulin-dependent diabetes mellitus: the environmental challenge. *Br Med J* 295:479–81, 1987
8. Borch-Johnsen K, Mandrup-Poulsen T, Zachau-Christiansen B, Joner G, Christy M, Kastrup K, Nerup J: Relation between breast-feeding and incidence of insulin-dependent diabetes mellitus. *Lancet* 2:1083–86, 1984
9. Glatthaar C, Whittall DE, Welborn TA, Gibson MJ, Brooks BH, Ryan MMP, Byrne C: Diabetes in Western Australian children: descriptive epidemiology. *Med J Austral* 148:117–23, 1988
10. Mayer EJ, Hamman RF, Gay EC, Lezotte DC, Savitz DA, Klingensmith GJ: Reduced risk of IDDM among breast-fed children. *Diabetes* 37:1625–32, 1988
11. Scott FW: Cow's milk and insulin-dependent diabetes mellitus: is there a relationship? *Am J Clin Nutr* 51:489–91, 1990
12. Blom L, Dahlquist G, Nystrom L, Sandstrom A, Wall S: The Swedish Childhood Diabetes Study: social and perinatal determinants for diabetes in childhood. *Diabetologia* 32:7–13, 1989
13. Kostraba JN, Dorman JS, LaPorte RE, Scott FW, Steenkiste AR, Gloninger M, Drash AL: Early infant diet and risk of insulin-dependent diabetes mellitus in blacks and whites: a matched case-control study. *Diabetes Care* 15:626–31, 1992
14. Virtanen SM, Rasanen L, Aro A, Lindrom J, Sippola H, Lounamaa R, Toivanen, Tuomilehto J, Akerblom HK, and Childhood Diabetes in Finland Study Group: Infant feeding in Finnish children <7 yr of age

with newly diagnosed IDDM. *Diabetes Care* 14:415–17, 1991
15. Hamman RF, Gay EC, Cruickshanks KJ, Cook M, Lezotte DC, Klingensmith GJ, Chase HP: Colorado IDDM Registry: incidence and validation of IDDM in children aged 0–17 yr. *Diabetes Care* 13:499–506, 1990
16. Bohme FG: Twenty censuses: Population and Housing Questions 1970–1980. Washington, DC, U.S. Dept. of Commerce, Bureau of the Census, 1979
17. Kostraba JN, Cruickshanks KJ, Neville TG, Lawler-Heavner J, Chase HP, Klingensmith GJ, Gay EC, Hamman RF: Clinical characteristics of IDDM in Hispanics and non-Hispanic whites: little evidence of heterogeneity by ethnicity. *Diabetes Care* 15:1303–309, 1992
19. *Colorado Vital Statistics 1988*. Colorado Department of Health, Health Statistics and Vital Records Division, Health Statistics Section, Denver
20. Danilovs J, Terasaki PI, Park MS, Ayoub G: B lymphocyte isolation by thrombin-nylon wool. In *Histocompatibility Testing 1980*. Terasaki PI, Ed. Los Angeles, CA, UCLA Tissue Typing Laboratory, 1980, p. 287–88
21. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnhein N: Enzymatic amplification of B-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–54, 1985
22. Horn GT, Bugawan TL, Long C, Erlich HA: Allelic sequence variation of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes susceptibility. *Proc Natl Acad Sci USA* 85:6012–16, 1988
23. Saiki RJ, Bugawan TL, Horn GT, Mullis KB, Erlich HA: Analysis of enzymatically amplified B-globin and HLA-DQ DNA with allele-specific oligonucleotide probes. *Nature* (Lond) 24:163–66, 1986
24. Todd JA, Fukui Y, Kitagawa T, Sasazuki T: The A3 allele of the HLA-DQA1 locus is associated with susceptibility to type 1 diabetes in Japanese. *Proc Natl Acad Sci USA* 87:1094–98, 1990
25. Todd JA, Mijovic C, Fletkcher J, Jenkins D, Bradwell AR, Barnett AH: Identification of susceptibility loci for insulin-dependent diabetes mellitus by transracial gene mapping. *Nature* (Lond) 338:587–89, 1989
26. Scraf S, Horn GT, Erlich HA: Direct cloning and sequence analysis of enzymatically amplified genomic sequences. *Science* 233:1076–78, 1986
27. SAS Institute Inc: *SAS User's Guide*. Statistics Version 5 Edition. Cary, NC, SAS Inst. Inc., 1985
28. Fort P, Lanes R, Dahlem S, Recker B, Weyman-Daum M, Pugliese M, Lifshitz F: Breast feeding and insulin-dependent diabetes mellitus in children. *J Am Coll Nutr* 5:439–41, 1986
29. Nigro G, Campea L, DeNovellis A, Orsini M: Breast-feeding and insulin-dependent diabetes mellitus (Letter). *Lancet* 1:467, 1985
30. Siemiatycki J, Colle E, Campbell S, DeWar RAD, Belmonte MM: Case-control study of IDDM. *Diabetes Care* 12:209–16, 1989
31. Elliot RB, Martin JM: Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? *Diabetologia* 26:297–99, 1984
32. Scott FW, Daneman D, Martin JM: Evidence for a critical role of diet in the development of insulin-dependent diabetes mellitus. *Diabetes Res* 7:153–57, 1988
33. Daneman D, Fishman L, Clarson C, Martin JM: Dietary triggers of insulin-dependent diabetes in the BB rat. *Diabetes Res* 5:93–97, 1987
34. Dahl-Jorgensen K, Joner G, Hanssen KF: Relationship between cow's milk consumption and incidence of IDDM in childhood. *Diabetes Care* 14:1081–83, 1991
35. Karjalainen J, Martin JM, Knip M, Ilonen J, Robinson BH, Savilahti E, Akerblom HK, Dosch H-M: A bovine albumin peptides as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med* 327: 302–307, 1992
36. Iyngkaran N, Robinson MJ, Sumithran E, Lam SK, Puthucheary SD, Yadav M: Cow's milk protein-sensitive enteropathy: an important factor in prolonging diarrhea of acute infective enteritis in early infancy. *Arch Dis Child* 53:150–53, 1978
37. Cunningham-Rundles C, Brandeis WE, Good RA, Day NK: Milk precipitins, circulating immune complexes, and IgA deficiency. *Proc Natl Acad Sci USA* 75:3387–89, 1978
38. Haaga JG: Reliability of retrospective survey data on infant feeding. *Demography* 25:307–14, 1988
39. Haggard EA, Brekstad A, Skard AG: On the reliability of the anamnestic interview. *J Abnor Soc Psychol* 61:311–18, 1960
40. Kark JD, Troya G, Friedlander Y, Slater PE, Stein Y: Validity of maternal reporting of breast-feeding history and the association with blood lipids in 17 year olds in Jerusalem. *J Epidemiol Community Health* 38:218–25, 1984
41. Huttly SRA, Barros FC, Victoria CG, Beria JU, Vaughan JP: Do mothers overestimate breast-feeding duration? An example of recall bias from a study in Southern Brazil. *Am J Epidemiol* 132:572–75, 1990
42. Vobecky JS, Vobecky J, Froda S: The reliability of the maternal memory in a retrospective assessment of nutritional status. J Clin Epidemiol 41:261–65, 1988
43. Lilienfeld AM, Lilienfeld DE: *Foundations of Epidemiology*. New York, Oxford Unv. Press, 1980, p. 217–18