This is to certify that the
thesis entitled

THE INFLUENCE OF EARLY COW'S MILK EXPOSURE ON THE OCCURRENCE OF CHILDHOOD ASTHMA AND ATOPY

presented by

Christopher Leon Fussman

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Epidemiology

Major Professor's Signature

July 28, 2004

Date

THE INFLUENCE OF EARLY COW'S MILK EXPOSURE ON THE OCCURRENCE OF CHILDHOOD ASTHMA AND ATOPY

By

Christopher Leon Fussman

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Epidemiology

2004

ABSTRACT

THE INFLUENCE OF EARLY COW'S MILK EXPOSURE ON THE OCCURRENCE OF CHILDHOOD ASTHMA AND ATOPY

By

Christopher Leon Fussman

The effect of cow's milk consumption on the development of atopy has been a matter of debate for several years. Due to the use of flawed study designs an accurate explanation for this possible association remains illusive. This thesis attempts to provide more information regarding this association through a longitudinal cohort study design.

The research conducted herein consists of a cohort of 696 newborns with parental history of atopy that was followed up from birth to 3 years of age. The main exposure in this study was newborn consumption of cow's milk and the three outcomes of interest were occurrence of asthma, wheezing, and allergic sensitization. Generalized estimation equation (GEE) models were used to analyze the concurrent, delayed, and reverse causation effects of cow's milk consumption on the above outcomes.

The GEE models indicate that cow's milk consumption can lead to an increased risk of asthma, wheezing, and allergic sensitization, but not all reached statistical significance. Additionally, asthma occurrence often resulted in reductions in cow's milk consumption in the following survey period (reverse causation). Mechanistic explanations for these findings on the development of atopy in children may involve the activity of specific proteins and/or essential fatty acids. To improve future results into this area of research, these potential explanations, as well as the possibility of reverse causation, should be taken into account.

ACKNOWLEDGEMENTS

I would like to thank all the research staff and participants of the Study on the Prevention of Allergy in Children in Europe (SPACE). For their timely and helpful advising throughout the development and completion of my thesis, I express my gratitude to Drs. Wilfried Karmaus, Michael Collins, and David Todem.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Over the past several decades, numerous reports have been published on the potential effects that early cow's milk exposure can have on the occurrence of asthma and other atopic manifestations, such as wheezing, allergic sensitization, eczema, and hay fever, in young children. This present study examines the influence of early cow's milk exposure on the development of asthma and other atopic manifestations in a cohort consisting of European newborns. This research was also conducted to identify known and unknown factors, other than consumption of cow's milk, which may affect childhood susceptibility to such atopic conditions.

Approximately 25% of all children in the United States will develop at least one atopic manifestation during their lifetime [1]. Asthma has been known as a complex syndrome that can affect both children and adults in several different ways [2]. Asthma usually manifests itself through airflow obstruction, bronchial hyperresponsiveness, and airway inflammation [2]. The majority of individuals who develop asthma do so during early childhood with 90% of all asthma cases diagnosed during the first six years of life [3]. Due to the non-specific clinically based definition of asthma, it can be very difficult to obtain an accurate diagnosis of asthma, as well as atopy, during early childhood. Even commonly used biomarkers for allergy, such as serum Immunoglobulin-E (IgE) and allergen skin prick tests (SPT), are not always correlated with clinical disease, thus making correct diagnosis even more difficult [4-6].

When focusing on other atopic disorders, diagnosis is equally challenging. Atopy is characterized by the production of IgE antibodies following allergen stimulation. When repeated allergen exposures occur over time, the condition known as atopy

manifests itself in the form of bronchial asthma, food allergy, atopic eczema, or hay fever [1, 7].

Asthma and other atopic conditions are becoming problems of increasing magnitude in today's world. The prevalence of these conditions has increased substantially over recent decades [8]. For instance, according to the 1980-1994 National Health Interview Surveys, asthma is currently a major cause of morbidity in the United States, being diagnosed in 7 to 15% of all children. Asthma accounts for an estimated 200,000 hospitalizations per year in U.S. children and the estimated cost of treating individuals with asthma under the age of 18 years is approximately $3.2 billion per year [9, 10].

When investigating factors that may affect the development of asthma and atopy in early childhood several potential variables need to be taken into account. Many genetic, neonatal, environmental, and nutritional factors can potentially influence the development of asthma and atopy in early childhood [11].

It has long been recognized that asthma and atopy seem to run in families, and that parental history of atopy establishes an increased risk of atopy in their offspring [12-19]. Previous studies indicate that history of atopy in both parents infers the greatest risk [14, 20]. These studies also suggest that the presence of maternal atopic history infers a much higher risk of infantile atopy compared to that of paternal atopy [14, 20]. Several studies have also shown asthma and atopy to be more prevalent in boys than in girls [21, 22]. Other factors that have been shown to affect the risk of asthma and atopy in childhood are maternal smoking and education, air pollution, birth weight, exposure to

inhaled allergens, as well as exposure to breast milk and cow's milk in the early years of life [12, 23-27].

Descriptions of the previous studies completed on this possible association are presented in Tables 1 and 2. The majority of previous studies in this area have indicated early cow's milk consumption as a risk factor for the development of asthma and atopy [28-34]. There have also been several published studies that indicate early cow's milk exposure as a risk factor for the development of allergic sensitization to cow's milk allergen, thus leading to the development of asthma in late childhood [35-41]. Additional studies completed recently show early cow's milk exposure to have no significant effect on the development of childhood asthma and other atopic manifestations [42-45].

Most of the previous studies above mention the work of Glaser and Johnstone, conducted in 1953, as being one of the first published reports on cow's milk exposure being indicated as a risk factor for the development of asthma and atopy in the first years of life. This study indicated that 52 to 65% of the control group participants fed cow's milk developed allergic conditions compared to only 15% of the cases, who were children with parental history of allergy and were fed only soy-based milk products [34]. Many researchers criticize these results due to the study being retrospective in design and also for including control groups that contained individuals in which major allergies had already developed, thus introducing several forms of bias. The studies completed since the Glaser and Johnstone report have attempted to avoid and build from the errors made through previous research. However, not all of the recent studies investigating this potential association have been able to avoid all such errors and biases.

Due to the fact that many of the studies showing early cow's milk exposure as either a risk factor or having no significant effect suffer from several limitations, their results should be interpreted with caution.

It has been suggested for many years by the majority of the medical community, based on previously mentioned research, that early cow's milk consumption can potentially be a risk factor for asthma and atopy, and therefore parents should have their newborns avoid the consumption of cow's milk until at or after 12 months of age [46]. Three recent European studies, however, indicate that this may not be the case at all, and early cow's milk consumption may actually be protective against asthma and atopic manifestations [47-49]. This research attempts to model the potential effects of early cow's milk exposure on the occurrence of asthma and atopy in early childhood while taking the time order of these events into account, and suggests new methods of analysis that should be implemented to more thoroughly explain the research completed on this potential association.

METHODS

Population

The cohort used in this research was assembled through a previous "Study on the Prevention of Allergy in Children in Europe" (SPACE) [50]. This initial study was a multi-center, population-based, randomized control study of children at high risk of allergy from the countries of Austria, Germany, Greece, Great Britain, and Lithuania. The objective of the SPACE study was to prevent sensitization to house dust mite and food allergens, as well as the development of atopic symptoms during infancy through

the use of mite and allergen impermeable mattress covers. The SPACE study consisted of three cohorts of participants: schoolchildren, toddlers, and newborns. The research for this thesis focused only on the newborn cohort, which includes newborns recruited from Austria, Great Britain, and Germany. The newborn cohort was used for this research because it was the only cohort of the three that had outcome and exposure information at each of the survey periods.

Prior to initiation of the SPACE study, the local ethical committees at each of the research sites approved the working protocol of the study. During recruitment, informed consent was obtained from the parents of each newborn prior to the collection of all measurements proposed in the study. Newborns were recruited into the SPACE study based on the atopic history of their parents. Through the recruitment process the parents were instructed to complete screening questionnaires for symptoms associated with the presence of allergic disease. If a history of bronchial asthma, atopic eczema, allergic rhinitis, or hay fever was reported by either of the parents, skin prick testing or serum IgE measurements were performed on the parents. If one or both of the parents showed positivity to the skin prick test (SPT) or serum IgE for at least one allergen out of the panel of five aeroallergens tested (*Dermatophagoides pteronyssinus*, *Dermatophagoides. farinae*, birch pollen, grass pollen, and cat dander) their newborn was eligible for the study. Additionally, specific IgE measurements of $\geq$ 1.43 kU/l to a specific aeroallergen were indicative of a positive serum IgE test. The only exclusion criteria that were implemented in this study were birth weights below 2500g and admission to a neonatal intensive care unit for longer than 7 days.

Through this recruitment process that took place from April 1997 to June of 1998, 696 newborn participants were recruited into the study and followed up through August of 2001.

Exposure Measurement

Attempts were made to follow each of the newborn participants up to 36 months of age (Figure 1). Following birth, short questionnaires were completed to ascertain information on the newborn, such as gender, birth weight, and current newborn health status. At six months of age the parents of each newborn were instructed to complete a more in-depth questionnaire dealing with a variety of exposures that their child may have been exposed to since birth. These questions targeted exposures in regard to the newborn's living environment, as well as the newborn's feeding habits. Specific questions dealt with issues of passive smoking, presence of chest infections, and consumption of different types of milk, such as breast milk, normal formula, hypoallergenic formula, and cow's milk. To ascertain information on the exposure variables at each of the follow-up surveys the questions were worded to develop a measure of the exposure that had occurred since the previous follow-up period. For example, the question regarding passive smoking exposure reads as follows: "Was your child exposed to household cigarette smoke in the last 6 months?" All other exposure variables were ascertained in the very same fashion.

A standardized questionnaire focusing on these exposures was also completed at each of the 12, 18, 24, and 36 month follow-up periods. For each survey period, the questionnaires were again developed so that they would obtain exposure information

since the previous survey period in order to develop a continuous and complete record of exposure throughout the first three years of life.

Depending on their contribution to the overall final models, several potential confounders were investigated through these analyses. Potential confounders were indicated through previous studies on this association and modeling was used to determine the most parsimonious models. The initial confounders included in the models were gender, history of parental atopy, passive smoking exposure, presence of chest infections, maternal education, exposure to breast milk, season of birth, country of residence, as well as normal and hypoallergenic formula use. Some of the potential confounders consisted of categorical data. For example, the parental atopy variable was categorized into three groups: maternal atopy, paternal atopy, and both parents atopic, with paternal atopy considered the reference group. Additionally, maternal education was divided into three categories ($\leq$ 10 years, 11-12 years, $\geq$ 13 years) with $\leq$ 10 years as the reference category.

Outcome Measurement

The three major outcomes of interest that were investigated through this study were the occurrence of self-reported wheezing, doctor's diagnosed asthma, and allergic sensitization to cow's milk protein. These outcomes were ascertained at each of the previously mentioned follow-up periods (Figure 1). Wheezing was measured at all of the survey periods, asthma was measured starting with the 12-month survey period, and cow's milk sensitization was only determined during the 12-month follow-up period. To ascertain the outcomes of asthma and wheezing at each of the follow-ups, questions regarding these conditions were placed into the standardized questionnaire completed by

the newborn's parents. Wheezing information was based on parental reports, while asthma was based on parental reports of medically diagnosed occurrences of this condition. The questions regarding the outcomes of asthma and wheezing were also geared to obtain information on the development of each outcome over the interval since the last survey period. For example, questions regarding asthma were based on this template: "In the last 6 months, was a doctor's diagnosis made in your child of asthma?" while the question used to ascertain wheezing was as follows: "Has your child had wheezing or whistling in the chest in the last 6 months?" The above questions were then adjusted to identify the particular time interval between each of the follow-up periods. Repeated measurements of these conditions allowed tracking of the progression of the diseases and conditions over the first three years of life.

At 12 months of age, allergic sensitization testing was completed through either specific IgE testing or through SPT. Due to different preferences toward allergen testing in the countries involved in the study, specific IgE testing was completed in Austria and Germany, while SPT was completed in Great Britain. These two methods were considered to be equivalent based on a study by Schuetze et al. [51]. The core group of allergens that were tested by both tests consisted of the mite allergens *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae (Der p* and *Der f)*, grass and tree pollen, cat and dog dander, as well as egg and cow's milk allergen. Similar testing was completed at 24 months of age, but this testing did not involve cow's milk allergen sensitization. Specific IgE concentrations against these allergens were determined through the use of the Magic Lite Test [50]. IgE test results were regarded as positive if the IgE values reached 1.43 kU/l or greater.

The skin prick testing was completed under common testing procedures described elsewhere [50]. In brief, each of the core allergens was applied individually to the forearm of the newborn with the aid of an ALK prick needle. Each wheal reaction was measured with a transparent ruler and the arithmetic mean was calculated. A positive test resulted when the mean wheal diameter scores were at least 2 mm larger than the negative controls that were run for each participant and the allergen wheal to histamine ratio was ≥ 0.5.

Statistical Analysis

The data from each of the survey periods was merged into one complete dataset based on a unique identification number created for each study participant.

In order to account for the repeated measurements, generalized estimation equation (GEE) models were used to estimate the potential effects of early cow's milk consumption on the occurrence of asthma, wheezing, and atopic sensitization to cow's milk protein in early childhood. GEE models are, basically, logistic regression models that can account for within-subject associations. Such models use a working correlation matrix, specifically indicated in the syntax of the modeling procedure, to estimate the coefficient estimates for the effects in the model. Then the models use what is termed as a robust or sandwich estimator of the variance/covariance matrix to calculate the standard errors for each of the coefficient estimates. Additionally, GEE models are capable of dealing with intermittent missing values (missing values intermixed with nonmissing values) by incorporating the "all available pairs" method, which uses data from all of the nonmissing pairs in the development of the final model, thus not excluding entire observations due to the presence of one missing value [52]. Asthma, wheezing, and

allergic sensitization to cow's milk were considered single outcomes; thus three separate groups of models were produced for each set of predictors.

For descriptive purposes, study population characteristics for each of the variables used in the study analyses were calculated prior to the development of the GEE models. Flow diagrams for cow's milk exposure and the outcomes of asthma and wheezing were also constructed to enable tracking of each newborn and their changes in exposure and outcome status throughout the entire follow-up period of the study.

For each GEE model, an auto regressive working correlation matrix was invoked. This particular matrix was used due to the assumption that each measurement of asthma, wheezing, and cow's milk exposure were dependent on the same measurement recorded at the previous follow-up period, and also due to the correlation between the repeated exposure measurements being a function of time. All statisical analyses were carried out using SAS software, version 8.2 [53]. All GEE analyses were completed through the use of the SAS GENMOD procedure.

For the models based on each of the three outcomes (asthma, wheezing, and allergic sensitization to cow's milk protein) investigated through this research, several different modeling strategies were used. Initially, concurrent models were used to assess the immediate effect of cow's milk on the occurrence of each of the above outcomes (Figure 2). These models took the cow's milk exposure from each of the periods and related these measurements to the outcome of interest during the same periods.

The second modeling strategy incorporated a delayed effects model that dealt with the possibility of a postponed effect of cow's milk exposure on the occurrence of each outcome (Figure 3). For this type of model the cow's milk exposure from one

period was related to the outcome measurement at the next follow-up. The repeated measurement nature of the data was also taken into account in this type of model. Additionally, due to the possibility that both the concurrent and delayed effects were actually occurring at the same time, models were also developed that included both aspects of cow's milk exposure (Figure 4).

A fourth modeling strategy focused on the potential for reverse causation in each of the developed models (Figure 5). The idea of reverse causation stems from previous studies, which investigated whether or not individual decreases in exposure over time could generate a false association between the outcome and the exposure [54-56]. This modeling strategy used a reverse delayed effects model to investigate whether the outcome of asthma, wheezing, or allergic sensitization at one period could significantly predict the cow's milk exposure status at the next follow-up period. The reverse causation model was used to provide further justification for any significant results obtained from the first three modeling strategies. Additionally, reverse causation was considered due to the suspicion that several outside influences, such as changes in the parental decisions on feeding habits of their children, could result in changes in exposure to cow's milk over time, thus distorting the results of the concurrent or delayed effects models.

A final modeling technique was used to investigate whether the specific current advice from the medical community to avoid cow's milk prior to one year of age could be supported (Figure 6) [57]. To do so, a new exposure variable was constructed to indicate whether or not the newborn had been exposed to cow's milk at or prior to 12 months of age. GEE models using this exposure variable were then created while controlling for

other potential confounders. Additional models of each modeling strategy were also run for each individual follow-up period in order to determine if cow's milk exposure had significant effects at any given time period.

Thus, this research involved the use of five modeling strategies that were incorporated into the GEE models. Each of the concurrent effects, six-month delayed effects, combined effects, reverse causation, and medical community recommendation modeling strategies were used to investigate this potential association. Additionally, the concurrent and six-month delayed effects models were stratified by survey period in order to investigate whether heterogeneity of effects existed over the course of the entire follow-up period.

The GEE models for each outcome produced effect coefficient estimates for each variable in the model, along with their 95% confidence intervals. These estimates were then transformed into equivalent estimates of odds ratios (along with the 95% confidence intervals of the odds ratio) to express the adjusted effect of each variable on the outcome specified in the model.

To reach the most parsimonious models for asthma, wheezing, and allergic sensitization, confounders were eliminated from all models based on a 10% rule of confounding. This rule indicates that if a change in the coefficient estimates between the models with and without the potential confounding variables is less than 10%, then this potential confounding variable can be eliminated from the final model.

Additionally, in order to determine if non-random losses to follow-up occurred over the entire study period, a GEE model was developed to determine if any of the predictors used in the models were able to significantly account for the loss to follow-up

that occurred at each of the survey periods. The loss to follow-up at each survey period was based on the absence of information regarding the outcomes of asthma, wheezing, and allergic sensitization. At any given survey period, if information on any of the outcomes was missing the loss to follow-up for this participant, at that particular period, was coded as affirmative. The losses for each survey period were them combined in a GEE model with loss to follow-up as the outcome.

RESULTS

Population characteristics for the potential confounding variables used in the GEE models are presented in tables 3 and 4. Table 3 focuses on the variables that were only measured once, while table 4 focuses on the time-dependent covariates. Out of the 696 total newborn participants 50.3% (n=350) were male and 49.7% (n=346) were female (Table 3). The majority (70.0%) of the newborns had birth weights between 3000 and 4000g. The majority (78%) of the study participants came from mothers with a medium level of education. Due to the fact that participant inclusion was based on the atopic history of the parents, this categorical variable (parental atopy) was also taken into account during analysis. The number of participants included in each parental atopy group was very similar. The highest frequency of parental atopy was reported for participants with maternal history of atopy, which made up 35.3% of the study population.

When investigating the time dependent covariates, the variables of passive smoking exposure and chest infections maintained relatively stable over the entire study period (Table 4). Passive smoking exposure during pregnancy and up to the six-month

follow-up was non-existent, but after this survey period the prevalence of passive smoking ranged from 17.5 to 24.9% for the remainder of the study. The presence of chest infections in the newborn participants was also fairly stable throughout the study period, ranging from 2.0 to 5.8%. Aspects of the feeding practices, however, changed markedly over time. No breastfeeding occurred after 24 months of age, with 2.6 to 10.1% of the newborns receiving breast milk in the first 24 months of life. The levels of breastfeeding in this study are much lower than what is expected for this region of the world [58].

The other formula-based feeding habits in the study participants continued through the first two years of life. For normal formula, consumption ranged from 10.9 to 48.7% with the presence of an overall decreasing trend over the first two years of life. The consumption of hypoallergenic formula followed a similar trend, with the greatest consumption percentage (34.6%) occurring in the first six months followed by a declining trend over the next three follow-up periods, resulting in only 5.2% of the study participants being exposed at 24 months of age. The remaining participants consumed other forms of milk, such as soy and goat's milk.

The prevalence of cow's milk exposure and its change over the study period, as well as for the outcomes of asthma and wheezing, are presented in figures 5-7. Due to the fact that less than 17% of the participants exposed to cow's milk at any given survey period were also exposed to breast milk or other formula-based feeding products during the same time period, there was minimal overlap in the different types of newborn feeding and the reference group for the cow's milk exposure variable was indicated as all those exposed to feeding products other than cow's milk. Newborn cow's milk exposure

occurred in only 9.9% of the study population at the six-month follow-up, but increased to 48.6% at twelve months of age. After twelve months of age the majority of the newborns participating in the study were exposed to cow's milk (Figure 7).

When following asthma occurrence over the same time intervals the percentages of asthma development were much lower compared to that of cow's milk consumption. Asthma occurrence ranged from 2.2 to 3.7% through the study follow-up, with the occurrence of asthma at the 36-month follow-up being the greatest (Figure 8). Overall, asthma development showed a relatively stable increase in occurrence with increasing age of the study participants.

The outcome of wheezing was not as stable over time. The prevalence of wheezing over the entire follow-up period ranged from 8.8 to 22.0% of the newborn population. The highest occurrence of wheezing occurred at the 12-month follow-up period. Overall, the occurrence of wheezing had a peak at 12 months of age followed by a gradual decline in occurrence at each subsequent follow-up period (Figure 9). Cow's milk allergen sensitization at 12 months of age occurred in approximately 5% of the population, while approximately 4% of the study population was sensitized to house dust mite allergen and 10% had a positive allergic sensitization to hen's eggs. These results were based on allergic sensitization testing that was completed in approximately 90% of the study population.

The asthma concurrent effects model showed no association between cow's milk consumption on the occurrence of asthma (OR=1.28, p=0.2607, Table 5). Of the other potentially important factors in the model only one indicated a significant association with asthma development. The presence of chest infections was indicative of a

significant increased risk of asthma (OR= 10.55, p=0.0095). In the initial GEE models for asthma, increasing years of maternal education was significantly protective against asthma development, but this effect disappeared when country of residence was included in the final model.

In the asthma delayed effects model, early cow's milk exposure again showed no significant association with asthma development. The presence of chest infections again gained significance in the delayed effects model (Table 6). When combining both concurrent and delayed effects similar inferences were obtained (Table 7).

Delayed effects models for asthma, stratified by survey period, indicated that a six-month delayed effect of cow's milk exposure was a risk factor for asthma development at the 12 and 18-month follow-ups, but at the 24 and 36-month follow-ups, children who were fed with cow's milk had less asthma (Figure 10). Due to the heterogeneity of effects in these two periods, delayed effects models for the 12-18 month follow-up, as well as the 24-36 month follow-up period were created. The results of these models indicated that during the 12-18 month follow-up, a delayed effect of cow's milk showed a moderate relative risk for asthma (OR=1.60, p=0.08), however, not statistically significant (Table 8). In contrast, the 24-36 month delayed exposure model documented the presence of a marginally significant protective effect (OR=0.67, p=0.08) on the development of asthma within these combined follow-up periods (Table 9).

Due to the presence of this protective effect of delayed cow's milk exposure for the 24-36 month delayed effects model, a reverse causation model was developed to see whether asthma would predict cow's milk exposure. This model indicated that the overall presence of asthma was indeed protective (OR=0.39, p=0.0105) against cow's

milk exposure at the next follow-up period (Table 10), consistent with the notion that the presence of asthma caused participants to stop consuming cow's milk. It is also important to note that due to the finding that country of residence did not substantially change (10% rule of confounding) the coefficient estimates for any of the predictors in the reverse causation model it was not included in the final model. Additionally, county of origin also did not substantially affect the coefficient estimates for the wheezing and allergic sensitization reverse causation models and thus were also eliminated from the final models, respectively.

The final model incorporating asthma as the outcome of interest investigated whether the recommendation to avoid cow's milk until one year of age could be supported by our data. No significant association between this exposure variable and the development of asthma was found (Table 11).

The models incorporating wheezing as the dependent variable revealed similarities as well as differences compared to that of the asthma models. In addition to the confounders included in the asthma models, breast milk exposure was also included in all the models with wheezing as the outcome. For the concurrent modeling strategy cow's milk exposure again had a non-significant protective effect (OR=0.94, p=0.56) on the occurrence of wheezing throughout the first three years of life (Table 12). Despite its non-significant protective effect of cow's milk exposure on wheezing, this model had heterogeneity of effects that occurred over each of the study intervals. Other variables that were significantly associated with wheezing occurrence were the presence of chest infections (OR= 3.5, p=<0.0001), consumption of normal formula (OR= 1.7, p= <0.0001) and hypoallergenic formula use (OR= 1.77, p= <0.0001, Table 12). Additionally, being a

female (OR= 0.62, p= 0.0004) was protective against wheezing development in the first three years of life. Similar to the asthma models, controlling for country of residence washed out the protective effect of increasing years of maternal education.

The six-month delayed effects model for wheezing presented several significant results. In the delayed effects model early cow's milk exposure was associated with a significant protective effect (OR= 0.75, p= 0.02) against the development of wheezing (Table 13). In addition to the same variables indicated as significant through the concurrent effects model, the delayed effects model also indicated passive smoking as a significant risk factor (OR= 1.45, p= 0.02) for wheezing occurrence in this study population. When combining the concurrent and delayed effects of cow's milk for the outcome of wheezing, all the significant results from the previous two models were again present (Table 14).

To investigate heterogeneity of effects, the delayed effects model for wheezing was also broken down into four separate models for each survey period. Because of the heterogeneity of effects that occurred over each of the study intervals, as well as the possibility of some random error, it was necessary to treat each individual follow-up period separately (Figure 10). The only separated interval that indicated a significant effect of cow's milk exposure of wheezing occurred at two years of age. This model showed cow's milk exposure as being a significant risk factor (OR= 2.27, p= 0.05) for the development of wheeze at this time period (Table 15).

In attempts to further explain the significant results indicated above, a reverse causation modeling strategy was used again, now with wheezing as the exposure and cow's milk consumption in the next follow-up as the outcome. Unlike the asthma reverse

causation model, no significant results were obtained relating wheezing to changes in cow's milk consumption patterns. This model indicated that wheezing was a weak risk factor (OR= 1.28, p= 0.0503) for consecutive cow's milk consumption (Table 16).

Again cow's milk exposure at or before 12 months of age was found to be a non-significant risk factor for the development of wheezing in the first year of life (OR= 1.01, p= 0.95), which is comparable to the results found for this same relationship with asthma as the outcome (Tables 11 and 17).

The models that included the outcome of allergic sensitization to cow's milk at 12 months of age provided few risk factors that reached statistical significance. For the concurrent effect of cow's milk consumption on cow's milk allergen sensitization the model indicated cow's milk to be a non-significant risk factor (OR= 1.89, p=0.14, Table 18). As for the other predictors that were included in this model, both the consumption of normal formula (OR= 0.36, p= 0.03) and being a female (OR= 0.47, p=0.05) were protective against the participant becoming sensitized to cow's milk allergen during the first three years of life. The remaining models for allergic sensitization to cow's milk allergen, incorporating the other modeling strategies, all developed similar results to that of the concurrent model (Tables 19-21).

To further explore the argument that cow's milk exposure in childhood is a risk factor for allergic sensitization, an additional model was developed using an exposure variable that combined the results from the remaining specific allergen tests completed through the SPACE study. Since information on the remaining core allergens was tested in both the 12 and 24-month follow-up periods, a repeated measurements (GEE) model could be constructed. This concurrent effects model indicated that cow's milk exposure

during the first three years of life had no important effect (OR = 0.88, p= 0.6668) on general allergic sensitization during childhood (Table 22). A reverse causation model was also developed using the overall sensitization information from both follow-up periods (12 and 24 months). This model indicated allergic sensitization at 12 months as exerting a non-significant protective effect (p= 0.2386) on cow's milk consumption at 24 months of age (Table 23).

In addition to the previous analyses, a GEE model was completed in order to investigate whether any of the predictors used in the models were able to significantly predict the loss to follow-up that occurred at each survey period (Table 24). Due to the non-significant p-values that were calculated for each predictor, loss to follow-up over the course of the study period was not due to the presence or absence of any one predictor, thus loss to follow-up was deemed to be negligible. No other significant attrition occurred over the course of the study.

DISCUSSION

The findings of this study indicate that when investigating the concurrent association of cow's milk consumption and asthma and other atopic outcomes, no significant results were found. The direction of effects remained similar throughout each of the models, and the models for both asthma and allergic sensitization to cow's milk at 12 months of age indicated a slight increased risk of these outcomes due to cow's milk consumption during early childhood. In contrast, cow's milk consumption was a non-significant protective factor against the development of wheezing during the first three years of life, but this model was also deemed to be inadequate due to the heterogeneity of effects across the

follow-up periods, indicating a similar trend to that of the delayed effects wheezing model (Figure 11). A non-significant protective effect was also indicated for cow's milk consumption on the presence of general allergic sensitization in the combined 12 and 24-month concurrent effects model.

The results of the models for all of the main outcomes incorporating a six-month delayed effect of cow's milk exposure developed similar results to that of the concurrent models, except for the model including wheezing as the outcome which gained statistical significance for the effect of exposure to cow's milk. Due to the strong evidence for heterogeneity of effects in the asthma and wheezing delayed effects models, the initial delayed models were deemed inadequate and thus separate models were produced grouping together the intervals with homogeneous effects for each outcome.

When investigating the delayed effects models stratified by survey period it was discovered that cow's milk exposure between 12 and 18 months was a non-significant risk factor for asthma, while consumption between 24 and 36 months of age was protective against asthma development (Figure 10). In addition, when interpreting the delayed effects models for wheezing that were stratified by survey period, cow's milk exposure showed a weak protective effect at 12 and 36 months, but at the other two survey periods (18 and 24 months) cow's milk was found to be a risk factor for wheezing (Figure 11). The only significant finding occurred with an increase risk for the development of wheezing at the 24-month survey period. The reverse causation model for asthma was able to provide further evidence into why protective effects were obtained for the 24-36 month delayed effects model. This model indicated that the protective delayed effect found for cow's milk exposure on the development of asthma at the 24 and

36-month follow-ups was potentially due to parents who stopped feeding their children cow's milk after the child's diagnosis of asthma, thus indicating that no true protective effect of cow's milk exists. No solid explanation was found for the presence of the non-significant delayed protective effect of cow's milk exposure on wheezing at 36 months of age, but this may have been influenced by the fact that approximately 46% of the wheezing individuals at this time period were study participants that had wheezing during an early period, had their symptoms subside, and then reappear in the 36-month follow-up (Figure 9). This finding is also supported by a previous study by Sherriff et al. that indicated that over 70% of the children in the study who developed wheezing in the first 6 months of life did not continue to have wheeze three years later [59]. A similar type of situation may have occurred in our study population.

The reverse causation model developed was able to suggest why significant protective results were obtained for the delayed asthma model. This model indicated that the protective delayed effect found for cow's milk exposure on the development of asthma at the 24 and 36-month follow-ups was a direct result of parents of children with asthma stopping their cow's milk consumption after diagnosis of asthma, thus indicating that no true protective effect of cow's milk exists. The reverse causation for general allergic sensitization also indicated a protective effect of allergic sensitization on cow's milk consumption at 24 months of age, but these results did not reach statistical significance. The wheezing reverse causation model actually indicated the opposite effects, in that participants diagnosed with wheezing continued or began consuming cow's milk after such a diagnosis, thus potentially furthering the development of this atopic disorder. Additionally, each model that investigated the effects of cow's milk

consumption prior to 12 months of age all indicated a non-significant increased risk of each of the outcomes. Taking the above findings into consideration, they could have resulted from the fact that the outcome of asthma was based on a parental report of an actual physician's diagnosis of asthma which would have most likely resulted in recommendations to avoid certain substances, such as cow's milk. With wheezing only being a self reported outcome, no such recommendations would have been given, thus not influencing the effected individuals to change their consumption habits.

Besides the cow's milk exposure variable, several of the other known risk factors in each of the models also developed significant associations with the outcome. For both the wheezing and cow's milk allergic sensitization models being a girl was significantly protective against the development of each outcome over the course of the study. The most significant and largest increased risk of asthma and wheezing was found for the presence of chest infections in the newborn participants. It is possible that chest infections may be a mediator of cow's milk exposure, e.g. children who were breastfed may have been exposed to less cow's milk, and also may have experienced fewer chest infections. To investigate these associations this variable was removed from the models in order to see if it had an effect on the coefficient estimates for cow's milk. No significant changes in the models resulted. Finally, both the use of hypoallergenic and normal formula in the first three years of life were significant risk factors for wheezing, while normal formula use was actually protective against positive cow's milk sensitization at 12 months of age. A potential explanation for why both formulas were risk factors in the wheezing models could be that newborns with wheezing in the first 12 months of life may switch to using formulas due to previous complications, thus the

formulas would be indicated as risk factors not because they caused the wheezing, but because the majority of the newborns switched to using primarily formula-based feeding methods. This explanation is supported by our data that shows that approximately 60% of newborns that develop wheezing in the first 12 months of life switched from cow's milk to the primary use of normal and hypoallergenic formulas.

In comparison to previous studies, the current one has both strengths and limitations. Many of the previous studies investigated this potential association through the use of retrospective or cross-sectional study designs [34, 36, 41, 49]. Regardless of the direction of the results obtained from these studies, any inferences made based on their results are weakened due to the potential biases that were introduced by these study designs. The studies that used retrospectively determined exposure and outcome variables were prone to recall bias, as well as misclassification bias [34, 36]. Some previous studies also investigated the participants at only one time period [41, 49]. Through this type of design the power of the study is low due to the inability to account for both the associations that occur between each participant, as well as the associations that occur within an individual subject over a series of repeated measurements. The analyses described in this thesis were able to account for both of the above associations. Due to the fact that the diagnosis of asthma is subject to a lack of validity, more than one measurement of exposure and outcome are needed to develop more reliable results. Other previous studies were limited by short follow-up periods. These studies were conducted over short time intervals lasting less than 3 years [28, 30, 31, 33, 34, 42, 43, 49]. Studies with short intervals don't give adequate time for the development of asthma and atopic manifestations and are unable to investigate delayed responses. Another issue

that limits the results produced by these studies are that some relied on small sample sizes of less than 300 participants with relatively low frequencies of asthma and atopy [28, 30-36, 42-45].

The study design used for this research has strength in that repeated measurements were taken over the first three years of life. A total of five exposure and outcome measurements for each participant were incorporated into the GEE models enabling both within and between subject associations to be accurately accounted for, thus increasing the power and quality of the study results. The presence of repeated measurements also allowed for the investigation into the potential effects of reverse causation, which is another strength of this thesis project.

An additional strength of this study was that at least one of the parents from each participant had a history of atopy. This allowed us to investigate the effect of cow's milk consumption in a group that was already at high risk for the development of asthma and other atopic disorders. Additionally, this study is strengthened by having a relatively high frequency of both exposures and outcomes. Unlike this study, other previous studies did not restrict their study populations to those only at high risk of asthma and atopy, which could have potentially washed out any association due to the presence of low risk individuals with a lower incidence of asthma and atopy [30, 34, 36, 38, 41, 43, 49].

One limitation of this study is that it was able to follow-up the participants until only three years of age. It has long been known that the development of asthma, wheezing, and other atopic disorders may take much longer than the first three years of life in order to develop. Further, it has also been noted that even if asthma and wheezing occurs in the first few years of life this doesn't necessarily mean that these disorders will

be present at some later age. This doesn't suggest that the presence of asthma before three years of age should be taken lightly, but simply indicates that asthma occurrence can vary over the first several years of life. The longitudinal nature of the data can partially challenge this limitation by being able to develop a trend for each outcome over the first years of life which could give some idea into the future of the disease, but one can not be sure that the participants' outcome status will not begin to fluctuate beyond the first three years of life. Other potential limitations of this study were that there was some loss to follow-up that occurred in the later stages of this follow-up study and also that the nature of the data was only binary, making it difficult to determine specific durations for the exposures and outcomes used in the models. You will always suspect some loss to follow-up in the later stages of a longitudinal study, but with this study there was some suspicion that the losses were not at random due to the fact that none of the risk factors or outcomes were able to account for this loss. Additionally, the binary nature of the data was also a limitation due to the fact that we desired to have the exact duration of the cow's milk exposure and of the outcomes that occurred prior to conversion (e.g. disease-free to asthma or asthma to disease-free). This would require both the date of cow's milk introduction and possibly cessation, as well as the date of disease onset.

When investigating the mechanistic explanations for the results of this study there are two explanations that have been indicated through previous studies. A group of explanations focus on the differences that specific proteins may have on the development of asthma and atopy [60-62]. These potential explanations also look into the controversy between specific and non-specific effects. One explanation assumes that proteins have a specific allergy-producing potential, while others suggest a cross-reactivity between

proteins [60-62] This cross-reactivity between proteins implies a synergistic modification in the atopic response to a particular protein due to the presence of other proteins from different types of foods. The answer to which effect (specific or non-specific) plays the most important role in the development of asthma and atopic manifestations remains uncertain.

Another explanation for the cow's milk/childhood asthma association focuses on the importance of essential polyunsaturated fatty acids as a potential mechanism of action. Essential fatty acids, such as linoleic acid and alpha-linolenic acid are necessary for proper development of the body's systems and are needed in the formation of eicosanoids, which are chemicals that regulate several body functions, including immune and inflammatory responses [63]. It is currently known that the essential polyunsaturated fatty acids, linoleic acid, linolenic acid, and arachadonic acid, are found in small, but significant amounts in cow's milk fat [64]. In a current study by Woods et al. these specific fatty acids were measured in a cross-sectional study comparing young adults with and without asthma. The results of this study indicated that the n-6 fatty acid dihomo γ-linolenic acid, an isomer of linolenic acid which is also present in cow's milk, was significantly associated with current asthma (OR= 1.30), ever asthma (OR= 1.34), and also doctors diagnosed asthma (OR= 1.25) [65]. These results thus provide a straight forward mechanism directly linking the fatty acids in cow's milk with the development of asthma and other atopic disorders.

To provide more evidence into this potential mechanism a study by Devereux et al. indicated that increasing number of pregnancies leads to the depletion of the essential fatty acids that are passed on to the newborn [66]. Additionally, through previous studies

it has been indicated that essential fatty acids are known to be capable of influencing Th cell responses [67]. With increased number of pregnancies it was also discovered that less asthma and atopy occurred in the children born at a higher order of pregnancy, thus depletion of essential fatty acids lead to a protective effect against asthma and atopy. Putting this evidence into the context of this thesis project would then indicate that the presence of essential fatty acids in newborns infers an increased risk of asthma and atopy in early childhood.

Another study has also suggested that there is more to this mechanism than just the simple presence of specific essential polyunsaturated fatty acids. A study by Yu and Bjorksten investigated the serum levels of fatty acids in mothers and their babies and also their relationship to the development of allergic disease [68]. It has been previously noted that the long-chain polyunsaturated fatty acid levels in the fetus and mother are highly associated due to the passage of certain fatty acids to the fetus via the placenta [69-72]. Due to this fact, Yu and Bjorksten investigated the possible existence of an abnormal metabolism of essential fatty acids in allergic mothers during pregnancy that could potentially affect the fatty acid composition and appearance of allergy in their offspring. The study compared levels of various fatty acids measured in the maternal blood at time of delivery and the umbilical venous blood just after delivery. For all of the non-allergic mothers the maternal levels for the majority of the essential fatty acids measured were significantly correlated to the same fatty acid levels in their offspring (cord serum). For instance, a significant positive correlation was for linoleic acid when comparing maternal and cord serum. Additionally, dihomo-γ-linolenic acid levels of non-allergic mothers also developed positive correlations with cord serum arachidonic

acid levels. In the allergic mothers and their babies none of the same correlations existed, indicating an altered fatty acid metabolism in allergic mothers that potentially could be passed on to their offspring. The mothers who had offspring that developed atopy in the first six years of life also had correlation patterns comparable to that of the allergic mothers. It was also discovered that the non-allergic participants had an inverse relationship between the levels of the essential fatty acid lenolenic acid, and its metabolic products arachadonic acid and C22:4, but the allergic participants did not develop this inverse relationship [68]. Taken together, these findings would indicate that allergic mothers and mothers who have children that develop allergy have an altered fatty acid metabolism that was passed onto their infants, thus indicating an association between altered fatty acid metabolism and the development of atopy. In addition, this study implies that mothers with a history of allergy and/or an altered fatty acid metabolism could be at risk of passing these traits onto their offspring, thus increasing the chances that their offspring will develop asthma and atopy. Within the present study population, consisting of approximately 60% of children with a maternal history of atopy, this explanation seems more feasible than the explanation suggesting that the simple consumption of specific fatty acids leads directly to the development of asthma and atopy.

To add more strength to the cow's milk consumption explanation a recent study by Zeyda et al. looked into the potential effects of poly-unsaturated fatty acids on the actions of T lymphocytes [73]. When treating a human line of T lymphocytes with poly-unsaturated fatty acids these actions lead to the significant reduction of interleukin (IL)-2 mRNA, thus leading to a decrease in the production of IL-2, a Th1 cytokine. The mRNA

expression in the majority of the other cytokines, especially the Th2 cytokine IL-4 were unaffected from the exposure to these fatty acids. This study suggests that both Th1 and Th2 cytokines can be affected by unsaturated fatty acids, but the greatest effects occur in the reduction of Th1 cytokines, disrupting the Th1/Th2 balance and creating a Th2 atopic response. Another cytokine that was inhibited by the fatty acid treatment was IL-13, a Th2 cytokine. The importance of this finding is that IL-13 is a known mediator of IgE production in patients with bronchial asthma [74]. This may indicate that the reduction in IL-13 due to fatty acids would help shift the early immune system response to more of a Th1 non-atopic response, but recent data demonstrates that IL-13 is of little importance in Th2 atopic responses compared to that of IL-4 [75]. In summary, these findings would suggest that polyunsaturated fatty acids have the potential to inhibit the production of IL-2 in T lymphocytes and shift the early immune system response to a more predominantly IL-4 based Th2 atopic response, thus increasing the occurrence of asthma and atopy in childhood.

It is also worth noting that not all previous studies propose the presence of poly-unsaturated fatty acids as being a risk factor for the development of asthma and other atopic manifestations. A study by Wijga et al. indicated that poly-unsaturated fatty acids may be a risk factor for the development of asthma and atopy, but saturated fatty acids may be protective against such outcomes [48].

The past studies investigating this explanation also focus on the issue of specific versus non-specific effects. Is it the fatty acids in the cow's milk that specifically leads to the formation of asthma/allergic sensitization or is it the presence of the essential poly-unsaturated fatty acids in general that leads to a non-specific triggering of several

different types of allergens, thus leading to the development of asthma? It is easy to see that there is not one accepted explanation into why cow's milk consumption may be a risk factor for or protective against the development of asthma and other atopic manifestations. In addition, the explanations involving specific proteins and fatty acids may go beyond the exposure of cow's milk. There are several other sources of fatty acids and proteins other than cow's milk, such as maternal exposure during pregnancy and other foodstuffs. It is also important to note that all of the above findings were developed from previous studies that did not take the effects of reverse causation into account, thus adding some uncertainty to these particular findings.

Overall the data generated through this thesis project suggest that early consumption of cow's milk can be a risk factor for asthma, wheezing, and allergic sensitization, though the association does not always reach statistical significance. It is worth mentioning that when investigations into this area of research develop significant protective effects of cow's milk exposure on asthma and atopy, the potential for reverse causation should be taken into account in order to determine if the effect is a true effect or just an effect manufactured by the model. The reverse causation results may indicate an influence of atopic manifestations on dietary habits, instead of the assumed effects of diet on the occurrence of asthma and other atopic disorders.

In conclusion, the present mechanistic explanations for these findings indicate that cow's milk could be associated with asthma and atopy due to the presence of essential fatty acids and/or specific proteins in cow's milk, through the inhibition of IL-2 due to fatty acid exposure in T lymphocytes, or even through the transfer of an altered fatty acid metabolism from mother to child. None of the above explanations are fully

accepted by the scientific community, but future studies may provide more evidence for one or more of the current suggested mechanisms.

Our research suggests that future studies first investigate for the presence of "reverse causation" in these potential associations. The scientific community must first accurately determine if these potential associations truly exist prior to developing the potential mechanisms by which they act. By taking reverse causation into account, future research will be one step closer to elucidating the true action of cow's milk consumption on the development of childhood asthma and atopy.

APPENDICES

Table 1: Studies Indicating Cow's Milk Consumption as a Risk Factor for Asthma, Atopy, and Allergic Sensitization

Asthma and Atopy:

Author(s)	Year	Location	Study Design
Glaser and Johnstone	1953	New York, NY	Retrospective, small sample, highly criticized
Johnstone and Dutton	1966	New York, NY	Prospective, restricted study sample, short duration
Kaufman and Frick	1976	San Francisco, CA	Prospective, restricted study sample, small sample size
Chandra, R.K.	1979	Canada	Prospective, short duration, non-restricted sample
Chandra, R.K.	1997	Canada	Prospective, long duration, restricted sample
Ram, F.S.F.	2003	London, England	Prospective, large sample size, restricted study sample
Schonberger, H.J.	2003	The Netherlands	Prospective, short duration, small sample size

Allergic Sensitization:

Author(s)	Year	Location	Study Design
Tikkanen, S. et al	2000	Finland	Cross-Sectional, small study sample, non-restricted sample
Novembre and Vierucci	2001	Italy	Prospective, large study sample, long duration
Rhodes, H.L. et al	2001	United Kingdom	Prospective, restricted study sample, small sample size
Arshad, S.H. et al	2001	United Kingdom	Prospective, long duration, non-restricted sample
Baena-Cagnani and Teijeiro	2001	Argentina	Prospective, long duration, restricted sample
Host, A. et al	2002	Denmark	Prospective, large sample size, non-restricted study sample
Host, A.	2002	Denmark	Prospective, large sample size, restricted study sample

Table 2: Studies Indicating No Effect and Protective Effects of Cow's Milk Consumption on the Development of Asthma and Atopy

No Effect:

Author(s)	Year	Location	Study Design
Van Asperen, A.S. et al	1984	New South Wales, Australia	Prospective, small study sample, restricted sample, low frequency of cow's milk consumption
Hattevig, G. et al	1989	Skovde, Sweden	Prospective, small study sample, short duration, non-restricted study sample
Poysa, L. et al	1991	Helsinki, Finland	Prospective, restricted study sample, small sample size, long study duration
Zeiger, R.S. et al	1995	San Diego, California	Prospective, long duration, restricted sample, small sample size

Protective Effect:

Author(s)	Year	Location	Study Design
Lindfors, A.T.B. et al	1992	Europe	Prospective, large study sample, restricted sample, long study duration
Reidler, J. et al	2001	Austria, Germany, Switzerland	Cross-Sectional, large study sample, short duration, non-restricted study sample
Wijga, A.H. et al	2003	The Netherlands	Prospective, restricted study sample, large sample size, long study duration

Table 3: Study Population Characteristics

Variable	Frequency	Percentage
Gender		
- Male	350	50.3
- Female	346	49.7
Birthweight		
- < 3000g	135	19.4
- ≥ 4000g	74	10.6
- ≥ 3000g and < 4000g	487	70.0
Season of Birth		
- Spring	187	26.9
- Summer	200	28.7
- Fall	154	22.1
- Winter	155	22.3
History of Parental Atopy		
- Maternal atopy	246	35.3
- Paternal atopy	234	33.6
- Both Parents atopic	216	31.1
Mother's Education		
- Normal (≤10 years)	159	22.8
- Medium (11-12 years)	377	54.2
- High (≥13 years)	160	23.0

Table 4: Study Population Characteristics for Repeated Measurements

	6 months		12 months		18 months		24 months		36 months	
Variable	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**
Passive Smoking										
- Yes	0	0.0	173	24.9	154	22.1	140	20.1	122	17.5
- No / Missing	696	100.0	523	75.1	542	77.9	556	79.9	574	82.5
Chest Infections										
- Yes	14	2.0	24	3.5	31	4.5	40	5.8	21	3.0
- No / Missing	682	98.0	672	96.5	665	95.5	656	94.2	675	97.0
Breastfeeding										
- Yes	70	10.1	48	6.9	70	10.1	18	2.6	-	-
- No / Missing	626	89.9	648	93.1	626	89.9	626	97.4	-	-
Normal Formula										
- Yes	279	40.1	339	48.7	182	26.2	76	10.9	-	-
- No / Missing	417	59.9	357	51.3	514	73.8	620	89.1	-	-
Hypoallergenic Formula										
- Yes	241	34.6	235	33.8	92	13.2	36	5.2	-	-
- No / Missing	455	65.4	461	66.2	604	86.8	660	94.8	-	-

Table 5: Asthma Model: Concurrent Effect of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.2470	0.2607	1.28	(0.83-1.97)
- No (ref.)				
Gender				
- Female	0.3473	0.4809	1.42	(0.54-3.72)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.6164	0.3720	1.85	(0.48-7.17)
- Both Parents Atopic	0.8654	0.2071	2.38	(0.62-9.11)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.5317	0.1021	1.70	(0.90-3.22)
- No (ref.)				
Chest Infections				
- Yes	2.3565	0.0095	10.55	(1.78-62.62)
- No (ref.)				
Mother's Education				
- High	-0.3478	0.6473	0.71	(0.16-3.13)
- Medium	0.0059	0.9905	1.01	(0.38-2.66)
- Normal (ref.)				
Normal Formula				
- Yes	-0.3120	0.2974	0.73	(0.41-1.32)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.3478	0.3965	0.71	(0.32-1.58)
- No (ref.)				
Country of Residence				
- Austria	0.7998	0.4825	2.23	(0.24-20.75)
- Great Britain	2.8947	0.0080	18.08	(2.13-153.56)
- Germany (ref.)				

Table 6: Asthma Model: Six-Month Delayed Effect of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.2366	0.2690	1.27	(0.38-1.93)
- No (ref.)				
Gender				
- Female	0.3024	0.5429	1.35	(0.51-3.59)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.5251	0.4577	1.69	(0.42-6.76)
- Both Parents Atopic	0.9024	0.1905	2.47	(0.64-9.52)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.6144	0.0691	1.84	(0.95-3.59)
- No (ref.)				
Chest Infections				
- Yes	2.3529	0.0083	10.52	(1.83-60.34)
- No (ref.)				
Mother's Education				
- High	-0.3895	0.6105	0.68	(0.15-3.03)
- Medium	-0.0761	0.8762	0.93	(0.36-2.42)
- Normal (ref.)				
Normal Formula				
- Yes	-0.1745	0.6072	0.84	(0.43-1.63)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.2789	0.4487	0.76	(0.37-1.56)
- No (ref.)				
Country of Residence				
- Austria	0.7449	0.5175	2.11	(0.22-20.10)
- Great Britain	2.6785	0.0123	14.56	(1.79-118.46)
- Germany (ref.)				

Table 7: Asthma Model: Concurrent and Delayed Effects of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk (Concurrent)				
- Yes	0.3198	0.1574	1.38	(0.88-2.14)
- No (ref.)				
Cow's Milk (Delay)				
- Yes	0.3356	0.0967	1.40	(0.94-2.08)
- No (ref.)				
Gender				
- Female	0.4264	0.4077	1.53	(0.56-4.20)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.6524	0.3826	1.92	(0.44-8.30)
- Both Parents Atopic	0.9395	0.2186	2.56	(0.57-11.42)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.5834	0.0739	1.79	(0.95-3.40)
- No (ref.)				
Chest Infections				
- Yes	2.2831	0.0149	9.81	(1.56-61.61)
- No (ref.)				
Mother's Education				
- High	-0.3226	0.6824	0.72	(0.15-3.40)
- Medium	0.0732	0.8871	1.08	(0.39-2.96)
- Normal (ref.)				
Normal Formula				
- Yes	-0.1515	0.6337	0.86	(0.46-1.60)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.2550	0.5299	0.77	(0.35-1.72)
- No (ref.)				
Country of Residence				
- Austria	0.7934	0.4865	2.21	(0.24-20.66)
- Great Britain	2.8205	0.0101	16.78	(1.96-143.89)
- Germany (ref.)				

Table 8: Asthma Model: Six-Month Delayed Effect of Cow's Milk Exposure (12-18 months)				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.4719	0.0827	1.60	(0.94-2.73)
- No (ref.)				
Gender				
- Female	-0.1771	0.6851	0.84	(0.36-1.97)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	-0.1168	0.8425	0.89	(0.28-2.82)
- Both Parents Atopic	0.6589	0.1821	1.93	(0.73-5.09)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	-0.1549	0.6363	0.86	(0.45-1.63)
- No (ref.)				
Chest Infections				
- Yes	2.1978	<0.0001	9.00	(3.12-26.03)
- No (ref.)				
Mother's Education				
- High	-1.9920	0.0025	0.14	(0.04-0.50)
- Medium	-1.5522	0.0017	0.21	(0.08-0.56)
- Normal (ref.)				

Table 9: Asthma Model: Six-Month Delayed Effect of Cow's Milk Exposure (24-36 months)				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	-0.4030	0.0833	0.67	(0.42-1.06)
- No (ref.)				
Gender				
- Female	-0.2846	0.4768	0.75	(0.34-1.65)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	-0.1260	0.7992	0.88	(0.33-2.33)
- Both Parents Atopic	0.2655	0.5927	1.30	(0.49-3.45)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.1701	0.6898	1.19	(0.51-2.73)
- No (ref.)				
Chest Infections				
- Yes	1.6578	0.0010	5.25	(1.95-14.13)
- No (ref.)				
Mother's Education				
- High	-0.9623	0.2087	0.38	(0.09-1.71)
- Medium	-0.0695	0.8774	0.93	(0.39-2.26)
- Normal (ref.)				

Table 10: Reverse Causation: Cow's Milk Exposure vs. Asthma at the next survey				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Asthma				
- Yes	-0.9392	0.0105	0.39	(0.19-0.80)
- No (ref.)				
Gender				
- Female	-0.2122	0.0794	0.81	(0.64-1.03)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	-0.0501	0.7291	0.95	(0.72-1.26)
- Both Parents Atopic	0.0017	0.9908	1.00	(0.75-1.34)
- Father Atopic (ref.)				
Mother's Education				
- High	0.9211	<0.0001	2.51	(1.77-3.57)
- Medium	0.6599	<0.0001	1.93	(1.50-2.50)
- Normal (ref.)				

Table 11: Asthma Model: Cow's Milk Exposure at or prior to 12 months of age				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.9705	0.2644	2.64	(0.48-14.51)
- No (ref.)				
Gender				
- Female	0.6342	0.2822	1.89	(0.59-5.99)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.6182	0.4836	1.86	(0.33-10.47)
- Both Parents Atopic	1.0756	0.2611	2.93	(0.45-19.14)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.6575	0.0553	1.93	(0.99-3.78)
- No (ref.)				
Chest Infections				
- Yes	2.1986	0.0413	9.01	(1.09-74.48)
- No (ref.)				
Mother's Education				
- High	-0.1147	0.8960	0.89	(0.16-4.97)
- Medium	0.0746	0.8972	1.08	(0.35-3.34)
- Normal (ref.)				
Normal Formula				
- Yes	-0.3249	0.3015	0.72	(0.39-1.34)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.2819	0.5033	0.75	(0.33-1.72)
- No (ref.)				
Country of Residence				
- Austria	0.6837	0.5554	1.98	(0.20-19.22)
- Great Britain	2.3457	0.0439	10.44	(1.07-102.28)
- Germany (ref.)				

Table 12: Wheeze Model: Concurrent Effect of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	-0.0638	0.5560	0.94	(0.76-1.16)
- No (ref.)				
Gender				
- Female	-0.4829	0.0004	0.62	(0.47-0.81)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.1223	0.4698	1.13	(0.81-1.57)
- Both Parents Atopic	0.2999	0.0746	1.35	(0.97-1.88)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.2439	0.0757	1.28	(0.98-1.67)
- No (ref.)				
Chest Infections				
- Yes	1.3919	<0.0001	4.02	(2.36-6.87)
- No (ref.)				
Mother's Education				
- High	0.1653	0.4190	1.18	(0.79-1.76)
- Medium	-0.0247	0.8886	0.98	(0.69-1.38)
- Normal (ref.)				
Breastfeeding				
- Yes	0.0028	0.9904	1.00	(0.64-1.58)
- No (ref.)				
Normal Formula				
- Yes	0.5320	<0.0001	1.70	(1.34-2.16)
- No (ref.)				
Hypoallergenic Formula				
- Yes	0.5726	<0.0001	1.77	(1.38-2.28)
- No (ref.)				
Country of Residence				
- Austria	-0.0410	0.8268	0.96	(0.66-1.39)
- Great Britain	0.8368	0.0001	2.31	(1.50-3.55)
- Germany (ref.)				

Table 13: Wheeze Model: Six-Month Delayed Effect of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	-0.2885	0.0220	0.75	(0.59-0.96)
- No (ref.)				
Gender				
- Female	-0.5864	0.0001	0.56	(0.41-0.75)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.0490	0.8010	1.05	(0.72-1.54)
- Both Parents Atopic	0.2760	0.1357	1.32	(0.92-1.89)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.3715	0.0150	1.45	(1.07-1.96)
- No (ref.)				
Chest Infections				
- Yes	1.5852	<0.0001	4.88	(2.77-8.61)
- No (ref.)				
Mother's Education				
- High	0.0190	0.9333	1.02	(0.65-1.59)
- Medium	-0.0181	0.9243	0.98	(0.68-1.43)
- Normal (ref.)				
Breastfeeding				
- Yes	0.1826	0.4671	1.20	(0.73-1.96)
- No (ref.)				
Normal Formula				
- Yes	0.4788	0.0007	1.61	(1.22-2.13)
- No (ref.)				
Hypoallergenic Formula				
- Yes	0.5571	0.0003	1.75	(1.29-2.36)
- No (ref.)				
Country of Residence				
- Austria	-0.1424	0.5139	0.87	(0.57-1.33)
- Great Britain	0.8714	0.0003	2.39	(1.48-3.85)
- Germany (ref.)				

Table 14: Wheeze Model: Concurrent and Delayed Effects of Cow's Milk				
Parameter	β Estimate	P-Value	OR	(95% Confidence Limits)
Cow's Milk (Concurrent)				
- Yes	0.0619	0.6364	1.06	(0.82-1.38)
Cow's Milk (Delay)				
- Yes	-0.2912	0.0204	0.75	(0.58-0.96)
Gender				
- Female	-0.5839	0.0001	0.56	(0.41-0.75)
Parental Atopy				
- Mother Atopic	0.0485	0.8030	1.05	(0.72-1.54)
- Both Parents Atopic	0.2743	0.1380	1.32	(0.92-1.89)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.3673	0.0166	1.44	(1.07-1.95)
- No (ref.)				
Chest Infections				
- Yes	1.5798	<0.0001	4.85	(2.75-8.57)
- No (ref.)				
Mother's Education				
- High	0.0162	0.9431	1.02	(0.65-1.59)
- Medium	-0.0195	0.9187	0.98	(0.67-1.43)
- Normal (ref.)				
Breastfeeding				
- Yes	0.1749	0.4901	1.19	(0.72-1.96)
- No (ref.)				
Normal Formula				
- Yes	0.4688	0.00144	1.60	(1.20-2.13)
- No (ref.)				
Hypoallergenic Formula				
- Yes	0.5652	0.0003	1.76	(1.30-2.39)
- No (ref.)				
Country of Residence				
- Austria	-0.1317	0.5496	0.88	(0.60-1.35)
- Great Britain	0.8868	0.0003	2.43	(1.49-3.94)
- Germany (ref.)				

Table 15: Wheeze Model: Six-Month Delayed Effect of Cow's Milk Exposure (at 24 months)				
Parameter	β Estimate	P-Value	OR	(95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.8185	0.0500	2.27	(1.00-5.14)
- No (ref.)				
Gender				
- Female	-0.4733	0.1011	0.62	(0.35-1.10)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.4577	0.1965	1.58	(0.79-3.17)
- Both Parents Atopic	0.3030	0.4191	1.35	(0.65-2.82)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.5875	0.0609	1.80	(0.97-3.33)
- No (ref.)				
Chest Infections				
- Yes	1.8433	<0.0001	6.32	(2.72-14.67)
- No (ref.)				
Mother's Education				
- High	0.2073	0.6120	1.23	(0.55-2.74)
- Medium	-0.1647	0.6387	0.85	(0.43-1.67)
- Normal (ref.)				
Breastfeeding				
-Yes	1.3622	0.0338	3.90	(1.11-13.74)
- No (ref.)				
Normal Formula				
- Yes	0.4345	0.3466	1.54	(0.62-3.82)
- No (ref.)				
Hypoallergenic Formula				
- Yes	0.2508	0.7288	1.28	(0.31-5.30)
- No (ref.)				
Country of Residence				
- Austria	-0.3118	0.3773	0.73	(0.37-1.46)
- Great Britain	0.4165	0.3005	1.52	(0.69-3.34)
- Germany (ref.)				

Table 16: Reverse Causation: Cow's Milk Exposure vs. Wheeze at the next survey				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Wheeze				
- Yes	0.2469	0.0503	1.28	(1.00-1.64)
- No (ref.)				
Gender				
- Female	-0.1602	0.0997	0.85	(0.70-1.03)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.0132	0.9100	1.01	(0.81-1.27)
- Both Parents Atopic	0.0262	0.8249	1.03	(0.81-1.29)
- Father Atopic (ref.)				
Mother's Education				
- High	0.5533	0.0001	1.74	(1.31-2.31)
- Medium	0.3362	0.0021	1.40	(1.13-1.73)
- Normal (ref.)				

Table 17: Wheeze Model: Cow's Milk Exposure at or prior to 12 months of age				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.0090	0.9493	1.01	(0.76-1.33)
- No (ref.)				
Gender				
- Female	-0.4811	0.0004	0.62	(0.47-0.81)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.1220	0.4714	1.13	(0.81-1.57)
- Both Parents Atopic	0.2981	0.0762	1.35	(0.97-1.87)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.2271	0.0901	1.25	(0.97-1.63)
- No (ref.)				
Chest Infections				
- Yes	1.3812	<0.0001	3.98	(2.34-6.77)
- No (ref.)				
Mother's Education				
- High	0.1625	0.4268	1.18	(0.79-1.76)
- Medium	-0.0251	0.8868	0.98	(0.69-1.38)
- Normal (ref.)				
Breastfeeding				
- Yes	0.0006	0.9981	1.00	(0.64-1.57)
- No (ref.)				
Normal Formula				
- Yes	0.5243	<0.0001	1.69	(1.34-2.14)
- No (ref.)				
Hypoallergenic Formula				
- Yes	0.5834	0.0002	1.79	(1.40-2.29)
- No (ref.)				
Country of Residence				
- Austria	-0.0284	0.8814	0.97	(0.67-1.41)
- Great Britain	0.8535	<0.0001	2.35	(1.54-3.58)
- Germany (ref.)				

Table 18: Allergic Sensitization Model: Concurrent Effect of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.63402	0.1389	1.89	(0.81-4.36)
- No (ref.)				
Gender				
- Female	-0.7568	0.0468	0.47	(0.22-0.99)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	-0.0979	0.8453	0.91	(0.34-2.42)
- Both Parents Atopic	0.8728	0.0616	2.39	(0.96-5.98)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.5607	0.1537	1.75	(0.81-3.78)
- No (ref.)				
Mother's Education				
- High	-0.4371	0.4476	0.65	(0.21-2.00)
- Medium	-0.4343	0.3630	0.65	(0.25-1.65)
- Normal (ref.)				
Normal Formula				
- Yes	-1.0315	0.0281	0.36	(0.14-0.90)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.1783	0.6606	0.84	(0.38-1.85)
- No (ref.)				
Country of Residence				
- Austria	0.0110	0.9822	1.01	(0.38-2.66)
- Great Britain	-0.6889	0.3750		(0.11-2.30)
- Germany (ref.)				

Table 19: Allergic Sensitization Model: Six-Month Delayed Effect of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.3079	0.5516	1.36	(0.49-3.75)
- No (ref.)				
Gender				
- Female	-0.7543	0.0462	0.47	(0.22-0.99)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	-0.0791	0.8751	0.92	(0.34-2.48)
- Both Parents Atopic	0.8701	0.0579	2.39	(0.97-5.87)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.5760	0.1356	1.78	(0.83-3.79)
- No (ref.)				
Mother's Education				
- High	-0.3818	0.4978	0.68	(0.23-2.06)
- Medium	-0.4153	0.3794	0.66	(0.26-1.67)
- Normal (ref.)				
Normal Formula				
- Yes	-0.8875	0.0616	0.41	(0.16-1.04)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.1838	0.6447	0.83	(0.38-1.82)
- No (ref.)				
Country of Residence				
- Austria	-0.1631	0.7127	0.85	(0.36-2.02)
- Great Britain	-0.7003	0.3606	0.50	(0.11-2.23)
- Germany (ref.)				

Table 20: Allergic Sensitization Model: Concurrent and Delayed Effects of Cow's Milk Exposure				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk (Concurrent)				
- Yes	0.6162	0.1501	1.85	(0.80-4.29)
- No (ref.)				
Cow's Milk (Delay)				
- Yes	0.1469	0.7749	1.16	(0.42-3.17)
- No (ref.)				
Gender				
- Female	-0.7555	0.0472	0.47	(0.22-0.99)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	-0.0925	0.8545	0.91	(0.34-2.45)
- Both Parents Atopic	0.8705	0.0613	2.39	(0.96-5.94)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.5551	0.1555	1.74	(0.81-3.75)
- No (ref.)				
Mother's Education				
- High	-0.4375	0.4478	0.65	(0.21-2.00)
- Medium	-0.4309	0.3661	0.65	(0.26-1.65)
- Normal (ref.)				
Normal Formula				
- Yes	-1.0235	0.0310	0.36	(0.14-0.91)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.1648	0.6832	0.85	(0.38-1.87)
- No (ref.)				
Country of Residence				
- Austria	0.0242	0.9613	1.02	(0.39-2.72)
- Great Britain	-0.6792	0.3783	0.51	(0.11-2.30)
- Germany (ref.)				

Table 21: Allergic Sensitization Model: Cow's Milk Exposure at or prior to 12 months of age				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	0.4982	0.2293	1.65	(0.73-3.71)
- No (ref.)				
Gender				
- Female	-0.7540	0.0475	0.47	(0.22-0.99)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	-0.1009	0.8401	0.90	(0.34-2.41)
- Both Parents Atopic	0.8779	0.0593	2.41	(0.97-5.99)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	0.5745	0.1412	1.78	(0.83-3.82)
- No (ref.)				
Mother's Education				
- High	-0.3872	0.4974	0.68	(0.22-2.08)
- Medium	-0.3972	0.4040	0.67	(0.26-1.71)
- Normal (ref.)				
Normal Formula				
- Yes	-0.9874	0.0344	0.37	(0.15-0.93)
- No (ref.)				
Hypoallergenic Formula				
- Yes	-0.1721	0.6704	0.84	(0.38-1.86)
- No (ref.)				
Country of Residence				
- Austria	-0.0534	0.9116	0.95	(0.37-2.43)
- Great Britain	-0.6935	0.3707	0.50	(0.11-2.28)
- Germany (ref.)				

Table 22: Concurrent Effect of Cow's Milk on Total Allergic Sensitization (All core allergens tested at 12 and 24 months)				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	-0.1229	0.6668	0.88	(0.51-1.55)
- No (ref.)				
Gender				
- Female	-0.4705	0.1024	0.62	(0.36-1.10)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.5149	0.1589	1.67	(0.82-3.43)
- Both Parents Atopic	0.3336	0.3859	1.40	(0.66-2.97)
- Father Atopic (ref.)				
Passive Smoking				
- Yes	-0.0171	0.9557	0.98	(0.54-1.80)
- No (ref.)				
Mother's Education				
- High	-0.3669	0.4439	0.69	(0.27-1.77)
- Medium	-0.4072	0.3200	0.67	(0.30-1.48)
- Normal (ref.)				
Normal Formula				
- Yes	-0.3969	0.2428	0.67	(0.35-1.31)
- No (ref.)				
Hypoallergenic Formula				
- Yes	0.2810	0.4084	1.32	(0.68-2.58)
- No (ref.)				
Country of Residence				
- Austria	-0.5953	0.1340	0.55	(0.25-1.20)
- Great Britain	0.8783	0.0727	2.41	(0.92-6.28)
- Germany (ref.)				

Table 23: Reverse Causation: Cow's Milk vs. Total Allergic Sensitization at the next survey period				
Parameter	β Estimate	P-Value	OR	Odds Ratio (95% Confidence Limits)
Cow's Milk Exposure				
- Yes	-0.3060	0.2386	0.74	(0.44-1.22)
- No (ref.)				
Gender				
- Female	-0.1906	0.0729	0.83	(0.67-1.02)
- Male (ref.)				
Parental Atopy				
- Mother Atopic	0.0810	0.5268	1.08	(0.84-1.39)
- Both Parents Atopic	-0.0207	0.8744	0.98	(0.76-1.27)
- Father Atopic (ref.)				
Mother's Education				
- High	0.7551	<0.0001	2.13	(1.56-2.91)
- Medium	0.4964	0.0001	1.64	(1.28-2.11)
- Normal (ref.)				

Table 24: Loss-to-follow-up vs. other predictors used in the analysis		
Parameter	β Estimate	P-Value
Gender		
- Female	0.0016	0.1538
- Male (ref.)		
Parental Atopy		
- Mother Atopic	0.0003	0.8429
- Both Parents Atopic	0.0015	0.1830
- Father Atopic (ref.)		
Passive Smoking		
- Yes	-0.0038	0.1527
- No (ref.)		
Chest Infections		
- Yes	0.0005	0.2730
- No (ref.)		
Mother's Education		
- High	0.0030	0.1799
- Medium	0.0033	0.1853
- Normal (ref.)		
Normal Formula		
- Yes	0.0018	0.1609
- No (ref.)		
Hypoallergenic Formula		
- Yes	0.0006	0.2746
- No (ref.)		
Breastfeeding		
- Yes	0.0006	0.2929
- No (ref.)		
Country of Residence		
- Austria	-0.0007	0.3724
- Great Britain	-0.0001	0.9339
- Germany (ref.)		

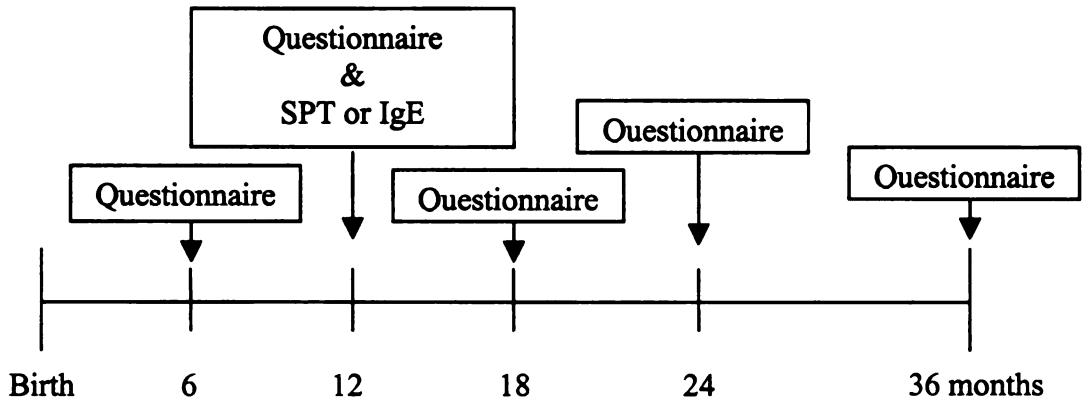
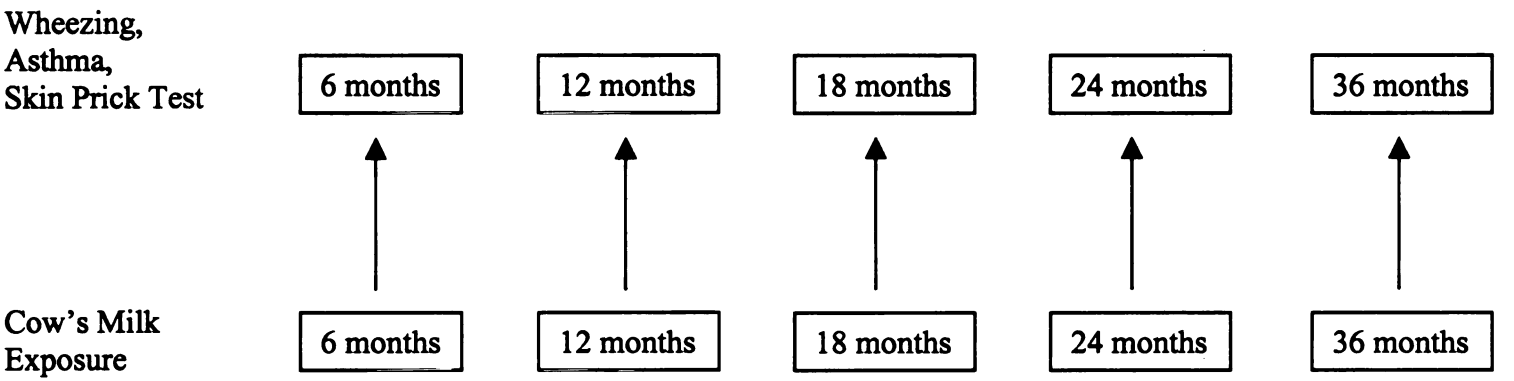


Figure 1: Timeline for the study follow-up period indicating time intervals at which exposure and outcome measurements were ascertained.

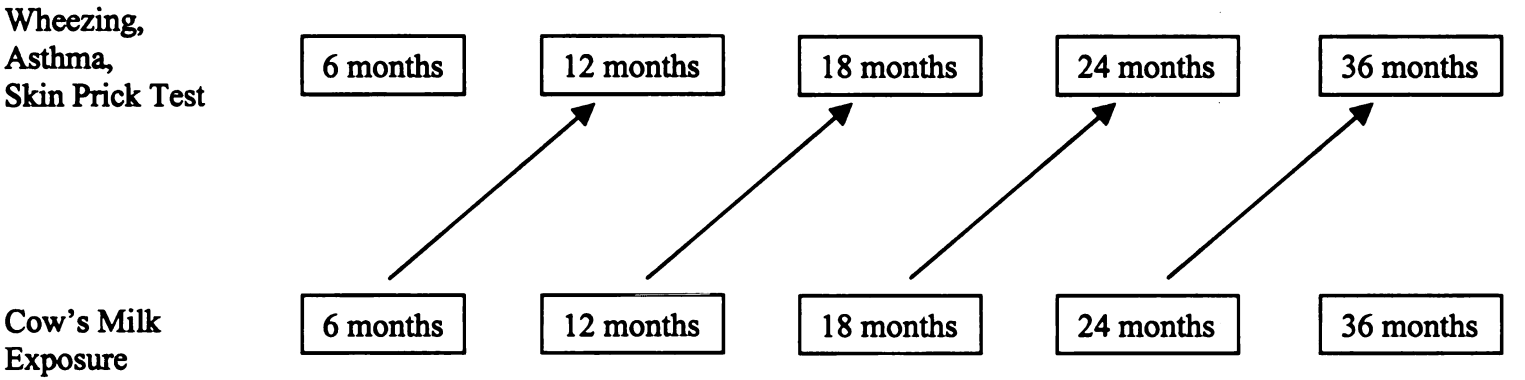
Concurrent effects models



* Model the association of the outcome at each time period with the exposure from the same time period.

Figure 2: Modeling Strategy for the Concurrent effects models

Delayed effects (6 months) models



* Model the association of the outcome at each time period with the exposure from the previous time period.

Figure 3: Modeling strategy for the six-month delayed effects models.

Combined effects (Concurrent and Delayed Effect)

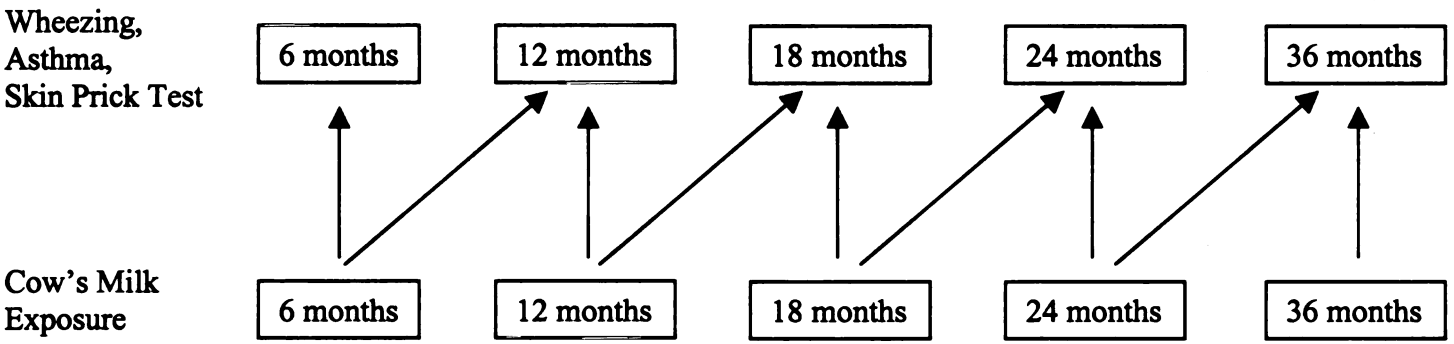
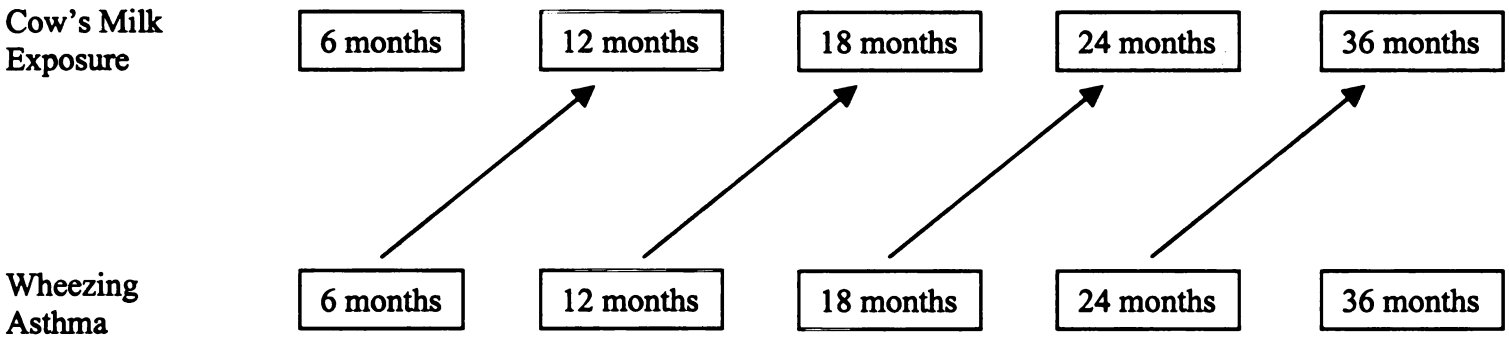


Figure 4: Modeling strategy for the combined effects models.

Reverse Causation Modeling



* Model the association of the exposure at one time period with the outcome from the previous time period.

Figure 5: Modeling strategy for the reverse causation models.

Medical Recommendation Modeling

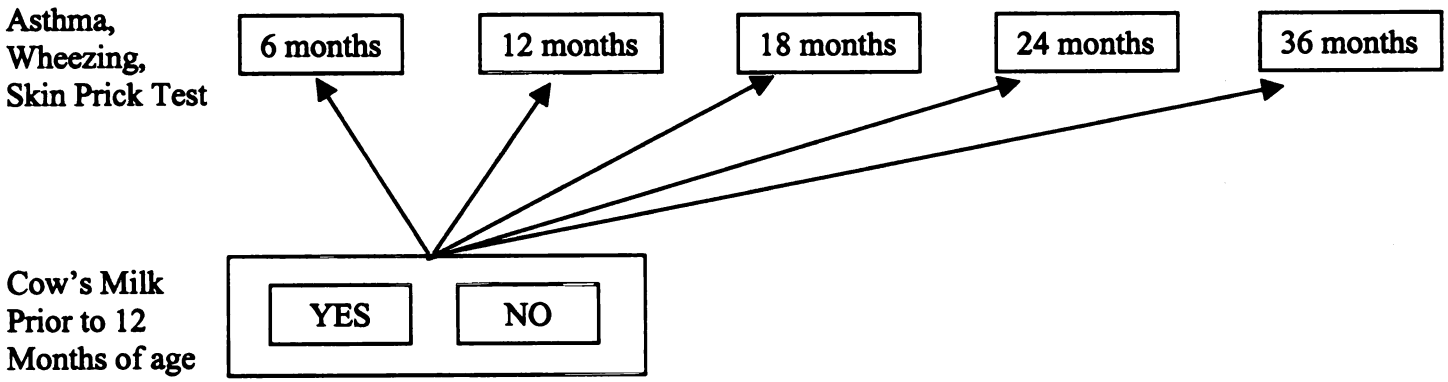


Figure 6: Modeling strategy for the medical recommendation models.

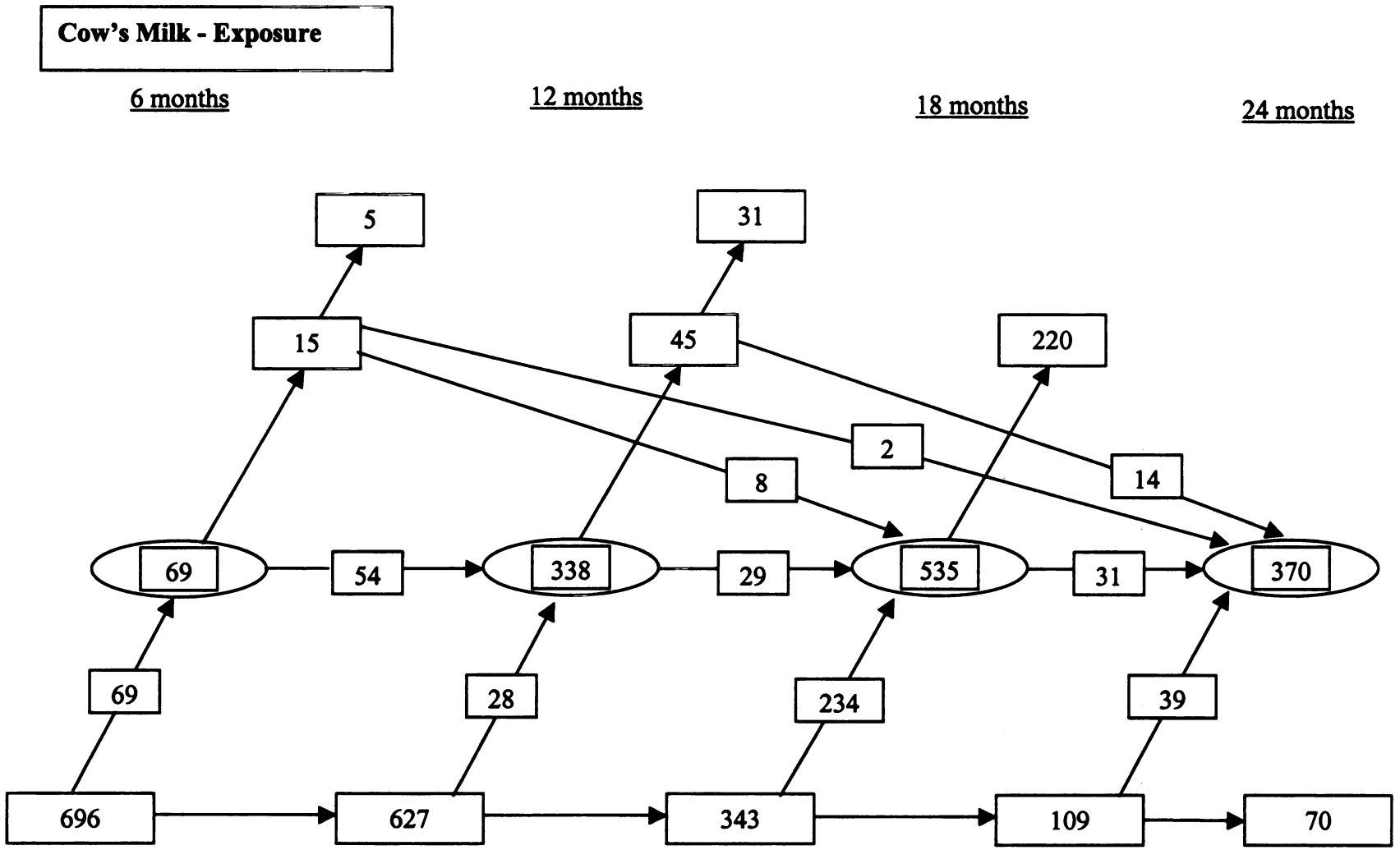


Figure 7: Occurrence of cow's milk consumption in the SPACE study population over the entire study period.

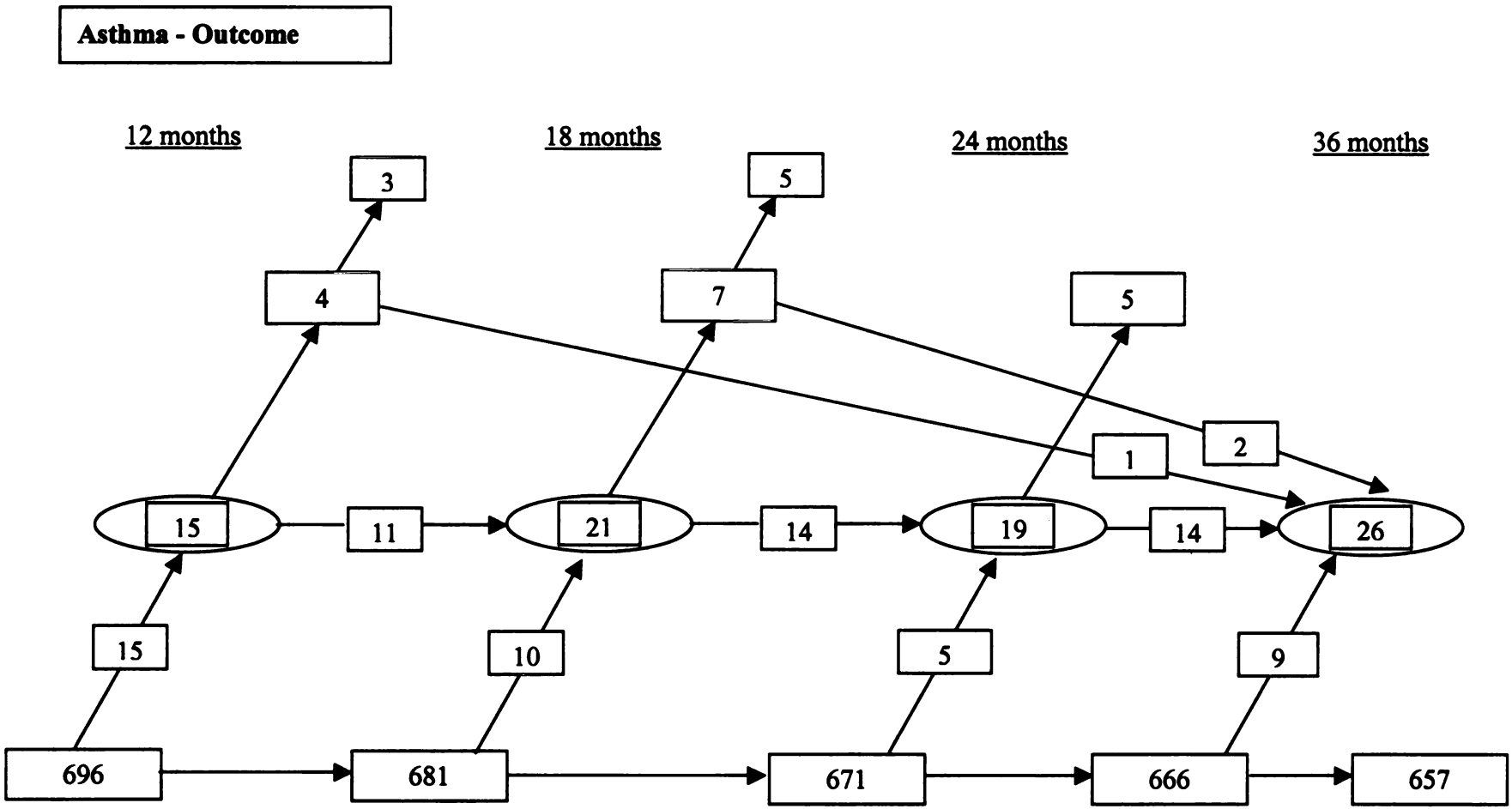


Figure 8: Occurrence of asthma in the SPACE study population over the entire study period.

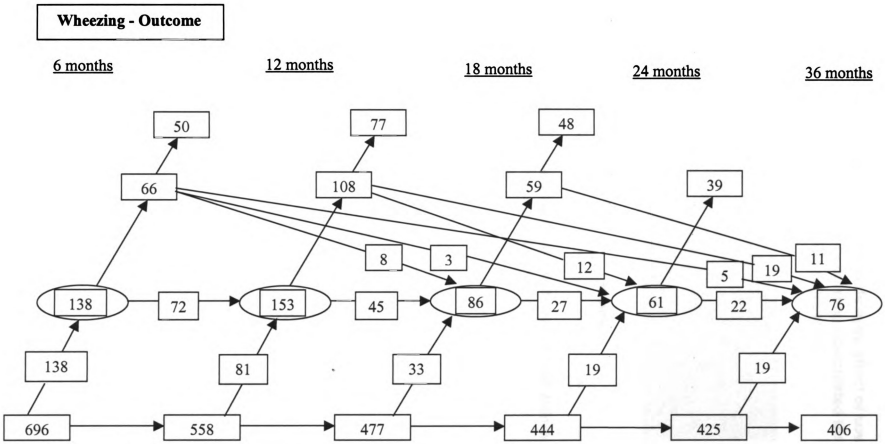


Figure 9: Occurrence of wheezing in the SPACE study population over the entire study period.

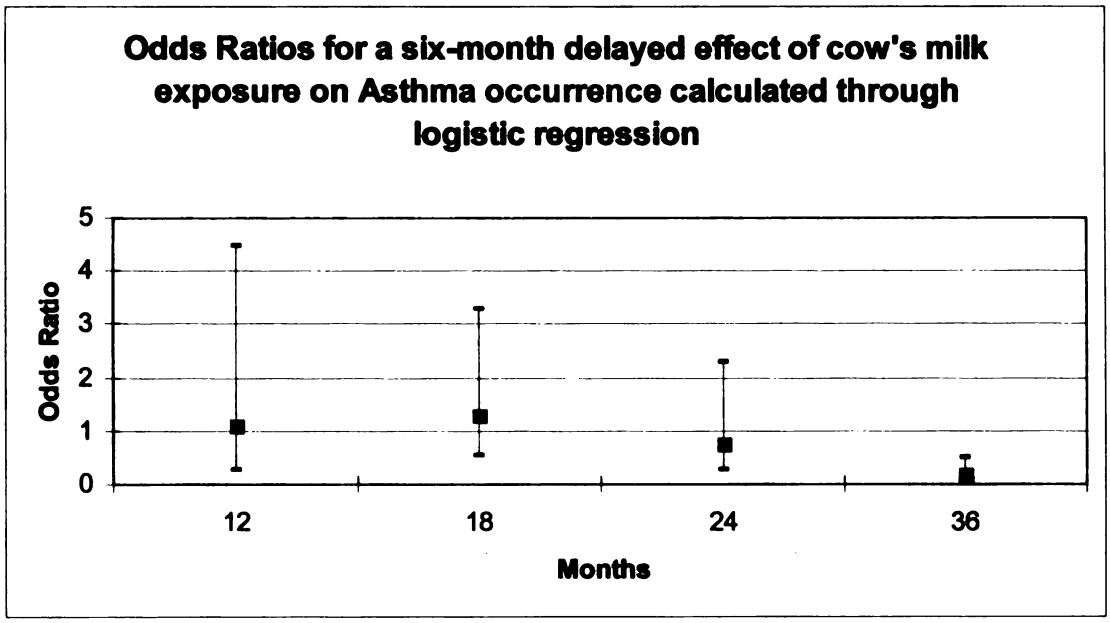


Figure 10: Odds ratios for the delayed effects models, with asthma as the outcome, for each separate time interval.

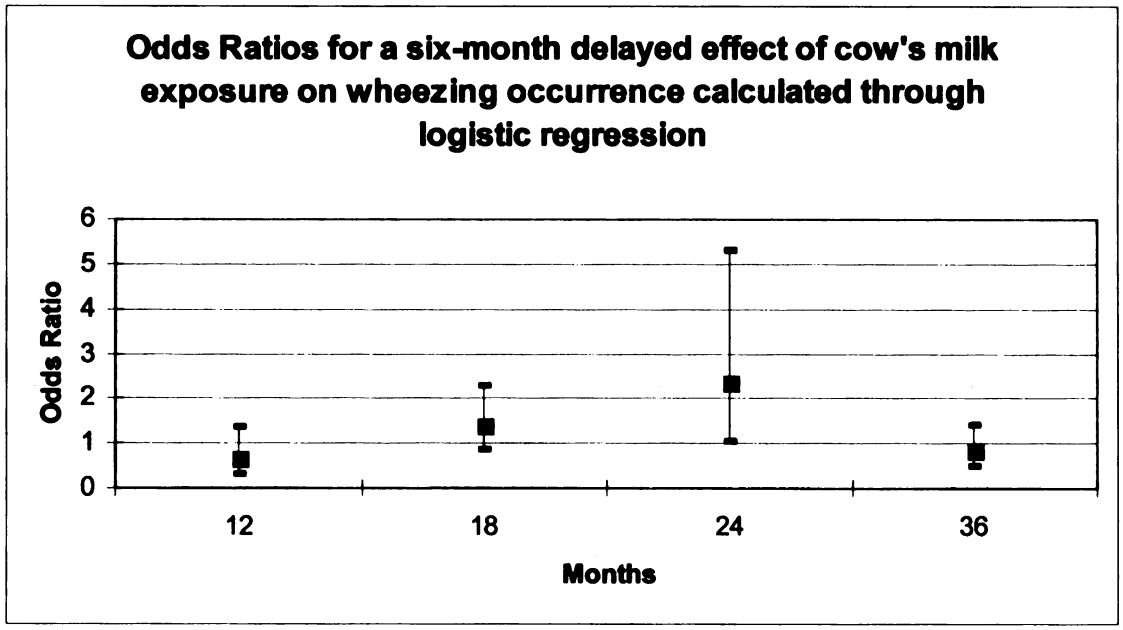


Figure 11: Odds ratios for the delayed effects models, with wheezing as the outcome, for each separate time interval.

REFERENCES

References

1. Kuehr, J., Frischer, T., Karmaus, W., et al. Clinical atopy and associated factors in primary-school pupils. Allergy, 1992. **47**(6): p. 650-655.

2. Busse, W.W. and Lemanske, R.F. Asthma. N Engl J Med, 2001. **344**(1): p. 350-362.

3. Weiss, S.T. Epidemiology and heterogeneity of asthma. Ann Allergy Asthma Immunol, 2001. **87**(1 Suppl 1): p. 5-8.

4. Burrows, B., Martinez, F., Halonen, M., et al. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med, 1989. **320**(1): p. 271-277.

5. Klink, M., Cline, M., Halonen, M., et al. Problems in defining the normal limits for serum IgE. J Allergy Clin Immunol, 1990. **85**(1): p. 440-444.

6. Ownby, D.R. Clinical significance of immunoglobulin E, in *Allergy: principles and practice*, E. Middleton, C.E. Reed, E.F. Ellis, et al., Editors. 1998, C V Mosby, Inc: St Louis, MO. p. 770-782.

7. Warner, J.O. Bronchial hyperresponsiveness, atopy, airway inflammation, and asthma. Pediatr Allergy Immunol, 1998. **9**(2): p. 56-60.

8. Beasley, R., Crane, J., Lai, C.K., et al. Prevalence and etiology of asthma. J Allergy Clin Immunol, 2000. **105**: p. S466-S472.

9. Taylor, W.R. and Newacheck, P.W. Impact of childhood asthma on health. Pediatrics, 1992. **90**(5): p. 657-662.

10. National Center for Environmental HealthE. Asthma's Impact on Children and Adolescents. 2003, Centers for Disease Control and Prevention.

11. Bracken, M.B., Belanger, K., Cookson, W.O., et al. Genetic and perinatal risk factors for asthma onset and severity: a review and theoretical analysis. Epidemiol Rev, 2002. **24**(2): p. 176-89.

12. Guilbert, T. and Krawiec, M. Natural history of asthma. Pediatr Clin North Am, 2003. **50**(3): p. 523-38.

13. Cantani, A. and Micera, M. Epidemiology of atopy in 220 children. Diagnostic reliability of skin prick tests and total and specific IgE levels. Minerva Pediatr, 2003. **55**(2): p. 129-37, 138-42.

14. Ruiz, R.G., Kemeny, D.M., and Price, J.F. Higher risk of infantile atopic dermatitis from maternal atopy than from paternal atopy. Clin Exp Allergy, 1992. **22**(8): p. 762-6.

15. Croner, J., Kjellman, N.I.M., Eriksson, B., et al. IgE screening in 1701 newborn infants and the development of atopic disease during infancy. Arch Dis Child, 1982. **57**: p. 364-368.

16. Businco, L., Marchetti, F., Pellegrini, G., et al. Predicitive values of cord blood IgE levels in 'at-risk' newborn babies and influence of type of feeding. Clin Allergy, 1983. **13**: p. 503-508.

17. Merrett, T.G., Burr, M.L., Butland, B.K., et al. Infant feeding and allergy: 12-month prospective study of 500 babies born into allergic families. Ann Allergy, 1988. **61**: p. 13-20.

18. Ruiz, R.G.G., Richards, D., Kemeny, D.M., et al. Neonatal IgE: a poor screen for atopic disease. Clin Exp Allergy, 1991. **21**: p. 467-472.

19. Hide, D.W., Arshad, S.H., Twiselton, R., et al. Cord serum IgE:an insensitive method for prediction of atopy. Clin Exp Allergy, 1991. **21**: p. 739-743.

20. Wright, A.L. and Taussig, L.M. Lessons from long-term cohort studies. Childhood asthma. Eur Respir J Suppl, 1998. **27**: p. 17s-22s.

21. Dodge, R.R. and Burrows, B. The prevalence and incidence of asthma and asthma-like symptoms in a general population sample. Am Rev Respir Dis, 1980. **122**(4): p. 567-575.

22. Weiss, S.T., Tosteson, T.D., Segal, M., et al. Effects of asthma on pulmonary function in children: a longitudinal population-based study. Am Rev Respir Dis, 1992. **145**: p. 58-64.

23. Anderson, H.R., Pottier, A.C., and Strachan, D.P. Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. Thorax, 1992. **47**: p. 537-542.

24. Peat, J.K., Tovey, E., Toelle, B.G., et al. House dust mite allergens. A major risk factor for childhood asthma in Australia. Am J Respir Crit Care Med, 1996. **153**: p. 141-146.

25. Platts-Mills, T.A. Dust mite allergens and asthma. A worldwide problem. J Allergy Clin Immunol, 1989. **83**: p. 416-427.

26. Wahn, U., Lau, S., Bergmann, R., et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. J Allergy Clin Immunol, 1997. **99**: p. 763-769.

27. Burr, M.L., Merrett, T.G., Dunstan, F.D., et al. The development of allergy in high-risk children. Clin Exp Allergy, 1997. **27**(11): p. 1247-53.

28. Schonberger, H.J., Maas, T., Dompeling, E., et al. Environmental exposure reduction in high-risk newborns: where do we start? Ann Allergy Asthma Immunol, 2003. **91**(6): p. 531-8.

29. Ram, F.S., Ducharme, F.M., and Scarlett, J. Cow's milk protein avoidance and development of childhood wheeze in children with a family history of atopy. Cochrane Database Syst Rev, 2002(3): p. CD003795.

30. Chandra, R.K. Prospective studies of the effect of breast feeding on incidence of infection and allergy. Acta Paediatr Scand, 1979. **68**(5): p. 691-4.

31. Kaufman, H.S. and Frick, O.L. The development of allergy in infants of allergic parents: a prospective study concerning the role of heredity. Ann Allergy, 1976. **37**(6): p. 410-5.

32. Chandra, R.K. Five-year follow-up of high-risk infants with family history of allergy who were exclusively breast-fed or fed partial whey hydrolysate, soy, and conventional cow's milk formulas. J Pediatr Gastroenterol Nutr, 1997. **24**(4): p. 380-8.

33. Johnstone, D.E. and Dutton, A.M. Dietary prophylaxis of allergic disease in children. N Engl J Med, 1966. **274**(13): p. 715-9.

34. Glaser, J. and Johnstone, D.E. Prophylaxis of allergic disease in newborn. JAMA, 1953. **153**: p. 620.

35. Rhodes, H.L., Sporik, R., Thomas, P., et al. Early life risk factors for adult asthma: a birth cohort study of subjects at risk. J Allergy Clin Immunol, 2001. **108**(5): p. 720-5.

36. Tikkanen, S., Kokkonen, J., Juntti, H., et al. Status of children with cow's milk allergy in infancy by 10 years of age. Acta Paediatr, 2000. **89**(10): p. 1174-80.

37. Novembre, E. and Vierucci, A. Milk allergy/intolerance and atopic dermatitis in infancy and childhood. Allergy, 2001. **56 Suppl 67**: p. 105-8.

38. Host, A., Halken, S., Jacobsen, H.P., et al. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. Pediatr Allergy Immunol, 2002. **13 Suppl 15**: p. 23-8.

39. Host, A. Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol, 2002. **89**(6 Suppl 1): p. 33-7.

40. Baena-Cagnani, C.E. and Teijeiro, A. Role of food allergy in asthma in childhood. Curr Opin Allergy Clin Immunol, 2001. **1**(2): p. 145-9.

41. Arshad, S.H., Tariq, S.M., Matthews, S., et al. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. Pediatrics, 2001. **108**(2): p. E33.

42. Van Asperen, P.P., Kemp, A.S., and Mellis, C.M. Relationship of diet in the development of atopy in infancy. Clin Allergy, 1984. **14**(6): p. 525-32.

43. Hattevig, G., Kjellman, B., Sigurs, N., et al. Effect of maternal avoidance of eggs, cow's milk and fish during lactation upon allergic manifestations in infants. Clin Exp Allergy, 1989. **19**(1): p. 27-32.

44. Poysa, L., Korppi, M., Remes, K., et al. Atopy in childhood and diet in infancy. A nine-year follow-up study. I. Clinical manifestations. Allergy Proc, 1991. **12**(2): p. 107-11.

45. Zeiger, R.S. and Heller, S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. J Allergy Clin Immunol, 1995. **95**(6): p. 1179-90.

46. Baker, S.S., Cochran, W.J., Greer, F.R., et al. American Academy of Pediatrics: Hypoallergenic Infant Formulas. Pediatrics, 2000. **106**(2): p. 346-349.

47. Lindfors, A.T.B., Danielsson, L., Enocksson, E., et al. Allergic symptoms up to 4-6 years of age in children given cow milk neonatally. BMJ, 1992. **47**: p. 207-211.

48. Wijga, A.H., Smit, H.A., Kerkhof, M., et al. Association of consumption of products containing milk fat with reduced asthma risk in pre-school children: the PIAMA birth cohort study. Thorax, 2003. **58**(7): p. 567-72.

49. Riedler, J., Braun-Fahrlander, C., Eder, W., et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet, 2001. **358**(9288): p. 1129-33.

50. Halmerbauer, G., Gartner, C., Schierl, M., et al. Study on the Prevention of Allergy in Children in Europe (SPACE): allergic sensitization at 1 year of age in a controlled trial of allergen avoidance from birth. Pediatr Allergy Immunol, 2003. **14**(1): p. 10-7.

51. Schuetze, G., Storm van's Gravesande, K., Sparhold, S., et al. Comparison between serial skin-prick tests and specific serum immunoglobulin E to mite allergens. Pediatr Allergy Immunol, 1999. **10**(2): p. 138-42.

52. SAS Institute Generalized Estimating Equations. 2004. (Accessed April 2004) http://v8doc.sas.com.

53. SAS Institute SAS/STAT Software. 2001, SAS Institute, Inc.: Cary, NC.

54. Mattes, J. and Karmaus, W. The use of antibiotics in the first year of life and development of asthma: which comes first? Clin Exp Allergy, 1999. **29**(6): p. 729-32.

55. Cullinan, P., Harris, J., Mills, P., et al. Early prescriptions of antibiotics and the risk of allergic disease in adults: a cohort study. Thorax, 2004. **59**(1): p. 11-5.

56. Greenland, S. and Brumback, B. An overview of relations among causal modelling methods. Int J Epidemiol, 2002. **31**(5): p. 1030-7.

57. American Academy of Pediatrics Committee on Nutrition: The use of whole cow's milk in infancy. Pediatrics, 1992. **89**(6): p. 1105-1109.

58. Grummer-Strawn, L.M. and Mei, Z. Does breastfeeding protect against pediatric overweight? Analysis of longitudinal data from the Centers for Disease Control and Prevention Pediatric Nutrition Surveillance System. Pediatrics, 2004. **113**(2): p. e81-6.

59. Sherriff, A., Peters, T.J., Henderson, J., et al. Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3(1/2) years. Int J Epidemiol, 2001. **30**(6): p. 1473-84.

60. Asero, R., Mistrello, G., Roncarolo, D., et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. Allergy, 2002. **57**(10): p. 900-6.

61. Azofra, J. and Lombardero, M. Limpet anaphylaxis: cross-reactivity between limpet and house-dust mite Dermatophagoides pteronyssinus. Allergy, 2003. **58**(2): p. 146-9.

62. Ragno, V., Giampietro, P.G., Bruno, G., et al. Allergenicity of milk protein hydrolysate formulae in children with cow's milk allergy. Eur J Pediatr, 1993. **152**(9): p. 760-2.

63. Salem, N., Simopoulos, A.P., Galli, C., et al. eds. Fatty acids and lipids from cell biology to human disease. Vol. 31(suppl). 1996. S1-S326.

64. National Dairy Council. Newer Knowledge of Dairy Foods: Milk. 2003. (Accessed March 2003) http://www.nationaldairycouncil.org.

65. Woods, R.K., Raven, J.M., Walters, E.H., et al. Fatty acid levels and risk of asthma in young adults. Thorax, 2004. **59**(2): p. 105-110.

66. Devereux, G., Barker, R.N., and Seaton, A. Antenatal determinants of neonatal immune responses to allergens. Clin Exp Allergy, 2002. **32**(1): p. 43-50.

67. Black, P.N. and Sharpe, S. Dietary fat and asthma: is there a connection? Eur Respri J, 1997. **10**: p. 6-12.

68. Yu, G. and Bjorksten, B. Serum levels of phospholipid fatty acids in mothers and their babies in relation to allergic disease. Eur J Pediatr, 1998. **157**: p. 298-303.

69. Friedman, Z., Danon, A., Lamberth, E.L., et al. Cord blood fatty acid composition in infants and in their mothers during the third trimester. J Pediatr, 1978. **92**: p. 461-466.

70. Hahn, P. Lipid metabolism and nutrition in the prenatal and postnatal period, in *Nutrition and development*, W. Winick, Editor. 1972, Wiley: New York. p. 99-134.

71. Monique, D.M.A.L., Adriana, C.V.H., Arnold, D.M.K., et al. Maternal fatty acids patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Br J Nutr, 1995. **74**: p. 55-68.

72. Nettleton, J.A. Are n-3 fatty acids essential nutrients for fetal and infant development? J Am Diet Assoc, 1993. **93**: p. 58-64.

73. Zeyda, M., Szekeres, A.B., Saemann, M.D., et al. Suppression of T Cell Signaling by Polyunsaturated Fatty Acids: Selectivity in Inhibition of Mitogen-Activated Protein Kinase and Nuclear Factor Activation. The Journal of Immunology, 2003. **170**: p. 6033-6039.

74. Van der Pouw Kraan, T.C., Van der Zee, J.S., Boeije, L.C., et al. The role of IL-13 in IgE synthesis by allergic asthma patients. Clin Exp Immunol, 1998. **111**: p. 129.

75. Fallon, P.G., Jolin, H.E., Smith, P., et al. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. Immunity, 2002. **17**: p. 7.