Early life protein intake: food sources, correlates, and tracking across the first 5 years of life

Citation of final article:

This is the accepted manuscript.

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The final version of this article, as published in volume 117 of Journal of the Academy of Nutrition and Dietetics, is available online from:
http://www.dx.doi.org/10.1016/j.jand.2017.03.016

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http://hdl.handle.net/10536/DRO/DU:30100217
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Article Type: Original Research

Keywords: infant; child; protein intakes; food sources; tracking

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Abstract: Background: High consumption of protein has been associated with accelerated growth and adiposity in early childhood.
Objective: This study aims to describe intake, food sources, correlates, and tracking of protein in young children.
Design: Secondary analysis of Melbourne Infant Feeding Activity and Nutrition Trial (InFANT); dietary data were collected using three 24-hour dietary recalls at ages 9 and 18 months, 3.5 and 5 years were used.
Participants/setting: First-time mothers and their parents (n=542) participated in an 18-month intervention to prevent childhood obesity and the cohort was followed-up with no intervention when children were age 3.5 and 5 years.
Main outcome measures: Protein intake, food sources, correlates, and tracking of protein.
Statistical analyses performed: Child and maternal correlates of protein intake were identified using linear regression and tracking of protein intake was examined using Pearson correlations of residualised protein scores between time points.
Results: Mean (SD) protein (g) intake was 29.7(11.0), 46.3(11.5), 54.2(13.8) and 60.0(14.8) at 9 and 18 months, 3.5 and 5 years, respectively. Protein intakes at all ages were two to three times greater than age appropriate Australian recommendations. The primary source of protein at 9 months was breast/formula milk. At later ages, the principal sources were milk/milk products, breads/cereals and meat/meat products. Earlier breastfeeding cessation, earlier introduction of solids, high dairy milk consumption (≥500ml) and high maternal education were significant predictors of high protein intake at various times (P<0.05). 'Slight' tracking was found for protein intakes at 9, 18 months and 5 years (r=0.16-0.21, P<0.01).
Conclusions: This study provides unique insights into food sources and correlates of young children's high protein intakes, and confirms that early protein intakes track slightly up to age 5. These finding have potential to inform nutrition interventions and strategies to address high protein intakes and protein-related obesity risk.
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Short Title: Protein intakes across early life

Word count for abstract: 296, for text: 4366

Authorship

KJC led the Melbourne InFANT program, collected the data, conceptualized the present study, guided the statistical analysis, drafted and edited the manuscript. GA conducted statistical analysis and edited the manuscript. JMZ contributed to interpretation of results and edited the manuscript. SAM conceptualized the study, guided statistical analysis, contributed to interpretation of results, and edited the manuscript. All authors critically reviewed and approved the final manuscript.
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Introduction

Overweight and obesity in early childhood remain highly prevalent, and a broad range of early-life risk factors have been identified. These include modifiable risk factors such as maternal pre-pregnancy BMI and excess maternal gestational weight gain, both correlated with increased gestational weight for age and subsequent risk of adiposity. Not surprisingly, early childhood diet is also an important risk factor. Contemporary evidence focuses on the associations between early life protein intakes and rapid infant growth (an important risk factor for subsequent adiposity across life), with the likely pathway the stimulating effect of protein on insulin-like growth factor (IGF-1).

A recently published UK study of >2000 infants reports that protein intake during infancy was predictive of body weight gain up to age 5 years. In that study a one percent increase in the percentage energy from protein was associated with a 0.04 increase in a child’s body mass index (BMI) and a 52 gram greater weight at any time point between age 21 months and 5 years. Further research indicates that higher protein intakes in infancy are associated with a 2 to 3 fold increase in the risk of obesity in adolescence and young adulthood. A recent systematic review provides additional confirmation that increased protein intakes (15-20% of total energy intake) in early childhood is associated with an increased risk of obesity in later life and that the upper level of a healthy intake remains to be determined.

In addition to the amount of protein consumed, the food source of protein (e.g. animal versus vegetable) in the diet may be an important determinant of rapid infant growth. Thorisdottir reports in an Icelandic study of infants that high intakes of animal protein, particularly dairy-based products, is associated with increased BMI in infants at 12 months of age. In addition, the timing of the introduction of higher protein intakes appears likely to be an important determinant of growth. Mihrshahi et al identified infant formula consumption as one of only two modifiable predictors (the other being parent feeding infants to a schedule) of rapid weight gain (birth to 4-7 months) in a sample of Australian infants and hypothesised that this growth was likely to have been stimulated by the increased protein intakes associated with formula feeding. In an older sample of children, Günther et al suggest that the transition from breast milk or infant formula to “family foods” at around 12 months is a critical phase for increased protein intake and subsequent childhood obesity.
Despite the potential contribution of high protein intakes to rapid infant growth and subsequent childhood obesity, many protein-dense foods are also nutrient-dense, and in some instances may be a child’s principal source of key nutrients such as calcium and iron.\textsuperscript{14,15} It is important therefore to ensure that any proposed reduction in protein intake will not compromise other essential nutrients. Identifying food sources of protein (and sources of other nutrients) is necessary to inform these considerations. In addition, describing modifiable correlates of high protein intakes in early life will identify appropriate targets for interventions seeking to reduce early protein intake. Given there is evidence that children’s diets track over time,\textsuperscript{16} it is important also to understand whether early protein intakes set a trajectory for higher protein intake across childhood.

Therefore the aims of this study were to describe dietary protein intakes, food sources, and modifiable child and maternal correlates of protein intake in a cohort of young Victorian (Australian) children over the first five years of life (9 and 18 months; 3.5 and 5 years). In addition, this study examines tracking of protein intake across these early years.

\section*{Materials and methods}

\textit{Study design and participants}

The Melbourne Infant Feeding Activity and Nutrition Trial (InFANT) Program was a cluster randomized controlled trial, involving first-time mothers attending parents’ groups, from when their infants were three to 20 months of age. This lifestyle intervention was conducted in 2008-2010 within Melbourne, Australia (population \~4 million), and the cohort was followed-up (no further intervention) when children were approximately 3.5 and 5 years of age. Primary aims of the Melbourne InFANT Program focused on reducing a range of children’s obesity-risk behaviors; there was no focus on protein consumption at that time and no difference in protein consumption was observed between trial arms (data not shown). The study design has been previously reported.\textsuperscript{17,18} Eighty six percent of eligible parents consented to participate (n=542). The Melbourne InFANT Program was approved by the Deakin University Human Research Ethics Committee (ID number: EC 175-2007) and the Victorian Government Department of Human Services, Office for Children, Research Coordinating Committee (Ref: CDF/07/1138). This study was deemed exempt under federal regulation 45 46.101 (b) CFR.

As there were no differences at any time in protein intakes between intervention and control group children, data for this paper has been pooled. We present data from children at approximately nine and 18- months; 3.5 and 5 years of age, herein referred to as time two (T2), three (T3), four (T4)
and five (T5) for consistency with other publications arising from these data. As outlined in Figure 1, data were excluded for children from non-first-time mothers (n=14) and those missing key baseline maternal variables (mother’s marital status, country of birth, education, employment, age, BMI) or missing data on age of breastfeeding cessation and starting solids (n=76). A total of 86 participants were excluded (some participants were excluded on more than one criteria), leaving an initial sample of 456 children. From this sample, further time point-specific exclusions were made to be consistent with our previously published data from this cohort. Children aged younger than seven months or older than 11 months of age were excluded from the T2 sample (n=14), while children younger than 16 months or older than 20 months were excluded from the T3 sample (n=32). Additionally, children lost to follow-up since baseline (T2 n=2; T3 n=14; T4 n=122; T5 n=123), children with fewer than three days of dietary recalls (T2 n=61; T3 n=89; T4 n=94; T5 n=97), children with outlier (+/- 3SD) total energy intakes (T2 n=1; T3 n=4; T4 n=3; T5 n=1), and children with missing BMI z-score data (T2 n=0; T3 n=15; T4 n=4; T5 n=0) were excluded from their respective time-specific sample. After exclusion of children who met one or more of these criteria the final analysis samples were n=381 for T2, n=321 for T3, n=237 for T4, and n=235 for T5.

Maternal factors

Self-administered paper-based questionnaires were provided to parents at the recruitment meeting and returned to program staff at the first InFANT Program session. These provided demographic and socioeconomic data at baseline (T1: children aged 3 months) including: maternal age, maternal employment, education level and country of birth. Maternal employment was dichotomized as not currently employed in paid work or currently employed full/part-time. Maternal education level was dichotomized as university educated or non-university educated. Country of birth was classified as born in Australia or Overseas. Mothers self-reported their current height and their pre-pregnancy weight, and from these measures body mass index (BMI) (kg/m²) were calculated.

Child dietary intake and feeding behaviors

Children’s diet was assessed by nutritionists trained by the researchers (KJC, SAM) using standardized telephone-administered, 5-pass 24-hour recalls. Study-specific food measurement books aided parents’ estimation of infants’ food consumption. Three non-consecutive days of dietary data (including one weekend day) were collected via a purpose designed computer program facilitating reporting fidelity and consistency between interviewers. Overall, 96% of telephone calls were unscheduled. Nutrient intakes were evaluated using the 2007 AUSNUT food composition database. Consistent with previous studies, breastfeeding was recorded as minutes spent...
breastfeeding, and breastmilk quantity was estimated using a conversion factor of 10mls per minute up to a maximum of 100 mls at any one feed. If breastmilk was expressed, volumes estimated by carer report were utilized. Single questions assessed the age in weeks at which solids were introduced, and at which breastfeeding was ceased.

To determine the contributions of protein from food groups, the 1688 individual food items consumed by children in the InFANT Program were grouped before analysis using the standard food groupings in the AUSNUT 2007 food coding system developed by Food Standards Australia New Zealand. The mean values and standard deviations of protein (g) consumed and percentage contribution to total protein intake were then calculated for each food group to determine the food sources of protein. These food groupings are described in Supplementary Table 1. Protein density was reported as protein in grams per 1000 kilojoules consumed. Intakes of calcium and iron (mg/day) at each time point were calculated and along with protein intake were compared to Australian Estimated Average Requirement (EAR) and Recommended Dietary intake (RDI).

Milk intake was further classified to examine the source and quantity of milk consumed in early childhood. Children’s primary source of milk at T2 (child aged 9 months) was classified according to whether the major source was breast, formula, or dairy milk. Where children derived more than two-thirds (66.7%) of their milk from a given source, this was determined to be their primary source of milk. Children with less than two-thirds of their milk from a single source had their primary source classified as ‘mixed’. Only four children had dairy milk as their primary milk source at this age and thus these children were combined with the formula group as these milk sources are more similar in protein than they are to breastmilk. Therefore, three categories of primary milk source were created: breastmilk, formula/dairy, and mixed. Finally, milk intake at T2 (child aged 18 months) was categorized according to recommendations in the Australian Infant Dietary Guidelines, which specify that children at ages 1-2 should not consume more than 500mls of milk per day, based on concerns that milk will displace other foods and in turn limit food variety and nutrient adequacy.

Child anthropometrics

Children’s height/length and weight without clothes were measured by trained staff at each time point. Height/length was measured to 0.1cm using a calibrated measuring mat (Seca 210, Seca Deutschland, Germany) or portable stadiometer (Invicta IPO955, Oadby, Leicester). Weight was measured to 10 grams using calibrated infant digital scales (Tanita 1582, Tokyo, Japan). The average of two measures was used in analyses. BMI (kg/m2) and BMI z-scores (zBMI) were calculated using WHO sex-specific BMI-for-age growth charts.
Descriptive statistics were used to summarize protein intake, food group contributions to total energy and protein intakes at all time points. For each of the time points between T2 and T5, a separate random-intercepts linear regression model, with participants nested within mothers’ group, was conducted to examine correlates of protein intake. The following predictor variables: child age, child sex, age of breastfeeding cessation, age when child began eating solids; and mothers’ pre-pregnancy BMI, age at baseline, whether working at baseline, education, and whether they were born in Australia were examined and were included simultaneously in the same model at each time point. All models also included child zBMI and intervention status as a covariate. Therefore, the associations of predictor variables with protein intake at each time point are independent of each other and of child zBMI. In the T2 model (children aged 18 months), children’s primary milk source (as described above) was also included as a predictor. In the T3 model, children’s consumption of more than 500mls of milk/day was also included.

To assess tracking of protein intakes across the time points, residualised protein intake scores were created by regressing children’s protein intake on their age, sex and total energy intake. Tracking of protein intake was assessed by examining Pearson product-moment correlations of these residualised protein scores between the different time points. This method has been widely used to assess the tracking of nutrient intakes. Protein intake at each time point was near normally distributed, and statistical assumptions were met for all models tested. Interpretation of these correlation coefficients was based on the following guidelines: <0.3 for slight tracking, 0.3–0.6 for moderate tracking and >0.6 for high tracking, consistent with our previous approach. Statistical analyses were conducted using Stata version 14.0 (StataCorp, TX) with statistical significance set at p<0.05.

Results

Sample characteristics

Among the initial sample (n=456), 53.3% of children were girls, 55.3% of mothers had a university education, 79.6% of mothers were born in Australia, and 8.8% of mothers were working. Mothers’ mean pre-pregnancy BMI and age was 24.3 (SD=5.1) kg/m² and 32.2 (SD=4.1) years respectively.

Protein intakes

Protein intakes (SD) grams/day and grams per kilogram body weight at each time point are presented in Table 1. Energy intakes (kJ), protein density (g/1000kJ) and the percentage of total
energy from protein are also presented. At T2, 95% of children exceeded the Australian
Recommended Dietary Intake (RDI) of 14g/d protein, and 99.4% of children exceeded the RDI of
1.08g/kg. At all other time points, these age-specific RDIs were exceeded by all children.

Iron and calcium intakes

Calcium intakes in this sample substantially exceeded the Australian Nutrient Reference Values
(Table 1). At 18 months and 3.5 years of age, mean calcium intakes were 759 (SD 220) mg and 732
(SD 239) mg respectively, thus more than double the Australian Estimated Average Requirement
(EAR) of 360mg and approximately 30% greater than the RDI (500mg) for 1-3 year olds. At 5
years of age, calcium intake was 767 (SD 271) mg which was much closer to both the EAR and RDI
for 4-8 year olds (520 and 700mg respectively).

While iron intakes were 20-50% greater than the EARs for equivalent ages (7mg at 0-6months; 4mg
at 1-3 years; 4mg at 4-8years), these intakes did not meet the RDIs at any age (11mg at 0-6months;
9 mg at 1-3 years, 10mg at 4-8years).

Food sources of protein

The primary food sources of protein changed across the first five years of life (Table 2). At T2 (age
9 months), infant formula was the predominant source of protein followed by milk and milk
products, breads/cereals, meat and poultry, and breast milk. Further analyses of the sources of milk
(breast milk, infant formula, dairy milk) at T2 showed that infant formula, and to a lesser degree
dairy milk were the predominant milk sources (providing more than 66.7% of all milk consumed)
for nearly two-thirds of infants at age 9 months (data not shown). At all other ages (age 18 months,
3.5 and 5 years), milk and milk products were the primary sources of protein, followed by
breads/cereals, meat and poultry and together these three groups of food contributed 66-71% of
total protein intake. Discretionary foods, such as cake/biscuits and processed meat were also major
protein sources at ages 3.5 and 5 years, each accounting for approximately 5% of total protein
intake by 5 years.

While protein intakes from breads/cereals at T3, T4 and T5 were more than double the consumption
at T2, the percentage of total protein from all milk decreased across ages from 47% at T2 to 26% at
T5, and the percentage of total protein from animal sources (meat, poultry, egg, fish and processed
meat all combined) increased from T2 and T3 (21%) to T4 and T5 (27-28%). Protein intake from
fruit and vegetables was consistent among all ages, providing 6-8% of total protein. Similarly, the
percentage of total protein from fish (3-4%) was consistent across all ages.
Correlates of protein density

Feeding choice (that is, the choice of breastfeeding or formula feeding) and the timing of the introduction of solids were all significantly correlated with protein density (Table 3). At T2, (child 9 months of age) both the earlier introduction of solids, and the primary milk source being formula/dairy or mixed (as opposed to the primary milk source being breastmilk) were associated with consuming significantly more protein per 1000kJ. At T3 (child aged 18 months), earlier cessation of breastfeeding, and the consumption of more than 500mls/day of dairy milk was significantly associated with higher protein density, while at T4 (child aged 3.5 years), later introduction of solids was associated with higher protein density.

At T4 (child age 3.5 years), a number of baseline maternal characteristics were also significantly associated with protein density, such that children of more highly educated mothers consumed diets with higher protein density, and higher maternal pre-pregnancy BMI and maternal employment at baseline were associated with lower protein density. No child or maternal characteristics were found to be associated with protein density at T5 (child age 5 years)

Tracking of protein intakes

Residualised protein intake at T2 was significantly associated with T3 ($r=0.16$, $p=0.007$) and T5 ($r=0.18$, $p=0.006$) scores, but not T4 scores ($r=-0.02$, $p=0.669$). Residualised protein intake at T3 was associated with both T4 ($r=0.24$, $p=.001$) and T5 scores ($r=0.21$, $p=.003$), while T4 and T5 scores were also significantly correlated ($r=0.26$, $p<.0005$).

Discussion

High protein intakes in young children are concerning given evidence of associations with early rapid growth, and in turn adiposity and its associated sequelae across the life course. Describing protein intakes across childhood, food sources of protein and the correlates of protein intakes provides insights which may enable public health action to address this important, and likely modifiable, predictor of child adiposity.

In this cohort of children, protein intakes were consistently and substantially in excess of existing recommendations. While mean protein intakes were double the RDI at 9 months of age, at all other ages, intakes were around three times the recommended intakes for similar ages, using both total...
grams per day and grams per kilogram of body weight. These findings are consistent with Australian national dietary data\textsuperscript{29} for 2-17 year olds, and with data internationally where reported intakes in infants and toddlers are 250-300\% greater than country-specific recommendations in the UK, Europe and the USA. \textsuperscript{30-32}

The current study elucidates the food sources of protein at several ages across early life, thus enabling identification of foods which may be targeted to reduce protein intakes in this life phase. Milk and milk products were the principle sources of protein across the first five years of life, peaking at 18 months of age when this food group provided nearly 40\% of all protein. This high consumption of milk and milk products was accompanied by the consumption of a range of other animal products: meats, poultry, fish, eggs and processed meats. While the relative contribution of each of these groups to total protein intakes varied across each time point, in total, meats, poultry, fish, eggs and processed meats were supplying around one quarter (23\%) of total protein consumed.

Consistent with milk and milk products, these animal protein foods also contribute important nutrients essential for healthy growth in childhood. In seeking to reduce protein intakes it is important that other key nutrients (e.g. calcium and iron) are not compromised. At all ages calcium intakes substantially exceeded the RDIs in this population and suggest a reduction in milk intake in the first three years of life is not likely to compromise children’s calcium intakes.

While it was not surprising to find that milk was the principal source of protein at T2 (9 months) a time when children are moving from a predominantly milk-based diet to family foods, it is important to note that infant formula was consumed by most infants at this time. Around half (46.7\%) of women reported they were breastfeeding when infants were nine months old, however many of these women frequently used a mixture of breast and formula feeding, thus increasing total protein intakes (as compared to breast feeding only). \textsuperscript{33}

At 18 months of age, breast, infant formula and toddler milk, each provided around 5\% of total milk consumed with dairy milk becoming the predominant milk source (average volume 335 ml, 85\% of all milk, data not shown). As previously reported\textsuperscript{14}, dairy milk was consumed by 90\% of 18 month olds and a quarter of these children consumed more than 500 ml per day. This high level of milk consumption is concerning as unmodified cows’ milk is a poor source of iron and is likely to displace potentially iron-rich foods, such as red meat. Indeed, this level of cow’s milk consumption in the first two years of life has been associated with substantially lower ferritin concentrations. \textsuperscript{34,35}

Furthermore, evidence suggests high intake of animal, especially dairy protein intake at 12 months is predictive of an unfavourable body composition in childhood. \textsuperscript{36}

Meat and meat products, particularly red meats are important sources of many nutrients and of
specific interest in early life are their contribution to heme iron intake. These nutrients are known to frequently be ‘at risk’ in this population. Previous analyses from the InFANT cohort identified that 33% of infants and 19% of toddlers were at risk of inadequate iron intakes, consistent with other research demonstrating that children aged 4-24 months did not consume enough iron-rich meat sources with processed meat intake displacing other sources. Given the importance of iron for young children’s growth and development, it is important to acknowledge that a reduction in iron rich sources of red meat as a means by which to reduce total protein is not recommended. There is however, good rationale to encourage parents to replace processed meats, which in this sample provided around 5% of protein at ages 3.5 and 5 years, with fresh meats. Processed meats are relatively poor sources of iron and have also been identified in this cohort as important contributors of sodium.

The findings of modifiable correlates of protein intake in this study provide useful leads for how we might appropriately focus public health messaging regarding young children’s protein intakes. The finding that at 9 months of age protein density was correlated both to earlier introduction of solids, and the primary milk source being formula/dairy or mixed (as opposed to the primary milk source being breastmilk), reinforces the importance of health messaging promoting increased duration of exclusive breastfeeding; and relatedly, delaying the introduction of both infant formula and solid foods (to around 6 months of age). These messages are broadly consistent with dietary guidelines in Australia, the UK and the USA. The Australian Infant Feeding Guidelines recommend limiting consumption of dairy milk to 500mls per day. As outlined by Cameron et al early cessation of breastfeeding (and in turn introduction of infant formula) and early introduction of solids are socioeconomically patterned, with mothers of lower socioeconomic position (SEP) more likely to engage these behaviours. Given the significantly increased risk of adiposity in children from lower SEP environments, these findings provide further support for the need to address the barriers to the adoption of these key health behaviours in at risk groups.

A number of baseline maternal characteristics emerged as predictors of protein density in later childhood. Maternal university education (a proxy for increased socioeconomic advantage) was directly associated with a child’s increased protein density at age 3.5 years, while lower protein density at this age was associated with higher maternal pre-pregnancy BMI and maternal employment at baseline, both potential proxies for lower maternal SEP. These associations may reflect financial capacity to purchase foods rich in protein but relatively lower in energy (e.g. lean chicken rather than chicken nuggets, fresh/tinned fish rather than fish fingers, lean meat versus sausages). It is also possible that the societal trend positing protein as a ‘good’ food to be consumed in preference to carbohydrates may be more strongly adopted by women with higher
levels of education. These findings are important and may indeed represent barriers to reducing
children’s protein intakes in higher SEP families. No baseline child and maternal correlates of
protein density were observed at T5 (child age 5 years). This may reflect the dilution of association
over time and/or unmeasured confounders and predictors, likely to change substantially over a
child’s early life.

Tracking
‘Slight’ tracking of protein intake was observed between most age groups. Intake at 9 and 18
months predicted intake at 5 years, although the tracking coefficients were low. Similarly, previous
studies also found ‘slight’ tracking of nutrient intakes starts early in preschool age. Tracking of
protein intake reinforces the premise that dietary behaviours expressed in early life may herald the
maintenance of these behaviours across life.

Strength and limitations
To our knowledge this is the first study to describe longitudinally, the protein intakes, food sources,
modifiable correlates and protein tracking during infancy and early childhood and thus provides
comprehensive data that will inform opportunities for safe protein reductions in early childhood. A
large and well retained sample and the assessment of dietary intake via three 24-hour recalls are
important strengths of this study design. However, the present study included only healthy full-term
infants and is thus not representative of nutritionally at-risk populations, such as preterm infants and
infants with low birth weight for gestational age. It is also important however to acknowledge
potential sources of bias including our over representation of university educated women and the
potential for reporting bias when collecting self-report data. Mis-reporting of dietary intake may be
due to a number of factors, such as a reliance on memory and inadequate recall and social
desirability. However, mis-reporting was not assessed in our study as there is currently a lack of
consensus in the literature in terms of the appropriate methods for this age group, and is
complicated by larger day-to-day variation in their dietary intake. However, the energy intakes of
3.5 and 5 years olds in the present study were lower than their national representative counterparts.
Although misreporting may be present, it may have little influence on our findings pertaining to
protein sources and examining tracking as misreporting may influence the same individuals over
time.

Conclusions:
The present study shows that young children’s protein intake exceeds national
recommendations across the first five years of life and are consistent with increased risk for
childhood adiposity. The early cessation of breast feeding alongside the early introduction of infant formula and solid foods were all correlated with children’s higher protein intakes. These findings provide further support for targeting these behaviours as part of a comprehensive approach to child obesity prevention policy and practice. Importantly, health care and policy advice regarding reductions in protein should be carefully considered with evidence here supporting a reduction in milk and milk products, but not meats (with the exception of processed meats).
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Figure 1. Flow chart showing the number of participants included in analyses at each time point. * Participants excluded due to missing data on baseline maternal variables and/or missing data on child age of breastfeeding cessation or starting solids; ** Participants excluded due to incomplete three day dietary recall data, extreme outlier total energy intake, and/or missing zBMI.
542 Participating children

Non-first time mothers: 14
*Other exclusions: 72

Baseline:
456 initial sample

T2: 454 participating children
Outside age range (7-11 months): 14
**Other exclusions: 59
→ 381 included in analysis

T3: 442 participating children
Outside age range (16-20 months): 32
**Other exclusions: 89
→ 321 included in analysis

T4: 334 participating children
**Other exclusions: 97
→ 237 included in analysis

T5: 333 participating children
**Other exclusions: 98
→ 235 included in analysis
Table 1. Protein intakes (grams/day and grams per kilogram of body weight), protein density (g/1000kJ) and the proportion (%) of energy from protein, energy intake (kilojoule/day), calcium and iron intakes (milligram/day) at T2 (age 9 months), T3 (age 18 months), T4 (age 3.5 years), and T5 (age 5 years) in the Melbourne Infant Feeding Activity and Nutrition Trial (InFANT) Program.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>T2 (n=381)</th>
<th>T3 (n=321)</th>
<th>T4 (n=237)</th>
<th>T5 (n=235)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean(SD)</td>
<td>Mean(SD)</td>
<td>Mean (SD)</td>
<td>Mean(SD)</td>
</tr>
<tr>
<td>Protein intake (g/d)</td>
<td>29.7(11)</td>
<td>46.3(11.5)</td>
<td>54.2(13.8)</td>
<td>60(14.8)</td>
</tr>
<tr>
<td>Protein per bodyweight (g/kg)</td>
<td>3.4(1.2)</td>
<td>4.1(1)</td>
<td>3.3(0.8)</td>
<td>3(0.8)</td>
</tr>
<tr>
<td>Protein density (g/1000kJ)</td>
<td>8.3(1.7)</td>
<td>10.5(1.6)</td>
<td>10.2(1.6)</td>
<td>10.2(1.6)</td>
</tr>
<tr>
<td>% of total energy from protein</td>
<td>14.1(2.9)</td>
<td>17.8(2.7)</td>
<td>17.3(2.7)</td>
<td>17.4(2.8)</td>
</tr>
<tr>
<td>Energy intake (kJ/d)</td>
<td>3513(835)</td>
<td>4421(832)</td>
<td>5329(1093)</td>
<td>5878(1220)</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>643(251)</td>
<td>759(220)</td>
<td>732(239)</td>
<td>767(271)</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>9.3(4.8)</td>
<td>6.5(2.4)</td>
<td>7.2(2.4)</td>
<td>8.1(2.6)</td>
</tr>
</tbody>
</table>
Table 2. Main protein food sources at T2 (age 9 months), T3 (age 18 months), T4 (age 3.5 years), and T5 (age 5 years) in the Melbourne Infant Feeding Activity and Nutrition Trial (InFANT) Program (food sources that provided at least 2% of total protein at one or more time points presented).

<table>
<thead>
<tr>
<th>Food group</th>
<th>T2 (n=381)</th>
<th>T3 (n=321)</th>
<th>T4 (n=237)</th>
<th>T5 (n=235)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%a</td>
<td>%a</td>
<td>%a</td>
<td>%a</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>Protein provided (g)</td>
<td>Protein provided (g)</td>
<td>Protein provided (g)</td>
<td>Protein provided (g)</td>
</tr>
<tr>
<td>Breads/cereals</td>
<td>94 (7.7)</td>
<td>10.7 (8.9)</td>
<td>3.2 (2.9)</td>
<td>100 (4.4)</td>
</tr>
<tr>
<td>Pasta</td>
<td>43 (2.3)</td>
<td>1.1 (2.1)</td>
<td>0.3 (0.6)</td>
<td>57 (1.4)</td>
</tr>
<tr>
<td>Infant cereals/products</td>
<td>75 (4.9)</td>
<td>3.7 (5.0)</td>
<td>1 (1.3)</td>
<td>21 (0.4)</td>
</tr>
<tr>
<td>Cakes/biscuits</td>
<td>48 (2.6)</td>
<td>0.8 (1.6)</td>
<td>0.2 (0.5)</td>
<td>88 (1.4)</td>
</tr>
<tr>
<td>Fruit</td>
<td>96 (5.1)</td>
<td>2.6 (2.5)</td>
<td>0.7 (0.7)</td>
<td>99 (0.9)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>93 (5.5)</td>
<td>5.6 (5.3)</td>
<td>1.6 (1.6)</td>
<td>89 (3.1)</td>
</tr>
<tr>
<td>Breast milk</td>
<td>44 (21.3)</td>
<td>15.7 (12.0)</td>
<td>1.6 (2.0)</td>
<td>10 (1.4)</td>
</tr>
<tr>
<td>Infant/toddler formula</td>
<td>70 (26.4)</td>
<td>32.2 (14.1)</td>
<td>7.2 (6.8)</td>
<td>13 (2.6)</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td>91 (8.1)</td>
<td>10.2 (11.5)</td>
<td>4.7 (4.8)</td>
<td>98 (32.4)</td>
</tr>
<tr>
<td>Beef, veal, lamb</td>
<td>62 (4.2)</td>
<td>3.0 (4.2)</td>
<td>9.0 (11.4)</td>
<td>31.1 (4.4)</td>
</tr>
<tr>
<td>Poultry</td>
<td>55 (3.9)</td>
<td>2.3 (3.9)</td>
<td>8.1 (11.3)</td>
<td>2.8 (4.5)</td>
</tr>
<tr>
<td>Egg and egg dishes</td>
<td>11 (1.0)</td>
<td>0.3 (1.0)</td>
<td>0.5 (1.7)</td>
<td>0.2 (0.6)</td>
</tr>
<tr>
<td>Fish</td>
<td>33 (1.8)</td>
<td>0.8 (1.8)</td>
<td>3.2 (6.3)</td>
<td>1.0 (2.1)</td>
</tr>
<tr>
<td>Processed meats</td>
<td>13 (0.8)</td>
<td>0.2 (0.8)</td>
<td>0.6 (2.2)</td>
<td>0.2 (1.0)</td>
</tr>
<tr>
<td>Infant foods</td>
<td>58 (4.7)</td>
<td>3.7 (4.7)</td>
<td>2.7 (4.4)</td>
<td>0.7 (1.2)</td>
</tr>
</tbody>
</table>

*a percentage of children who consumed the food
Table 3. Child and maternal correlates of protein density (g/1000kJ) at T2 (age 9 months), T3 (age 18 months), T4 (age 3.5 years), and T5 (age 5 years) in the Melbourne Infant Feeding Activity and Nutrition Trial (InFANT) Program.

<table>
<thead>
<tr>
<th></th>
<th>T2 (n=381) (95% CI)</th>
<th>p</th>
<th>T3 (n=321) (95% CI)</th>
<th>p</th>
<th>T4 (n=237) (95% CI)</th>
<th>p</th>
<th>T5 (n=235) (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Child factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child age</td>
<td>0.29</td>
<td>(0.10, 0.47)</td>
<td>0.002</td>
<td>0.10</td>
<td>(-0.08, 0.27)</td>
<td>0.07</td>
<td>0.02</td>
<td>(-0.02, 0.16)</td>
</tr>
<tr>
<td>Child sex female (ref cat = male)</td>
<td>0.11</td>
<td>(-0.21, 0.42)</td>
<td>0.512</td>
<td>0.13</td>
<td>(-0.20, 0.46)</td>
<td>0.449</td>
<td>0.27</td>
<td>(-0.12, 0.66)</td>
</tr>
<tr>
<td>Child age at breastfeeding cessation</td>
<td>-0.03</td>
<td>(-0.08, 0.02)</td>
<td>0.267</td>
<td>-0.05</td>
<td>(-0.08, -0.02)</td>
<td>0.003</td>
<td>-0.02</td>
<td>(-0.06, 0.01)</td>
</tr>
<tr>
<td>Child age when started solids</td>
<td>-0.30</td>
<td>(-0.49, -0.12)</td>
<td>0.002</td>
<td>-0.01</td>
<td>(-0.23, 0.22)</td>
<td>0.951</td>
<td>0.36</td>
<td>(0.10, 0.62)</td>
</tr>
<tr>
<td>Primary milk source (ref = breastmilk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula/dairy</td>
<td>0.62</td>
<td>(0.03, 1.20)</td>
<td>0.038</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>1.15</td>
<td>(0.48, 1.83)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child consumed &gt; 500g/day of dairy milk at T2</td>
<td>0.40</td>
<td>(0.01, 0.79)</td>
<td>0.044</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maternal factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>0.00</td>
<td>(-0.04, 0.03)</td>
<td>0.773</td>
<td>0.00</td>
<td>(-0.03, 0.03)</td>
<td>0.913</td>
<td>-0.04</td>
<td>(-0.09, 0.00)</td>
</tr>
<tr>
<td>Age at baseline</td>
<td>-0.01</td>
<td>(-0.05, 0.03)</td>
<td>0.644</td>
<td>0.03</td>
<td>(-0.01, 0.07)</td>
<td>0.142</td>
<td>-0.01</td>
<td>(-0.06, 0.04)</td>
</tr>
<tr>
<td>Working at baseline</td>
<td>-0.29</td>
<td>(-0.86, 0.28)</td>
<td>0.321</td>
<td>0.14</td>
<td>(-0.50, 0.78)</td>
<td>0.664</td>
<td>-0.80</td>
<td>(-1.51, -0.08)</td>
</tr>
<tr>
<td>Tertiary educated at baseline</td>
<td>0.09</td>
<td>(-0.24, 0.43)</td>
<td>0.581</td>
<td>0.29</td>
<td>(-0.06, 0.65)</td>
<td>0.105</td>
<td>0.57</td>
<td>(0.15, 0.99)</td>
</tr>
<tr>
<td>Born overseas</td>
<td>-0.13</td>
<td>(-0.52, 0.27)</td>
<td>0.530</td>
<td>-0.04</td>
<td>(-0.45, 0.38)</td>
<td>0.864</td>
<td>0.03</td>
<td>(-0.50, 0.56)</td>
</tr>
</tbody>
</table>
Supplementary Table 1. Milk intakes at T2 (age 9 months) according to primary milk source in the Melbourne Infant Feeding Activity and Nutrition Trial (InFANT) Program.

<table>
<thead>
<tr>
<th></th>
<th>66.7-100%</th>
<th>66.7-100%</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>breastmilk</td>
<td>formula/dairy milk</td>
<td>(n=128)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Total milk</td>
<td>440.8</td>
<td>133.6</td>
<td>720.9</td>
</tr>
<tr>
<td>Dairy milk</td>
<td>19.3</td>
<td>35.0</td>
<td>34.1</td>
</tr>
<tr>
<td>Infant formula</td>
<td>14.8</td>
<td>41.3</td>
<td>680.0</td>
</tr>
<tr>
<td>Breastmilk</td>
<td>406.8</td>
<td>126.8</td>
<td>6.7</td>
</tr>
</tbody>
</table>
CONFLICT OF INTEREST

All authors declare no conflict of interest.
Funding Disclosure

The senior author is supported by an NHMRC Career Development Fellowship Level 2, ID1104636 and was previously supported by an ARC Future Fellowship (2011-2015, FT100100581).
### STROBE Statement—Checklist of items that should be included in reports of cohort studies

<table>
<thead>
<tr>
<th>Item No</th>
<th>Recommendation</th>
<th>Line numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title and abstract</strong></td>
<td>1</td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Provide in the abstract an informative and balanced summary of what was done and what was found</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
</tr>
<tr>
<td><strong>Objectives</strong></td>
<td>3</td>
<td>State specific objectives, including any prespecified hypotheses</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td>4</td>
<td>Present key elements of study design early in the paper</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) For matched studies, give matching criteria and number of exposed and unexposed</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable</td>
</tr>
<tr>
<td></td>
<td>8*</td>
<td>For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Describe any efforts to address potential sources of bias</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Explain how the study size was arrived at</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>(a) Describe all statistical methods, including those used to control for confounding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Describe any methods used to examine subgroups and interactions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Explain how missing data were addressed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) If applicable, explain how loss to follow-up was addressed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Describe any sensitivity analyses</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>13*</td>
<td>(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Give reasons for non-participation at each stage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Consider use of a flow diagram</td>
</tr>
<tr>
<td></td>
<td>14*</td>
<td>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Indicate number of participants with missing data for each variable of interest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Summarise follow-up time (eg, average and total amount)</td>
</tr>
<tr>
<td></td>
<td>15*</td>
<td>Report numbers of outcome events or summary measures overtime</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>(a) Give unadjusted estimates and, if applicable, confounder-adjusted</td>
</tr>
</tbody>
</table>
estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included

| Other analyses | 17 | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses | 190-242 |

**Discussion**

| Key results | 18 | Summarise key results with reference to study objectives | 245-249 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 332-349 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 251-331 |

**Generalisability**

| 21 | Discuss the generalisability (external validity) of the study results | 337-341 |

**Other information**

| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | Page 14 |

*Give information separately for exposed and unexposed groups.*