Very early introduction of semisolid foods in preterm infants does not increase food allergies or atopic dermatitis

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Conflicts of interest: none

Funding sources: This work was supported by research grants from the Alma and K.A. Snellman Foundation, the Finnish Medical Association, the Allergy Research Foundation and the Finnish Pediatric Research Foundation.

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Word count: 3480

Number of figures: 3

Number of tables: 3

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Introduction

The optimal age for the introduction of solid foods for infants has long been a controversial issue. National and international recommendations have varied from prolonged avoidance diets to early introduction approaches over the past decades.[1] Current accumulating research evidence favours early rather than delayed introduction of solid foods because of its advantages in promoting oral tolerance and preventing disease.[2] Furthermore, diversity of diet before the age of one year seems to be associated with lower incidence of food allergies.[3] It has been suggested that the probable optimal age for tolerance induction in normal full-term infants will fall within 3–7 months of age.[4, 5]

The few available published nutritional recommendations for preterm infants (PT; born before the 37th gestational week or with birth weight under 2,500g) differ from those for term-born infants.[6, 7] PT infants have limited nutrient stores[6] and an increased need for energy[8], protein[9,10], long-chain polyunsaturated fatty acids[11] and micronutrients.[12-15] Some authors have recommended the introduction of complementary foods at three to four months of corrected age[6,16], whereas others suggest, as a general rule, not to introduce them before the corrected age of four months and only when the infant has acquired the ability to eat.[17, 18] However, there is still a lack of consensus and data regarding post-discharge nutrition of preterm infants, especially those that are extremely preterm (EP, preterm infants born before the 28th gestational week). In a recent systematic review, the timing of complementary feeding in preterm infants was defined as *early* when it started before the corrected gestational age of 15 weeks, *intermediate* from 15 to 23 weeks and *late* if it started after 24 weeks.[7]

Despite the immaturity of preterm infants, there is no evidence that early feeding practices increase the incidence of atopic diseases. In contrast, clinical experience and a few published studies seem to show that preterm children have even fewer food allergies and a smaller

occurrence of atopic dermatitis than term-born children do.[19, 20] This supports the hypothesis that early complementary feeding is a protective factor rather than a risk factor for the development of these diseases.

If the swallowing behaviour is mature enough, it is a common clinical practice in our unit at Oulu University Hospital to introduce semisolid complementary foods to the diet of all preterm infants at as early as 3 months of chronological age. Therefore, all preterm infants may start their first semisolid foods before their term-equivalent ages, and the EP infants even before their calculated due date.

In this large retrospective cohort study on preterm infants and their age- and sex matched term-born control children from the general population, our hypothesis was that prematurity and the early introduction of complementary feeding with semisolid foods does not increase the prevalence of food allergies or atopic dermatitis by the ages of one and two years. We also describe here the exceptionally early feeding practice for preterm infants that currently prevails.

Methods

Our study is a single-centre, retrospective cohort study including age- and sex-matched control children. The primary outcome of the study was the difference in the timing of complementary feeding between preterm and full-term infants. The secondary outcomes were the incidences of food allergies and atopic dermatitis by the ages of one and two years.

Subjects

The Oulu University Hospital is a tertiary hospital with approximately 4,000 births per year; it has a modern university hospital level neonatal intensive care unit (NICU). All the preterm infants born in Oulu University Hospital (or admitted from the other hospitals in the Oulu

University Hospital District) between 1 January 2008 and 31 December 2012 were included. They were identified from the electronic patient registry of Oulu University Hospital using ICD-10 codes for low birth weight and prematurity. The preterm infants were grouped according to their gestational age as *late preterm* (LP, born from 32 to <37 gestational weeks), *very preterm* (VP, born from 28 to <32 gestational weeks) and *extremely preterm* (EP, born <28 gestational weeks) infants. Clinical data over the period of hospital stay and on all the follow-up visits in the hospital outpatient clinic and at the primary care public health centres (PHC) were obtained from hospital and PHC medical patient records up to the age of two years of chronological age. The data included: birth date; gestational weeks at birth; weight and length at the time of birth and at the age of one year; possible causes of prematurity; delivery method; use of breast milk; age at introduction of cow's milk formulas; age at introduction of various foods; and the symptoms and doctor-defined diagnoses of food allergy and atopic dermatitis. For this study, *cow's milk formula* means the standard formula; it does not include any special formulas designed for preterm infants (hydrolysed formulas or those based on amino acids). *Atopic dermatitis*[21] is defined as either doctor-diagnosed atopic dermatitis or eczema with classical history, location and characteristics described in the clinical patient records. Doctor-diagnosed food allergy was based on the clinical patient records. All preterm infants with follow-up data for less than up to the age of 12 months of chronological age were excluded from the analyses.

For each preterm infant, a chronological age- and sex-matched term-born control subject was identified by using the PHC child health clinics' electronic patient registry in the city of Oulu, Finland. The search was carried out using the birth date of a preterm infant; the closest available term infant of the same gender was chosen as the control. From the medical patient records, clinical data were obtained regarding regular follow-up visits in the public child health clinic, monthly during the first year of life and at the ages of 18 and 24 months. The

collected data included: birth date; gestational weeks at birth; weight and length at the time of birth and at the ages of 12, 18 and 24 months; duration of breastfeeding; age at introduction of cow's milk formulas; age at introduction of various foods; and the symptoms and doctor-defined diagnoses of food allergy and atopic dermatitis.

The study was carried out according to national and international ethical principles, regulations and laws regarding biomedical research. The study protocol was approved by Oulu University Hospital, the Ethical committee of North Ostrobothnia's hospital district and the City of Oulu. The data were collected retrospectively from medical records; there was no direct contact with patients or families. All the data were stored and analysed anonymously, without personal identification data.

Statistical methods

Student's t-test was used to analyse normally-distributed continuous variables, and Mann-Whitney U-test or Kruskal-Wallis analysis was used in cases of skewed distributions. Differences in the distribution of individuals among groups were tested with Chi-square statistics unless any expected value was less than five, when Fisher's exact test was used. A two-tailed P value of 0.05 was considered to indicate statistical significance. All the data were analysed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp. Released 2013. Armonk, NY, USA). The exact or estimated age (in months) when each of the foods were introduced to the diet of a child was defined from patient records. For preterm infants, the time of food introduction was calculated for both chronological and corrected ages.

Results

In the five years from 2008 to 2012, 935 preterm infants were born and/or cared for in the NICU of Oulu University Hospital (Figure 1). Of these, 471 were not followed up throughout the first year of life and were therefore excluded from the analysis—follow-up data were

unavailable either because there was no prematurity-related need for extended follow-up or because the patient had moved out of the Hospital's catchment area after discharge. Of these excluded infants, by far the most (83.9%) were LP, while 11.9% were VP and only 4.2% EP. Altogether, 464 preterm infants were included and, for 250 of these, follow-up data up to the age of 24 months were also available. Controls from the general population were initially identified for all 935 preterm infants. The up-to-12-months data were analysed for the 464 cases and their controls, and the up-to-24-months data for the 250 cases and their controls. Baseline characteristics of the children are shown in Table 1.

Timing of food introduction

The results for the introduction of various foods according to *the chronological age from birth* are shown in Table 2 and Figure 2. The median chronological age from birth at the introduction of semisolid food (of any kind) was 3.0 months (interquartile range (IQR) 3.0–4.0 months) in all preterm infants, compared to 4.0 months (IQR 4.0–5.0 months) in controls ($p<0.001$). The chronological age at introduction varied between specific foods; it was significantly earlier in preterm infants for fruits, vegetables and meats ($p<0.001$ for each food) but did not differ for oatmeal or other cereal ($p=0.117$) or for cow's milk formulas ($p=0.114$). Among the preterm infants, the chronological age at introduction was independent of the degree of prematurity.

The results for the introduction of various foods according to *the corrected age* of the preterm infants are shown in Table 3 and Figure 3. For all preterm infants, semisolid food (of any kind) was introduced at the median corrected age of 1.4 months (IQR 0.8–2.1 months). Analysis by degree of prematurity showed that the introduction of semisolid food (of any kind) began at the median corrected age of 1.9 months (IQR 1.5–2.9 months) among the LP

121 infants, 0.9 months (IQR 0.6–1.3 months) among the VP infants and 0.1 months (IQR -0.05–
122 0.5 months) among the EP infants.

123

124 *The incidence of food allergy and atopic dermatitis*

125 The overall cumulative incidences of various food allergies (FAs) among the entire control
126 population (*n*=935) were 3.3% for any FA, 1.9% for milk, 0.2% for wheat, 1.5% for egg and
127 0.3% for any other food by the age of 12 months, and 4.1%, 1.9%, 0.2%, 2.4% and 1.2% by
128 the age of 24 months (*n*=905). Correspondingly, the cumulative incidence of atopic dermatitis
129 among the entire control population was 10.1% by the age of 12 months and 14.8% by the age
130 of 24 months.

131 The comparisons of cumulative incidences of food allergies and atopic dermatitis between the
132 preterm infants and their controls are shown in Table 3. Among the food allergies, only the
133 cumulative incidence of egg allergy differed statistically significantly by the age of 12 months
134 (0.2% vs. 2.4%, p=0.006), although there was only one preterm infant who was diagnosed
135 with egg allergy. By the age of 24 months, this difference between the 250 preterm infants
136 with follow-up data available and their controls had ceased to be statistically significant (0.4%
137 vs. 2.0%, *p*=0.122). There were no significant differences in the cumulative incidence of food
138 allergies between LP, VP or EP infants and controls by the age of one and two years. The
139 mode of delivery, caesarean section vs. vaginal, had no impact on the incidences of food
140 allergy or atopic eczema among the preterm infants (data not shown).

141 Among the preterm infants, any semisolid food was introduced significantly later into the diet
142 of those who developed any food allergy (median corrected age 2.3 months, IQR 1.2-2.8) than
143 in those who did not (1.4, IQR 0.8-2.1) (p=0.031). The age at the introduction of cow's milk

formula was not related to the development of cow's milk allergy or any food allergy among the preterm infants (data not shown).

The cumulative incidence of atopic dermatitis was 17.7% by the age of 12 months and 19.6% by the age of 24 months among the preterm infants; it did not differ significantly from the controls (14.9% by the age of 12 months, p=0.29; 14.9% by the age of 24 months, p=0.23). The only significant difference in the cumulative incidence of atopic dermatitis was between EP infants and controls by the age of 24 months (35.3 vs. 14.9%, *p=0.039*).

The incidence of atopic dermatitis was not related to the age at the introduction of cow's milk formula or any semisolid foods among the preterm infants (data not shown). However, cow's milk formula was introduced significantly earlier and cereals significantly later in those controls who developed atopic dermatitis than in those who did not [median 1.0 months (IQR 0-2.0) vs. 1.5 (0-5.0), p=0.023; and 6.0 (6.0-6.0) vs. 6.0 (5.0-6.0, p=0.006, respectively].

Discussion

Among preterm infants, the ability to suck liquids or swallow solid food and other oral motor skills, as well as keeping an adequate position during feeding, varies widely.[22, 23] At our institution, experienced nurses and a speech therapist follow the developmental progress and readiness of preterm infants to swallow semisolid foods without respiratory or positional problems; they check weekly during the hospital stay and after home discharge before they recommend the introduction of complementary semisolid foods.[20] If the preterm infant has problems in coordination of swallowing and breathing, complementary food is given as gruel. We recently reported that even EP babies (n=19) learn the various feeding skills earlier than, or at the same corrected ages as, full-term infants.[22] Now, looking at the data furnished by this large cohort of preterm infants at our institution, we report that the introduction of semisolid

foods occurs at the median age of three months from birth among all preterm babies, independently of the degree of prematurity. Therefore, the median corrected age at the time of semisolid food introduction, based on the calculated due date (that is, the developmental age of preterm babies) was only 1.4 months, and most strikingly 0.1 months (IQR -0.05–0.5 months) among the most immature babies (<28th gestational week). This early feeding practice will enhance the growth of preterm babies and provides diverse stimulus for oral sensory and oral motor development in terms of new textures and tastes. It is important, however, that the feeding technique used is based on the developmental stage of a preterm infant. Our experience is that preterm babies usually gain the needed skills for semisolid complementary feeding earlier than their term peers and the skills for eating solid foods at the same corrected age (i.e. developmental age). Furthermore, we have seen no increase in feeding problems among preterm infants starting semisolids early compared to those who start these complementary foods later.

Oral tolerance is generally accepted as a key mechanism to maintain the balance between body defence and food antigens.[24] Other factors, such as microbiota and other routes of exposure to the antigens, e.g. via skin, may also have a role in the development of tolerance or sensitization.[25] The physiological period of maturation may involve an optimal time-frame for antigen presentation in infants.[4, 26] This accords with the perinatal and postnatal programming and maturation of the gut-associated lymphoid tissue (GALT); the likely critical tolerogenic window in term-born babies has been suggested to reside between three and seven months of life.[4, 5, 27] The results from recent intervention studies support this hypothesis.[28-30] For preterm babies, the optimal age for the introduction of complementary foods, in terms of the development of oral tolerance, is not yet known. In a newborn baby, both the epithelial barrier and GALT are characteristically incomplete. The epithelial barrier may remain fragile and leaky up to two years of age in humans[5, 31], but crypt formation and acquisition of adult

properties begins as early as the 11th or 12th gestational week, and the intestine reaches maturity by around the 22nd gestational week.[32] Furthermore, immoderate influx of macromolecules across the epithelium is prevented by *gut closure*, the establishment of a more mature epithelial barrier. This occurs within a few days after birth in humans.[26] Although the preterm infants' immaturity means that they have higher intestinal permeability at birth, they seem to adapt rapidly postnatally.[33-35] In a Dutch study study, the preterm infants (26th to 36th gestational weeks) had higher intestinal permeability during the first two days of life but there was no difference between preterm and term infants three to six days later.[36] After birth, the size of the Peyer's patches (PPs) increases and the germinal centres develop in line with normal physiological maturation by encountering foreign material.[37, 38] Taken together, it is logical to assume that the GALT of preterm babies is ready to encounter foreign proteins from the 23rd gestational week onwards and to begin the maturation process very soon after birth.

Our results provide indirect evidence that, despite general immaturity, the GALT of preterm infants is ready to encounter food proteins to promote tolerance at 3–4 months chronological age. We report here that the cumulative incidence of any doctor-diagnosed food allergy by the age of one year was 3.2% among all preterm infants and did not differ from that observed in the age- and sex-matched full-term control children from the general population. Strikingly, none of the 53 EP infants had any food allergies, and only three of the 125 VP infants had cow's milk allergy (but no other food allergies) by the age of one year, despite the very early introduction of complementary feeding. Furthermore, we observed that any semisolid food was introduced significantly later into the diet of those preterm infants who developed any food allergy. Only a few other studies into food allergy in preterm infants have been published. The results of the Canadian birth cohort study suggest that no association exists between gestational age and the development of food allergies mediated by Immunoglobin E

(IgE).[19] They report the cumulative incidences of any food allergy to be 3.5% in term infants, 4.2% in LP infants, 1.2% in VP infants and 8.1% in EP infants by the age of seven years. In the Japanese study, 10.2% of LP infants and 7.1% of VP and EP infants (<32 gestational weeks) had a food allergy by the age of 18 months, compared to 11.0% of the term-born children.[39]

The cumulative incidence of doctor-diagnosed atopic dermatitis in our entire control population (*n*=935) was 15%, both by the age of one and by the age of two years. This is comparable to the prevalence reported in other studies for this age group.[40, 41] Several reports have shown similar or lower incidences of atopic dermatitis in preterm infants compared to full-term children during the early years of life.[41-47] Extremely preterm infants have also been reported to have a decreased risk for atopic dermatitis when compared to infants born with greater gestational ages.[20] Our present results are in line with other studies, as the cumulative incidence of atopic dermatitis was 18% by the age of 12 months and 20% by the age of 24 months among all preterm infants; it did not differ significantly from the incidence among the age- and sex-matched controls. Therefore, it is evident that the very early complementary feeding practice currently used in our unit does not increase the incidence of atopic dermatitis in preterm infants.

The major weakness of our study is its retrospective design, based on the regular medical records. The data regarding complementary feeding and the symptoms and diagnoses related to food allergy and atopic dermatitis are therefore somewhat incomplete. This holds especially true for the control children from the general population, as the hospital records for the preterm infants were more accurate. However, the overall incidence of food allergy and atopic dermatitis are consistent with prior studies, suggesting that our diagnoses may be fairly accurate despite these limitations. We found the cumulative incidence of any doctor-defined food allergy (based on clinical history and/or open oral food challenge) to be 3.3% by the age

of one year and 4.1% by the age of two years, among 935 control children from the general population. These figures align well with the reported prevalence of 2–7% in children under five years of age in other European countries and in the U.S..[48] Because of retrospective design of the study, the exact data on the timing of each specific food was heterogeneously available. In addition, the size of the study population is limited taken into account the low prevalence of specific food allergies. Therefore, no definite conclusions related to the timing of a specific food and subsequent development of respective allergy (e.g. egg introduction and egg allergy) can be drawn.

In conclusion, we describe here a complementary feeding practice for preterm babies that starts much earlier than that employed for full-term controls. We find that it is safe and does not increase the risk of food allergies or atopic dermatitis even among the most preterm infants. It seems evident that the GALT of preterm infants is ready to encounter food proteins and to begin the maturation process within 3–6 months from birth, regardless of the corrected age. It is probable that the key determining factor for the window of opportunity to develop oral tolerance is the time outside the womb rather than the corrected age (i.e. developmental age) of the baby. Our results shed new light on the current discussion on the optimal timing of complementary feeding in general, as even the most preterm infants seem to be immunologically ready to encounter foreign food proteins considerably earlier than their term peers.

Introduction

The optimal age for the introduction of solid foods for infants has long been a controversial issue. National and international recommendations have varied from prolonged avoidance diets to early introduction approaches over the past decades.[1] Current accumulating research evidence favours early rather than delayed introduction of solid foods because of its advantages in promoting oral tolerance and preventing disease.[2] Furthermore, diversity of diet before the age of one year seems to be associated with lower incidence of food allergies.[3] It has been suggested that the probable optimal age for tolerance induction in normal full-term infants will fall within 3–7 months of age.[4, 5]

The few available published nutritional recommendations for preterm infants (PT; born before the 37th gestational week or with birth weight under 2,500g) differ from those for term-born infants.[6, 7] PT infants have limited nutrient stores[6] and an increased need for energy[8], protein[9,10], long-chain polyunsaturated fatty acids[11] and micronutrients.[12-15] Some authors have recommended the introduction of complementary foods at three to four months of corrected age[6], whereas others suggest, as a general rule, not to introduce them before the corrected age of four months and only when the infant has acquired the ability to eat.[16, 17] However, there is still a lack of consensus and data regarding post-discharge nutrition of preterm infants, especially those that are extremely preterm (EP, preterm infants born before the 28th gestational week). In a recent systematic review, the timing of complementary feeding in preterm infants was defined as *early* when it started before the corrected age of 15 weeks, *intermediate* from 15 to 23 weeks and *late* if it started after 24 weeks.[7]

Despite the immaturity of preterm infants, there is no evidence that early feeding practices increase the incidence of atopic diseases. In contrast, clinical experience and a few published studies seem to show that preterm children have even fewer food allergies and a smaller

occurrence of atopic eczema than term-born children do.[18, 19] This supports the hypothesis that early complementary feeding is a protective factor rather than a risk factor for the development of these diseases.

If the swallowing behaviour is mature enough, it is a common clinical practice in our unit at Oulu University Hospital to introduce semisolid complementary foods to the diet of all preterm infants at as early as 3 months of chronological age. Therefore, all preterm infants may start their first semisolid foods before their term-equivalent ages, and the EP infants even before their calculated due date.

In this large retrospective cohort study on preterm infants and their age- and sex matched term-born control children from the general population, our hypothesis was that prematurity and the early introduction of complementary feeding with semisolid foods does not increase the prevalence of food allergies or atopic dermatitis by the ages of one and two years. We also describe here the exceptionally early feeding practice for preterm infants that currently prevails.

Methods

Our study is a single-centre, retrospective cohort study including age- and sex-matched control children. The primary outcome of the study was the difference in the timing of complementary feeding between preterm and full-term infants. The secondary outcomes were the incidences of food allergies and atopic dermatitis by the ages of one and two years.

Subjects

The Oulu University Hospital is a tertiary hospital with approximately 4,000 births per year; it has a modern university hospital level neonatal intensive care unit (NICU). All the preterm infants born in Oulu University Hospital (or admitted from the other hospitals in the Oulu

University Hospital District) between 1 January 2008 and 31 December 2012 were included. They were identified from the electronic patient registry of Oulu University Hospital using ICD-10 codes for low birth weight and prematurity. The preterm infants were grouped according to their gestational age as *late preterm* (LP, born from 32 to <37 gestational weeks), *very preterm* (VP, born from 28 to <32 gestational weeks) and *extremely preterm* (EP, born <28 gestational weeks) infants. Clinical data over the period of hospital stay and on all the follow-up visits in the hospital outpatient clinic and at the primary care public health centres (PHC) were obtained from hospital and PHC medical patient records up to the age of two years. The data included: birth date; gestational weeks at birth; weight and length at the time of birth and at the age of one year; possible causes of prematurity; delivery method; use of breast milk; age at introduction of cow's milk formulas; age at introduction of various foods; and the symptoms and doctor-defined diagnoses of food allergy and atopic dermatitis. For this study, *cow's milk formula* means the standard formula; it does not include any special formulas designed for preterm infants (hydrolysed formulas or those based on amino acids). *Atopic dermatitis* is defined as either doctor-diagnosed atopic dermatitis or eczema with classical history, location and characteristics described in the clinical patient records. Doctor-diagnosed food allergy was based on the clinical patient records. All preterm infants with follow-up data for less than up to the age of 12 months were excluded from the analyses.

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the ages of 12, 18 and 24 months; duration of breastfeeding; age at introduction of cow's milk formulas; age at introduction of various foods; and the symptoms and doctor-defined diagnoses of food allergy and atopic dermatitis.

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Statistical methods

Student's t-test was used to analyse normally-distributed continuous variables, and Mann-Whitney U-test or Kruskal-Wallis analysis was used in cases of skewed distributions. Differences in the distribution of individuals among groups were tested with Chi-square statistics unless any expected value was less than five, when Fisher's exact test was used. A two-tailed P value of 0.05 was considered to indicate statistical significance. All the data were analysed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp. Released 2013. Armonk, NY, USA). The exact or estimated age (in months) when each of the foods were introduced to the diet of a child was defined from patient records. For preterm infants, the time of food introduction was calculated for both chronological and corrected ages.

Results

In the five years from 2008 to 2012, 935 preterm infants were born and/or cared for in the NICU of Oulu University Hospital (Figure 1). Of these, 471 were not followed up throughout the first year of life and were therefore excluded from the analysis—follow-up data were unavailable either because there was no prematurity-related need for extended follow-up or

because the patient had moved out of the Hospital's catchment area after discharge. Of these excluded infants, by far the most (83.9%) were LP, while 11.9% were VP and only 4.2% EP. Altogether, 464 preterm infants were included and, for 250 of these, follow-up data up to the age of 24 months were also available. Age- and sex-matched full-term control children from the general population were initially identified for all 935 preterm infants. The up-to-12-months data were analysed for the 464 cases and their controls, and the up-to-24-months data for the 250 cases and their controls. Baseline characteristics of the children are shown in Table 1.

Timing of food introduction

The results for the introduction of various foods according to *the chronological age from birth* are shown in Table 2 and Figure 2. The median chronological age from birth at the introduction of semisolid food (of any kind) was 3.0 months (interquartile range (IQR) 3.0–4.0 months) in all preterm infants, compared to 4.0 months (IQR 4.0–5.0 months) in controls ($p<0.001$). The chronological age at introduction varied between specific foods; it was significantly earlier in preterm infants for fruits, vegetables and meats ($p<0.001$ for each food) but did not differ for oatmeal or other cereal ($p=0.117$) or for cow's milk formulas ($p=0.114$). Among the preterm infants, the chronological age at introduction was independent of the degree of prematurity.

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121 infants, 0.9 months (IQR 0.6–1.3 months) among the VP infants and 0.1 months (IQR -0.05–
122 0.5 months) among the EP infants.

123

124 *The incidence of food allergy and atopic dermatitis*

125 The overall cumulative incidences of various food allergies (FAs) among the entire control
126 population (*n*=935) were 3.3% for any FA, 1.9% for milk, 0.2% for wheat, 1.5% for egg and
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136 infants with follow-up data available and their age- and sex matched control children had
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138 differences in the cumulative incidence of food allergies between LP, VP or EP infants and
139 controls by the age of one and two years. The mode of delivery, caesarean section vs. vaginal,
140 had no impact on the incidences of food allergy or atopic eczema among the preterm infants
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142 Among the preterm infants, any semisolid food was introduced significantly later into the diet
143 of those who developed any food allergy (median corrected age 2.3 months, IQR 1.2-2.8) than
144 in those who did not (1.4, IQR 0.8-2.1) (p=0.031). The age at the introduction of cow's milk

formula was not related to the development of cow's milk allergy or any food allergy among the preterm infants (data not shown).

The cumulative incidence of atopic dermatitis was 17.7% by the age of 12 months and 19.6% by the age of 24 months among the preterm infants; it did not differ significantly from the age- and sex-matched controls (14.9% by the age of 12 months, p=0.29; 14.9% by the age of 24 months, p=0.23). The only significant difference in the cumulative incidence of atopic dermatitis was between EP infants and controls by the age of 24 months (35.3 vs. 14.9%, *p=0.039*).

The incidence of atopic dermatitis was not related to the age at the introduction of cow's milk formula or any semisolid foods among the preterm infants (data not shown). However, cow's milk formula was introduced significantly earlier and cereals significantly later in those control children who developed atopic dermatitis than in those who did not [median 1.0 months (IQR 0-2.0) vs. 1.5 (0-5.0), p=0.023; and 6.0 (6.0-6.0) vs. 6.0 (5.0-6.0, p=0.006, respectively].

Discussion

Among preterm infants, the ability to suck liquids or swallow solid food and other oral motor skills, as well as keeping an adequate position during feeding, varies widely.[20, 21] At our institution, experienced nurses and a speech therapist follow the developmental progress and readiness of preterm infants to swallow semisolid foods without respiratory or positional problems; they check weekly during the hospital stay and after home discharge before they recommend the introduction of complementary semisolid foods.[20] If the preterm infant has problems in coordination of swallowing and breathing, complementary food is given as gruel. We recently reported that even EP babies (n=19) learn the various feeding skills earlier than,

or at the same corrected ages as, full-term infants.[20] Now, looking at the data furnished by this large cohort of preterm infants at our institution, we report that the introduction of semisolid foods occurs at the median chronological age of three months from birth among all preterm babies, independently of the degree of prematurity. Therefore, the median corrected age at the time of semisolid food introduction, based on the calculated due date (that is, the developmental age of preterm babies) was only 1.4 months, and most strikingly 0.1 months (IQR -0.05–0.5 months) among the most immature babies (<28th gestational week). This early feeding practice will enhance the growth of preterm babies and provides diverse stimulus for oral sensory and oral motor development in terms of new textures and tastes. It is important, however, that the feeding technique used is based on the developmental stage of a preterm infant. Our experience is that preterm babies usually gain the needed skills for semisolid complementary feeding earlier than their term peers and the skills for eating solid foods at the same corrected age (i.e. developmental age). Furthermore, we have seen no increase in feeding problems among preterm infants starting semisolids early compared to those who start these complementary foods later.

Oral tolerance is generally accepted as a key mechanism to maintain the balance between body defence and food antigens.[22] Other factors, such as microbiota and other routes of exposure to the antigens, e.g. via skin, may also have a role in the development of tolerance or sensitization.[23] The physiological period of maturation may involve an optimal time-frame for antigen presentation in infants.[4, 24] This accords with the perinatal and postnatal programming and maturation of the gut-associated lymphoid tissue (GALT); the likely critical tolerogenic window in term-born babies has been suggested to reside between three and seven months of life.[4, 5, 25] The results from recent intervention studies support this hypothesis.[26-28] For preterm babies, the optimal age for the introduction of complementary foods, in terms of the development of oral tolerance, is not yet known. In a newborn baby, both the epithelial barrier

and GALT are characteristically incomplete. The epithelial barrier may remain fragile and leaky up to two years of age in humans[5, 29], but crypt formation and acquisition of adult properties begins as early as the 11th or 12th gestational week, and the intestine reaches maturity by around the 22nd gestational week.[30] Furthermore, immoderate influx of macromolecules across the epithelium is prevented by *gut closure*, the establishment of a more mature epithelial barrier. This occurs within a few days after birth in humans.[24] Although the preterm infants' immaturity means that they have higher intestinal permeability at birth, they seem to adapt rapidly postnatally.[31-33] In a Dutch study study, the preterm infants (26th to 36th gestational weeks) had higher intestinal permeability during the first two days of life but there was no difference between preterm and term infants three to six days later.[34] After birth, the size of the Peyer's patches (PPs) increases and the germinal centres develop in line with normal physiological maturation by encountering foreign material.[35, 36] Taken together, it is logical to assume that the GALT of preterm babies is ready to encounter foreign proteins from the 23rd gestational week onwards and to begin the maturation process very soon after birth.

Our results provide indirect evidence that, despite general immaturity, the GALT of preterm infants is ready to encounter food proteins to promote tolerance at 3–4 months chronological age. We report here that the cumulative incidence of any doctor-diagnosed food allergy by the age of one year was 3.2% among all preterm infants and did not differ from that observed in the age- and sex-matched full-term control children from the general population. Strikingly, none of the 53 EP infants had any food allergies, and only three of the 125 VP infants had cow's milk allergy (but no other food allergies) by the age of one year, despite the very early introduction of complementary feeding. Furthermore, we observed that any semisolid food was introduced significantly later into the diet of those preterm infants who developed any food allergy. Only a few other studies into food allergy in preterm infants have been

219 published. The results of the Canadian birth cohort study suggest that no association exists
220 between gestational age and the development of food allergies mediated by Immunoglobin E
221 (IgE).[18] They report the cumulative incidences of any food allergy to be 3.5% in term infants,
222 4.2% in LP infants, 1.2% in VP infants and 8.1% in EP infants by the age of seven years. In
223 the Japanese study, 10.2% of LP infants and 7.1% of VP and EP infants (<32 gestational
224 weeks) had a food allergy by the age of 18 months, compared to 11.0% of the term-born
225 children.[37]

226 The cumulative incidence of doctor-diagnosed atopic dermatitis in our entire control
227 population (*n*=935) was 15%, both by the age of one and by the age of two years. This is
228 comparable to the prevalence reported in other studies for this age group.[38, 39] Several reports
229 have shown similar or lower incidences of atopic dermatitis in preterm infants compared to
230 full-term children during the early years of life.[40-45] Extremely preterm infants have also been
231 reported to have a decreased risk for atopic dermatitis when compared to infants born with
232 greater gestational ages.[19] Our present results are in line with other studies, as the cumulative
233 incidence of atopic dermatitis was 18% by the age of 12 months and 20% by the age of 24
234 months among all preterm infants; it did not differ significantly from the incidence among the
235 age- and sex-matched controls. Therefore, it is evident that the very early complementary
236 feeding practice currently used in our unit does not increase the incidence of atopic dermatitis
237 in preterm infants.

238 The major weakness of our study is its retrospective design, based on the regular medical
239 records. The data regarding complementary feeding and the symptoms and diagnoses related
240 to food allergy and atopic dermatitis are therefore somewhat incomplete. This holds
241 especially true for the control children from the general population, as the hospital records for
242 the preterm infants were more accurate. However, the overall incidence of food allergy and
243 atopic dermatitis are consistent with prior studies, suggesting that our diagnoses may be fairly

accurate despite these limitations. We found the cumulative incidence of any doctor-defined food allergy (based on clinical history and/or open oral food challenge) to be 3.3% by the age of one year and 4.1% by the age of two years, among 935 control children from the general population. These figures align well with the reported prevalence of 2–7% in children under five years of age in other European countries and in the U.S..[46] Because of retrospective design of the study, the exact data on the timing of each specific food was heterogeneously available. In addition, the size of the study population is limited taken into account the low prevalence of specific food allergies. Therefore, no definite conclusions related to the timing of a specific food and subsequent development of respective allergy (e.g. egg introduction and egg allergy) can be drawn. To confirm the results obtained in this retrospective study, well-designed prospective studies are needed. Furthermore, our study is a single centre study on a homogeneous Caucasian population and therefore needs to be repeated prospectively also in other ethnic groups.

In conclusion, we describe here a complementary feeding practice for preterm babies that starts much earlier than that employed for full-term controls. We find that it is safe and does not increase the risk of food allergies or atopic dermatitis even among the most preterm infants. It seems evident that the GALT of preterm infants is ready to encounter food proteins and to begin the maturation process within 3–6 months from birth, regardless of the corrected age. It is probable that the key determining factor for the window of opportunity to develop oral tolerance is the time outside the womb rather than the corrected age (i.e. developmental age) of the baby. Our results shed new light on the current discussion on the optimal timing of complementary feeding in general, as even the most preterm infants seem to be immunologically ready to encounter foreign food proteins considerably earlier than their term peers. Further prospective studies are warranted to confirm our results and investigate the optimal preventive feeding practices and their mechanisms in preterm and full-term infants.

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Table 1. Baseline characteristics of the 464 preterm infants and their controls.

	LP	*VP*	*EP*	*All preterm*	*Controls*
n (% of all preterm)	286 (62)	125 (27)	53 (11)	464	464
Male sex, n (%)	157 (55)	72 (58)	27 (51)	256 (55)	256 (55)
Birth weight (g), mean (min-max)	2258 (950-4915)	1419 (760-2300)	820 (440-1190)	1868 (440-4915)	3582 (2550-5165)
Delivery mode caesarean section, n (%)	163 (57)	90 (72)	40 (76)	293 (63)	N.A.
Antenatal corticosteroid, n (%)	160 (56)	111 (89)	48 (91)	319 (69)	N.A.
i.v. antibiotic, days median (IQR)	3.0 (0-5)	7.0 (5-11)	22.0 (13.5-41.5)	5.0 (3-8)	N.A.

EP, extremely preterm (<28th gestational weeks); LP, late preterm (born from 32th to <37th gestational weeks); VP, very preterm (born from 28th to <32th gestational weeks). N.A., data not available.

Table 2. The timing of the introduction of regular cow's milk formula and solid/semisolid food in 464 preterm infants and in their controls.

A. The timing according to the chronological (calendar) age

	LP (n=286)	**VP (n=125)**	**EP (n=53)**	**All preterm (n=464)**	**Controls (n=464)**	**p-value***
Regular cow's milk formula	0.5 (0-1) (n=207)	1.5 (1-3) (n=102)	4 (2-6) (n=41)	1 (0.5-2) (n=250)	1 (0-4) (n=275)	0.114
Any solid/semisolid food	3 (3-4) (n=221)	3 (3-3.5) (n=123)	3 (3-4) (n=53)	3 (3-4) (n=398)	4 (4-5) (n=303)	<0.001
Fruits	3 (3-4) (n=213)	3 (3-3.5) (n=121)	3 (3-4) (n=53)	3 (3-4) (n=387)	4 (4-5) (n=303)	<0.001
Vegetables	3 (3-4) (n=217)	3 (3-3.5) (n=123)	3 (3-4) (n=53)	3 (3-4) (n=393)	4 (4-5) (n=303)	<0.001
Any meat	5 (5-5) (n=169)	5 (4-5) (n=119)	5 (4.5-5) (n=51)	5 (4.5-5) (n=339)	6 (5-6) (n=166)	<0.001
Oatmeal or other cereal	6 (5-6) (n=113)	6 (5-6.5) (n=107)	6 (5.5-7) (n=47)	6 (5-6) (n=267)	6 (5-6) (n=178)	0.117

B. The timing according to the corrected age

	LP (n=286)	**VP (n=125)**	**EP (n=53)**	**All preterm (n=464)**	**Controls (n=464)**	**p-value***
Regular cow's milk formula	-1 (-1-0) (n=207)	-1 (-1-0.5) (n=102)	0.5 (-1-2) (n=41)	-0.5 (-1-4.5) (n=250)	1.5 (0-4.5) (n=272)	<0.001
Any solid/semisolid food	2 (1.5-3) (n=221)	2 (0.5-1.5) (n=123)	0 (-0-0.5) (n=53)	1.5 (1-2) (n=398)	4.5 (4-5) (n=302)	<0.001
Fruits	2 (1.5-3) (n=213)	1 (0.5-1.5) (n=121)	0 (-0-0.5) (n=53)	1.5 (1-2) (n=387)	4.5 (4-5) (n=302)	<0.001
Vegetables	2 (1.5-3) (n=217)	1 (0.5-1.5) (n=123)	0.5 (0.5-0.5) (n=53)	1.5 (1-2) (n=392)	4.5 (4-5) (n=302)	<0.001
Any meat	3.5 (3-4) (n=169)	2.5 (2-3) (n=119)	1.5 (1-2) (n=51)	3 (2-3.5) (n=339)	6 (5-6) (n=166)	<0.001
Oatmeal or other cereal	4 (3.5-5) (n=113)	3.5 (2.5-4) (n=107)	2.5 (2-3.5) (n=47)	3.5 (3-4.5) (n=267)	5.5 (5-6) (n=178)	<0.001

Data is presented as median age in months (interquartile range). EP, extremely preterm (<28th gestational weeks); LP, late preterm (born from 32th to <37th gestational weeks); VP, very preterm (born from 28th to <32th gestational weeks). * the difference between all the preterm babies and their controls.

Table 3. The cumulative incidences of doctor-diagnosed food allergies and atopic eczema among the 464 preterm infants and their controls by the age of 1 and 2 years.

A. The cumulative incidences by the chronological age of 1 year

	LP (n=286)	**VP (n=125)**	**EP (n=53)**	**All preterm (n=464)**	**Controls (n=464)**	**p-value***
Atopic eczema, n (%)	52 (18.2)	21 (16.8)	9 (17)	82 (17.7)	69 (14.9)	0.286
Any food allergy, n (%)	12 (4.2)	3 (2.4)	0	15 (3.2)	17 (3.7)	0.856
Cow's milk	10 (3.5)	3 (2.4)	0	13 (2.8)	9 (1.9)	0.518
Other food	3 (0.9)	0	0	1 (0.6)	16 (3.4)	0.004

B. The cumulative incidences by the chronological age of 2 years

	LP (n=180)	**VP (n=53)**	**EP (n=17)**	**All preterm (n=250)**	**Controls (n=464)**	***p-value****
Atopic eczema, n (%)	35 (19.4)	8 (15.1)	6 (35.3)[§]	49 (19.6)	37 (14.9)[§]	0.192
Any food allergy, n (%)	6 (3.3)	3 (5.7)	0	9 (3.6 %)	9 (3.6)	1.000
Cow's milk	5 (2.8)	3 (5.7)	0	8 (3.2 %)	4 (1.6)	0.382
Other food	1 (0.6)	0	0	1 (0.4 %)	5 (2)	0.122

EP, extremely preterm (<28th gestational weeks); LP, late preterm (born from 32th to <37th gestational weeks); VP, very preterm (born from 28th to <32th gestational weeks). N.A., not applicable. *the difference between all preterm and controls

Table 1. Baseline characteristics of the 464 preterm infants and their age and sex matched full-term control children.

	LP	*VP*	*EP*	*All preterm*	*Full-term controls*
n (% of all preterm)	286 (62)	125 (27)	53 (11)	464	464
Male sex, n (%)	157 (55)	72 (58)	27 (51)	256 (55)	256 (55)
Birth weight (g), mean (min-max)	2258 (950-4915)	1419 (760-2300)	820 (440-1190)	1868 (440-4915)	3582 (2550-5165)
Delivery mode caesarean section, n (%)	163 (57)	90 (72)	40 (76)	293 (63)	N.A.
Antenatal corticosteroid, n (%)	160 (56)	111 (89)	48 (91)	319 (69)	N.A.
i.v. antibiotic, days median (IQR)	3.0 (0-5)	7.0 (5-11)	22.0 (13.5-41.5)	5.0 (3-8)	N.A.

EP, extremely preterm (<28th gestational weeks); LP, late preterm (born from 32th to <37th gestational weeks); VP, very preterm (born from 28th to <32th gestational weeks). N.A., data not available.

Table 2. The timing of the introduction of regular cow's milk formula and solid/semisolid food in 464 preterm infants and in their age- and sex-matched full-term control children.

A. The timing according to the chronological (calendar) age

	LP (n=286)	**VP (n=125)**	**EP (n=53)**	**All preterm (n=464)**	**Full-term controls (n=464)**	**p-value***
Regular cow's milk formula	0.5 (0-1) (n=207)	1.5 (1-3) (n=102)	4 (2-6) (n=41)	1 (0.5-2) (n=250)	1 (0-4) (n=275)	0.114
Any solid/semisolid food	3 (3-4) (n=221)	3 (3-3.5) (n=123)	3 (3-4) (n=53)	3 (3-4) (n=398)	4 (4-5) (n=303)	<0.001
Fruits	3 (3-4) (n=213)	3 (3-3.5) (n=121)	3 (3-4) (n=53)	3 (3-4) (n=387)	4 (4-5) (n=303)	<0.001
Vegetables	3 (3-4) (n=217)	3 (3-3.5) (n=123)	3 (3-4) (n=53)	3 (3-4) (n=393)	4 (4-5) (n=303)	<0.001
Any meat	5 (5-5) (n=169)	5 (4-5) (n=119)	5 (4.5-5) (n=51)	5 (4.5-5) (n=339)	6 (5-6) (n=166)	<0.001
Oatmeal or other cereal	6 (5-6) (n=113)	6 (5-6.5) (n=107)	6 (5.5-7) (n=47)	6 (5-6) (n=267)	6 (5-6) (n=178)	0.117

B. The timing according to the corrected age

	LP (n=286)	**VP (n=125)**	**EP (n=53)**	**All preterm (n=464)**	**Full-term controls (n=464)**	**p-value***
Regular cow's milk formula	-1 (-1-0) (n=207)	-1 (-1-0.5) (n=102)	0.5 (-1-2) (n=41)	-0.5 (-1-4.5) (n=250)	1.5 (0-4.5) (n=272)	<0.001
Any solid/semisolid food	2 (1.5-3) (n=221)	2 (0.5-1.5) (n=123)	0 (-0-0.5) (n=53)	1.5 (1-2) (n=398)	4.5 (4-5) (n=302)	<0.001
Fruits	2 (1.5-3) (n=213)	1 (0.5-1.5) (n=121)	0 (-0-0.5) (n=53)	1.5 (1-2) (n=387)	4.5 (4-5) (n=302)	<0.001
Vegetables	2 (1.5-3) (n=217)	1 (0.5-1.5) (n=123)	0.5 (0.5-0.5) (n=53)	1.5 (1-2) (n=392)	4.5 (4-5) (n=302)	<0.001
Any meat	3.5 (3-4) (n=169)	2.5 (2-3) (n=119)	1.5 (1-2) (n=51)	3 (2-3.5) (n=339)	6 (5-6) (n=166)	<0.001
Oatmeal or other cereal	4 (3.5-5) (n=113)	3.5 (2.5-4) (n=107)	2.5 (2-3.5) (n=47)	3.5 (3-4.5) (n=267)	5.5 (5-6) (n=178)	<0.001

Data is presented as median age in months (interquartile range). EP, extremely preterm (<28th gestational weeks); LP, late preterm (born from 32th to <37th gestational weeks); VP, very preterm (born from 28th to <32th gestational weeks). * the difference between all the preterm babies and their full-term controls.

Table 3. The cumulative incidences of doctor-diagnosed food allergies and atopic eczema among the 464 preterm infants and their age- and sex-matched full-term control children by the age of 1 and 2 years.

A. The cumulative incidences by the chronological age of 1 year

	LP (n=286)	**VP (n=125)**	**EP (n=53)**	**All preterm (n=464)**	**Full-term controls (n=464)**	**p-value***
Atopic eczema, n (%)	52 (18.2)	21 (16.8)	9 (17)	82 (17.7)	69 (14.9)	0.286
Any food allergy, n (%)	12 (4.2)	3 (2.4)	0	15 (3.2)	17 (3.7)	0.856
Cow's milk	10 (3.5)	3 (2.4)	0	13 (2.8)	9 (1.9)	0.518
Other food	3 (0.9)	0	0	1 (0.6)	16 (3.4)	0.004

B. The cumulative incidences by the chronological age of 2 years

	LP (n=180)	**VP (n=53)**	**EP (n=17)**	**All preterm (n=250)**	**Full-term controls (n=250)**	***p-value****
Atopic eczema, n (%)	35 (19.4)	8 (15.1)	6 (35.3)[§]	49 (19.6)	37 (14.9)[§]	0.192
Any food allergy, n (%)	6 (3.3)	3 (5.7)	0	9 (3.6 %)	9 (3.6)	1.000
Cow's milk	5 (2.8)	3 (5.7)	0	8 (3.2 %)	4 (1.6)	0.382
Other food	1 (0.6)	0	0	1 (0.4 %)	5 (2)	0.122

EP, extremely preterm (<28th gestational weeks); LP, late preterm (born from 32th to <37th gestational weeks); VP, very preterm (born from 28th to <32th gestational weeks). N.A., not applicable. *the difference between all preterm and full-term

Legends for the figures

Figure 1. Flowchart of the study population and analyses.

Figure 2. The chronological age at the time of the introduction of regular cow's milk formula and various semisolid or solid foods among preterm infants and controls. *p-value <0.001 when compared to the controls.

Figure 3. The corrected age (based on the calculated due date) at the time of the introduction of regular cow's milk formula and various semisolid or solid foods among preterm infants and the controls. **A.** All preterm children in comparison to the control children. **B.** Preterm children grouped according to the degree of prematurity. LP, late preterm; VP, very preterm; EP, extremely preterm. $^{*}p<0.001$ and $^{\S}p=0.001$ when compared to the controls.

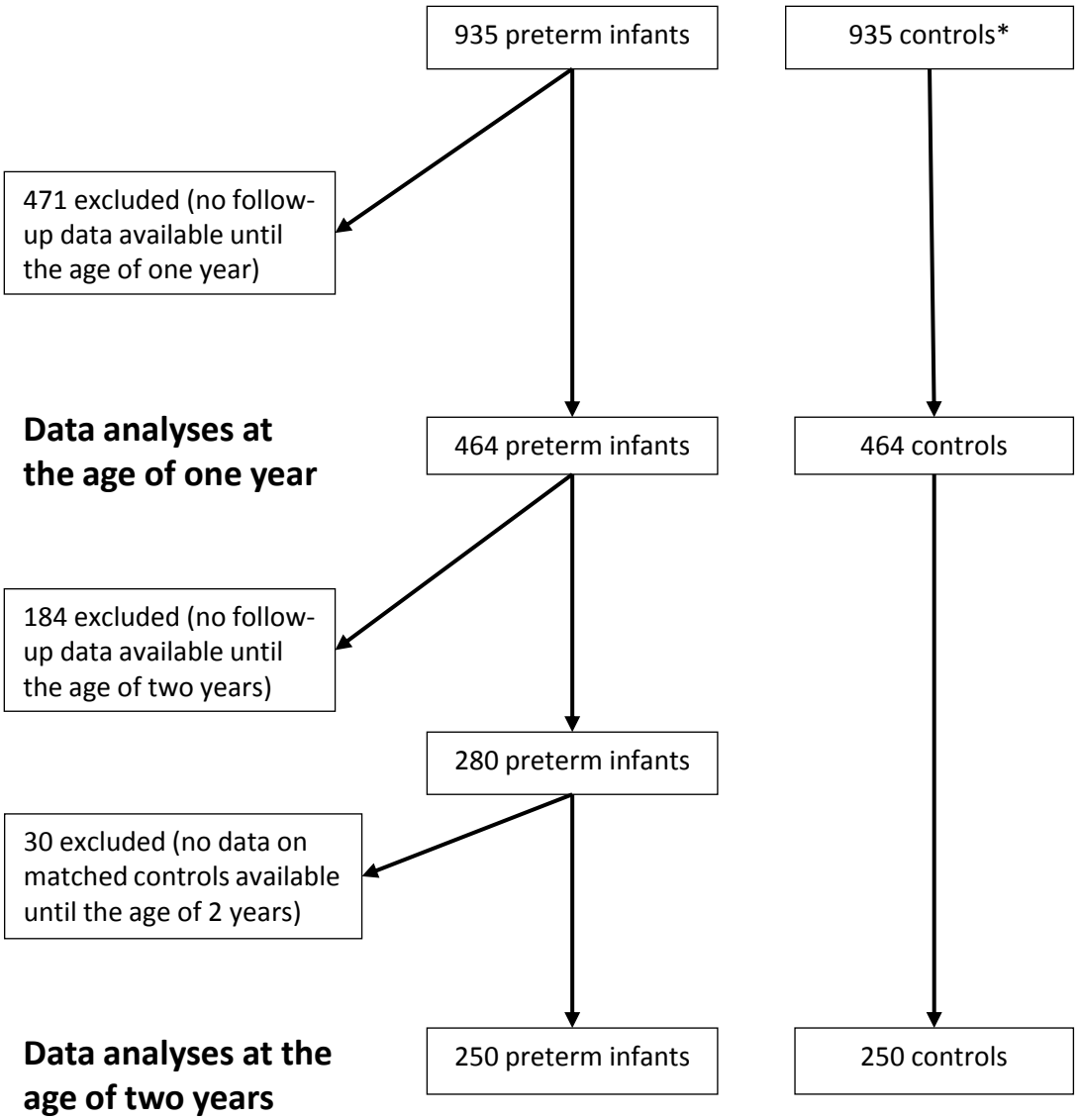
Figure Legend

Figure 1. Flowchart of the study population and analyses.

Figure 2. The calendar age from birth at the time of the introduction of regular cow's milk formula and various semisolid or solid foods among preterm infants and the age- and sex matched full-term control children. *p-value* <0.001 when compared to the controls.

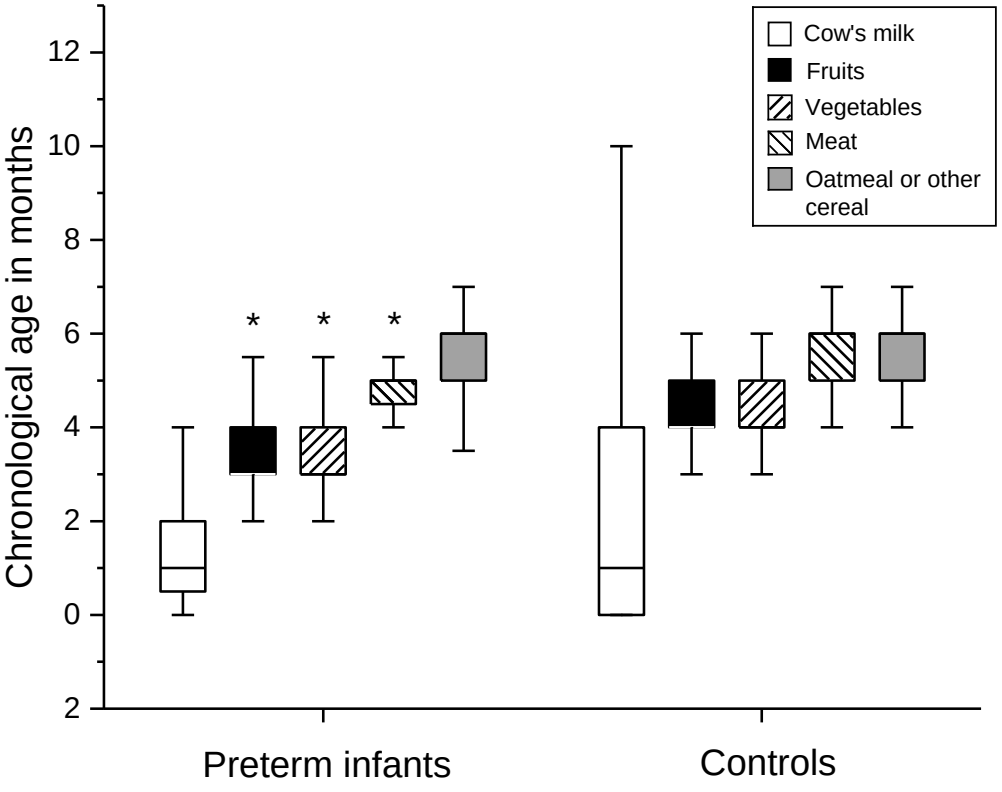
Figure 3. The corrected age (based on the calculated due date) at the time of the introduction of regular cow's milk formula and various semisolid or solid foods among preterm infants and the age- and sex-matched full-term control children. **A.** All preterm children in comparison to the control children. **B.** Preterm children grouped according to the degree of prematurity. LP, late preterm; VP, very preterm; EP, extremely preterm. $^{*}p<0.001$ and $^{\S}p=0.001$ when compared to the controls.

Figure 1.


935 preterm infants
935 controls*
471 excluded (no follow-up data available until the age of one year)
Data analyses at the age of one year
464 preterm infants
464 controls
184 excluded (no follow-up data available until the age of two years)
280 preterm infants
30 excluded (no data on matched controls available until the age of 2 years)
Data analyses at the age of two years
250 preterm infants
250 controls

Figure

Figure 2.



Figure

Figure 3.

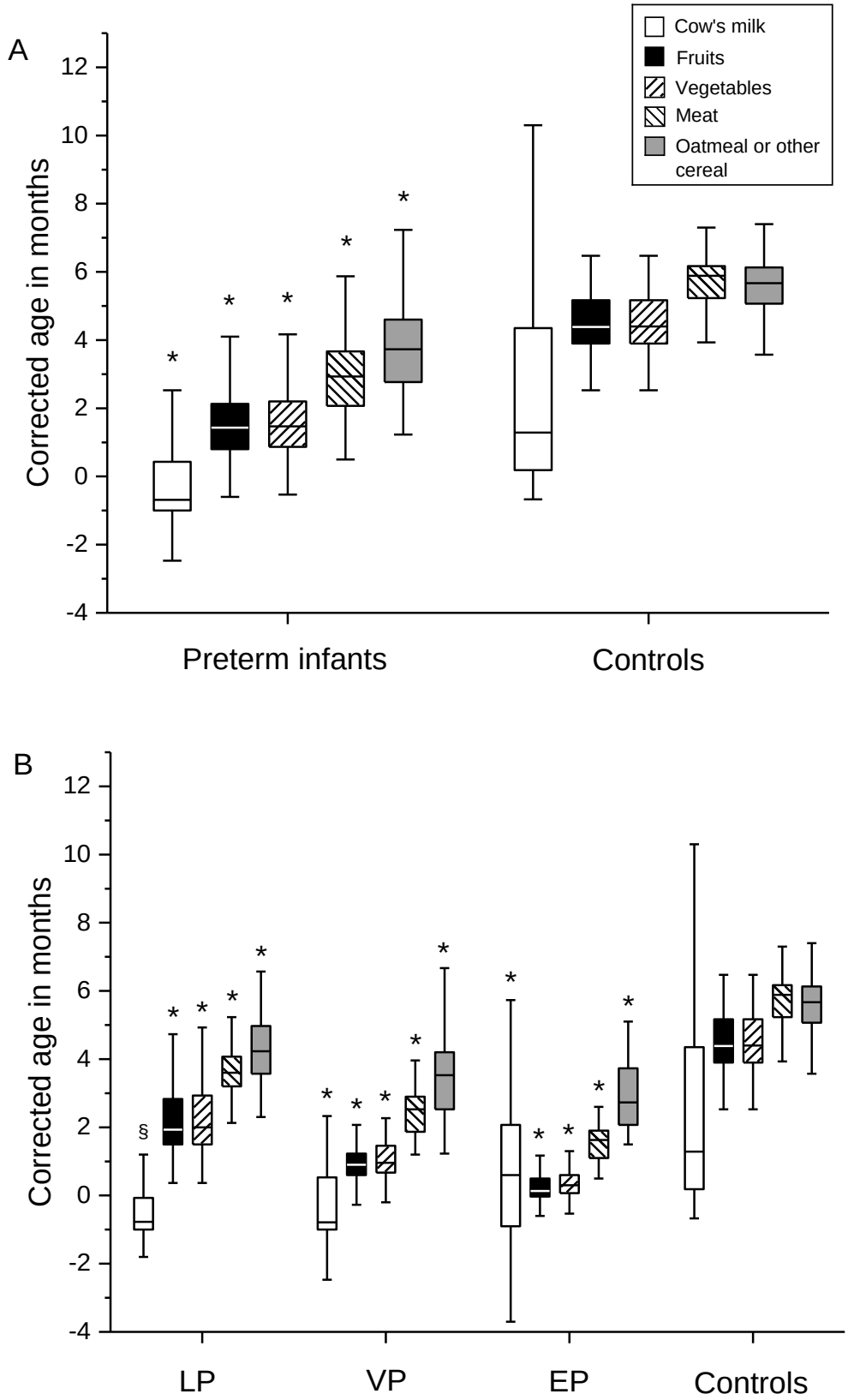


Figure 1.

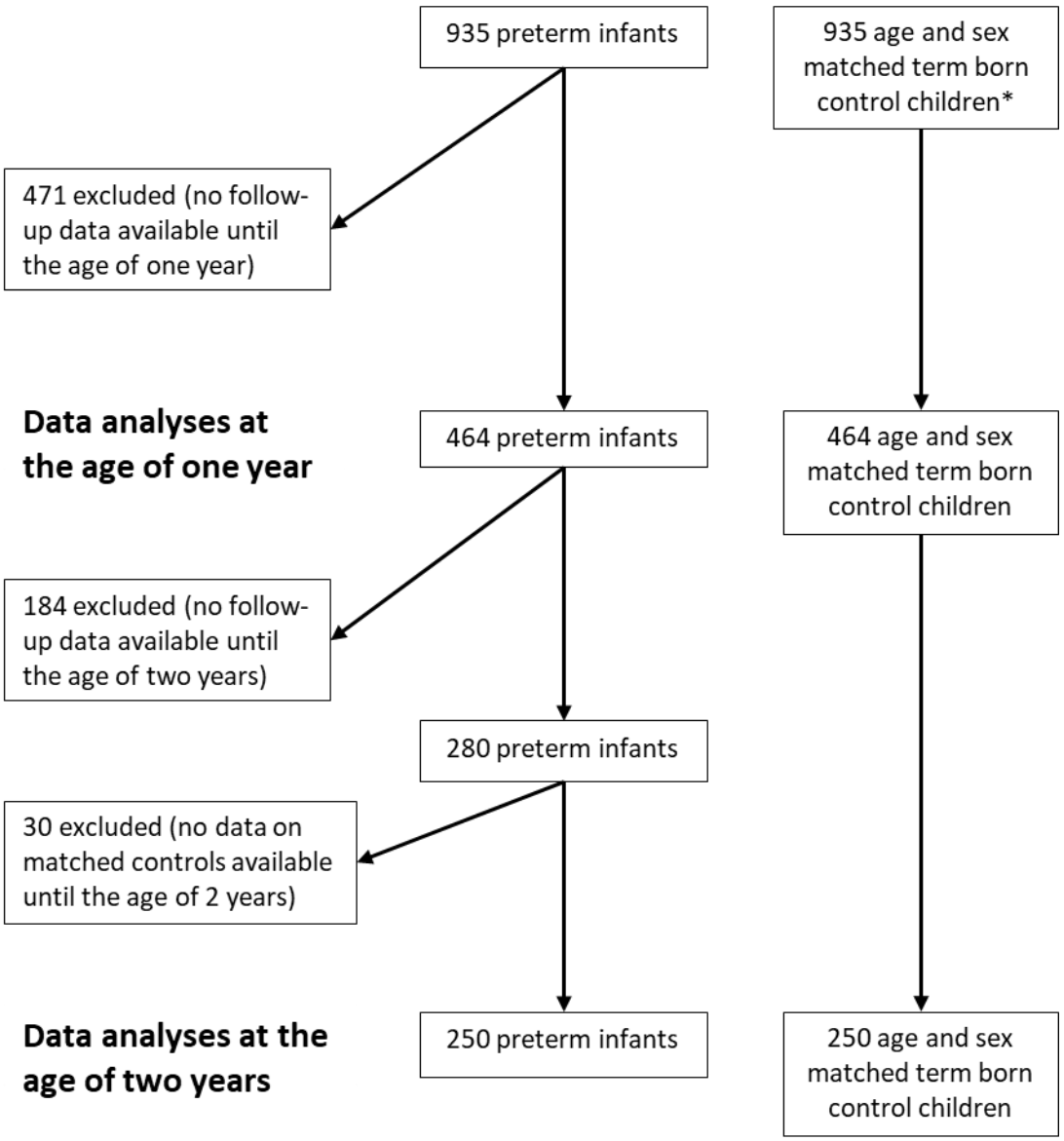

935 preterm infants
935 age and sex matched term born control children*
471 excluded (no follow-up data available until the age of one year)
Data analyses at the age of one year
464 preterm infants
464 age and sex matched term born control children
184 excluded (no follow-up data available until the age of two years)
280 preterm infants
30 excluded (no data on matched controls available until the age of 2 years)
Data analyses at the age of two years
250 preterm infants
250 age and sex matched term born control children

Figure 2

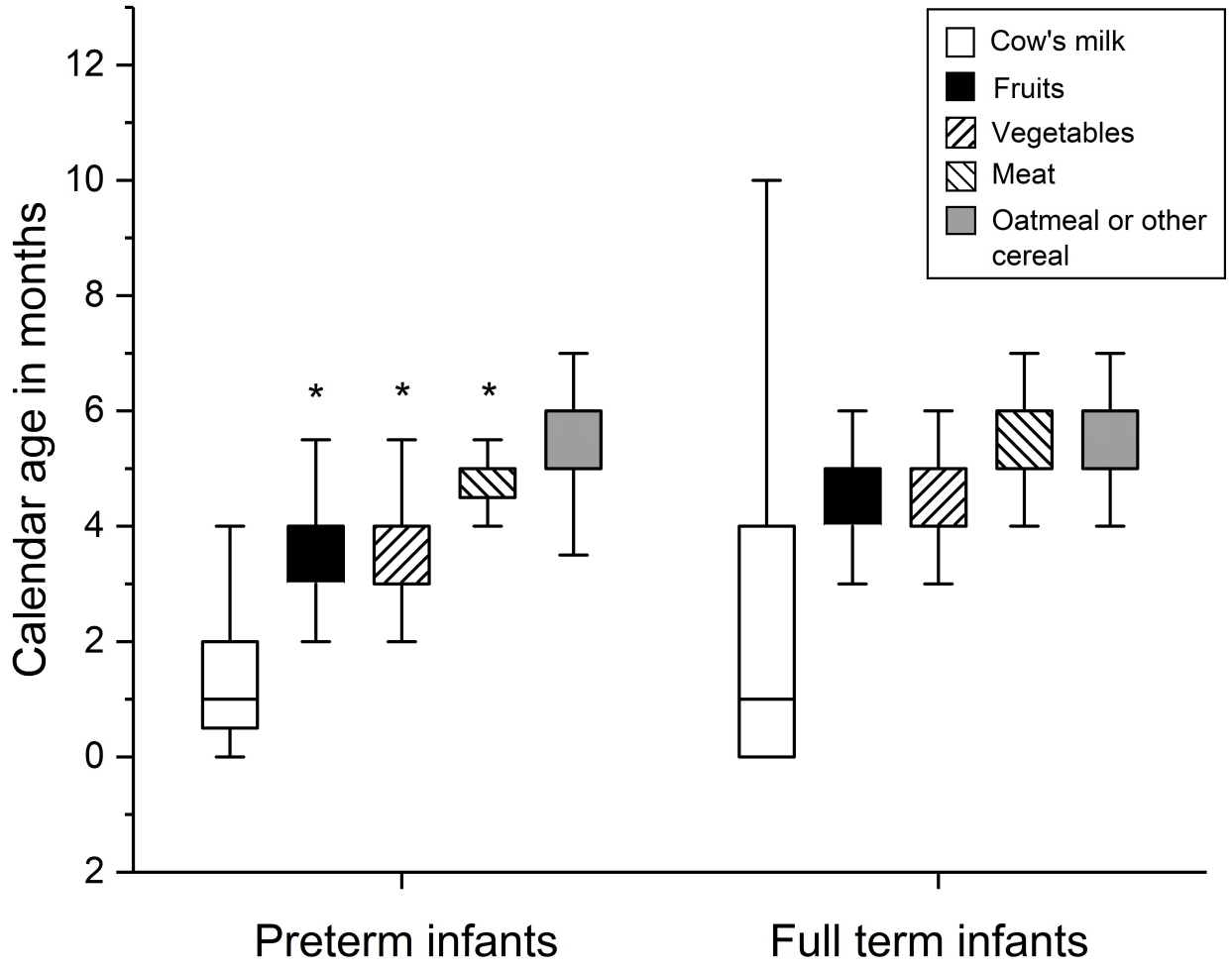
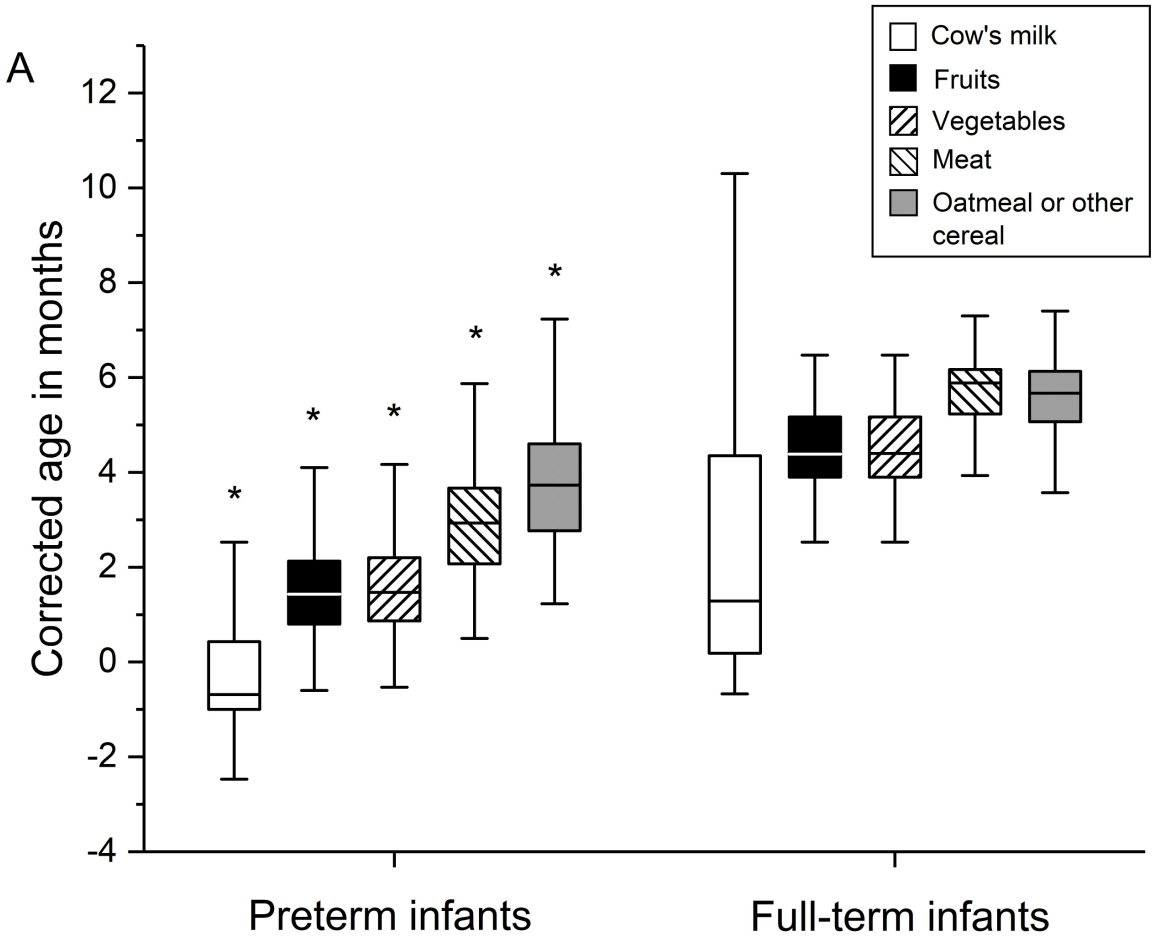
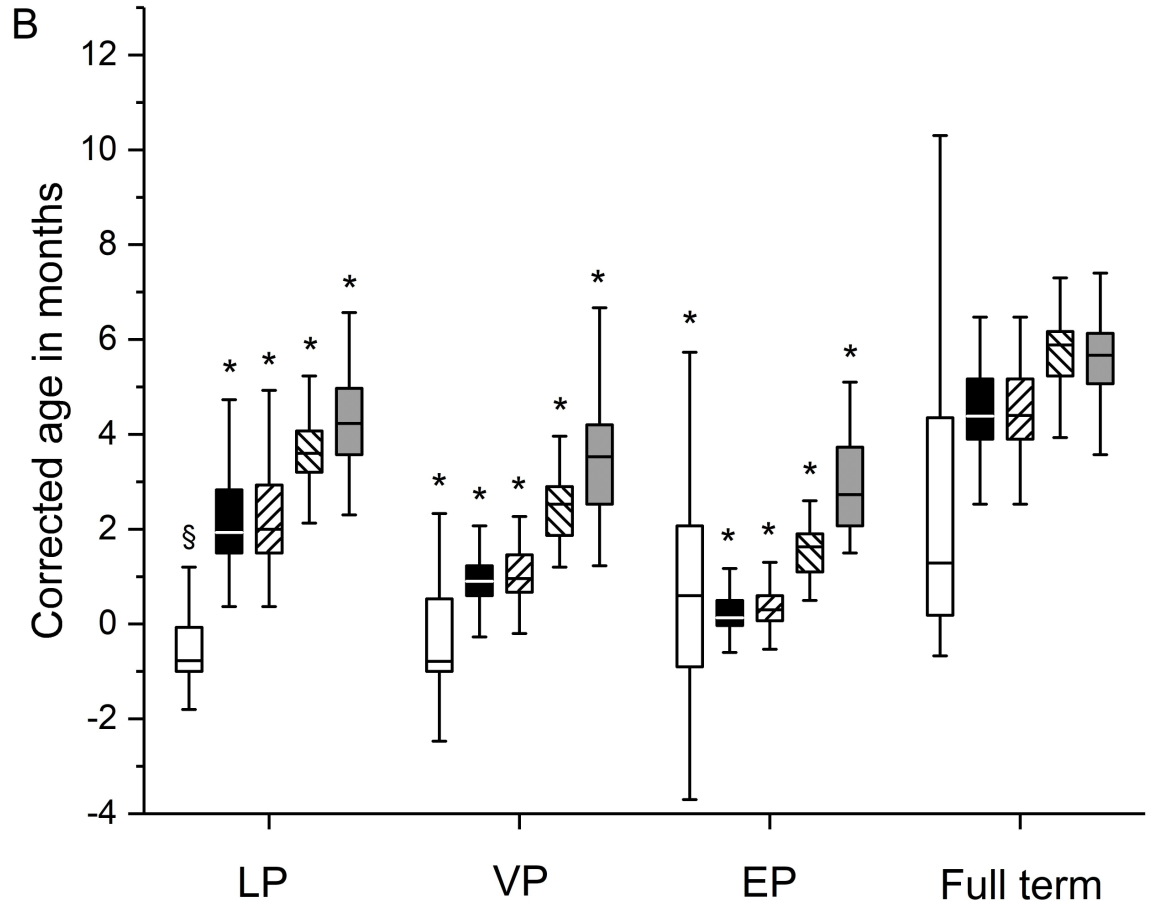

Cow's milk
Fruits
Vegetables
Meat
Oatmeal or other cereal
Calendar age in months
12
10
8
6
4
2
0
2
*
*
*
Preterm infants
Full term infants

Figure 3


A
Cow's milk
Fruits
Vegetables
Meat
Oatmeal or other cereal
Corrected age in months
12
10
8
6
4
2
0
-2
-4
*
Preterm infants
Full-term infants


B
Corrected age in months
12
10
8
6
4
2
0
-2
-4
§
*
LP
VP
EP
Full term