BREASTFEEDING AND PROTEIN INTAKE INFLUENCE BODY COMPOSITION FROM INFANCY TO ADULTHOOD

Melecia Jeseica Wright

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Approved by:
Linda Adair
Allison Aiello
Barry Popkin
Michelle Mendez
Daniela Sotrez-Alvarez
ABSTRACT

Melecia Jeseica Wright: Breastfeeding and protein intake influence body composition from infancy to adulthood
(Under the direction of Linda Adair)

Background

Although there is intriguing evidence that breastfeeding (BF) and protein intake (PI) may influence contemporaneous nutritional status and long-term body composition, few studies have explored the nuances of these age-specific associations. Dietary and anthropometric data obtained from 3080 individuals from the Cebu (Philippines) Longitudinal Health and Nutrition birth cohort (1983-2005) were used to clarify the role of protein in influencing body size across the life course.

Methodology

We examined i) the influence of breastfeeding and PI on contemporaneous body mass index (BMI = kg/m²) from birth to 22y using random-effects longitudinal regression models, ii) whether age-specific PI from 2-22y relate differentially to later body composition (22y) using multivariable regression models, and iii) how longitudinal patterns of PI from 2-22y relate to young adult body composition using latent growth curve analyses and multivariable regression models.

Results

i) BF was associated with better nutritional status (higher BMI) in infancy and BF duration was inversely associated with BMI thereafter. Total complementary protein and
complementary animal protein were positively associated with infant BMI Z-score (zBMI), while plant protein intake was inversely associated with infant zBMI. In post-infancy analyses, animal protein was associated with higher BMI. ii) Participants were classified into 4 mutually exclusive trajectories characterized by normal consumers, high consumers in infancy, usually-high consumers and always-high consumers. Compared to the normal consumers, always-high and usually-high consumers had lower predicted BMI, lean mass and fat mass at 22y. iii) Excess PI at age 2 was positively associated with BMI and lean mass at age 22y, while excess PI at ages 11, 15 and 22 were inversely associated with later lean mass, fat mass and BMI.

**Conclusion**

BF and PI significantly contribute to concurrent BMI across the life course. While age-specific PI and trajectories of PI were differentially related to later body composition, recent PI or PI pattern was most strongly related to adulthood outcomes. PI modifies risk of both current and long-term malnutrition. These findings support the optimization of early diets for promoting current and future health.
To my father, Ivan, for teaching me how to be resolute,
To my mother, Neva, for teaching me how to be strong,
To my sister, Venecia, for teaching me to persevere,
To my brothers, Elian and Ivan Jr., who remind me to laugh,
And to my husband, Gregory Harris, for assuring me that I can.
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Gregory Harris, thank you for your unwavering encouragement and support. To the Wright family, si mi ya! from Rose Heights tu di werl!
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CHAPTER 1. INTRODUCTION

Background

Nearly one-third of the human population suffers from some form of malnutrition. (1) Overnutrition (obesity) is major risk factor for mortality. (2) Globally, nearly 39% of all adults are either overweight or obese and 42 million children under 5y are overweight, so halting the obesity epidemic is a priority for mitigating deaths due to non-communicable chronic diseases. (3) On the other end of the spectrum is undernutrition, which accounts for 45% of child deaths (3.1 million child deaths) each year. (4) It is well established that diet is one key, modifiable determinant of both forms of malnutrition. The macronutrient protein, for example, has been shown to promote growth and development in infancy, thus reducing risk of undernutrition, but also potentially increases risk of obesity in later life.

Clarifying how dietary factors such as protein intake affect nutritional status across the life course is necessary to inform evidence-based interventions that reduce malnutrition. Conventionally, proximal dietary and lifestyle habits are thought to be modifiable risk factors for disease, and so modifying adult behaviors, for example, may mitigate risk of adult lifestyle diseases. (5) In contrast to this orthodox perspective, recent literature proposes that exposures throughout the entire life course may independently, synergistically or cumulatively influence chronic disease risk. (6,7) In fact, this life course approach to understanding disease evolved from the developmental origins of health and disease (DOHaD) body of literature (8) that revealed how fetal and early life undernutrition may promote later chronic disease risk. (9,10) Indeed, biological and epidemiological literature increasingly provide nuanced evidence for age-specific
associations of protein intake with contemporaneous body composition as well as long term adiposity. Studying body size, specifically BMI, fat mass and lean mass, is important since it is strongly related to cardiovascular disease, a leading cause of global morbidity and mortality even in low- and middle-income settings.\(^{(11)}\) These developmental perspectives have far-reaching implications--if protein intake promotes adequate nutritional status and its consequences persist into adulthood then optimizing nutrition during infancy may mitigate both current and future morbidity. Furthermore, clarifying its role in modifying adult disease risk in adulthood potentially provides an actionable point of intervention for reversing or mitigating obesity risk.

A unifying life course framework\(^{(12,13)}\) forms the basis of this series of three longitudinal analyses of the association of protein intake with body size. Firstly, we examined how dietary protein was associated with body mass index (BMI) from birth to 22y, while paying special attention to modifications by breastfeeding status, protein source and age. Next, we classified longitudinal protein intake patterns from 2 to 22y and then described the association of these patterns with body composition at age 22y. Finally, we estimated how age-specific intakes of protein were related to body composition at age 22y.

The Cebu Longitudinal Health and Nutrition Survey (CLHNS) birth cohort was used to explore these associations. The CLHNS is a unique longitudinal dataset with multiple repeated measures of diet and anthropometry collected for 3080 infants at birth, bimonthly for the first 2 years of life, and again when participants were an average of 8, 11, 15, 19 and 22y of age.\(^{(14)}\) In recent years, what began as a low-income, undernourished cohort has seen rapid industrialization and economic development\(^{(15)}\) and concomitant shifts towards a westernized, obesogenic diet\(^{(16)}\). Thus the CLHNS is ideal for investigating the role of protein in modifying malnutrition in a low- and middle-income country (LMIC) experiencing a nutrition transition.
Specific Aims

This dissertation was designed to clarify the associations of dietary protein intake with body size by answering three primary research questions:

1) Is dietary protein intake associated with contemporaneous BMI? Random-effects longitudinal regression models estimated:
   a) The association of protein intake from complementary foods with infant BMI, with special attention paid to modifications by age, protein source and breastfeeding frequency, and
   b) The association of complementary protein with post-infancy BMI, with special attention paid to modifications by age, protein source and breastfeeding history.

We hypothesized that there were significant associations between protein intake and contemporaneous BMI and that these associations would be modified by age, animal vs vegetable protein source and breastfeeding. For example, protein intake in infancy would be positively associated with BMI but these associations would reverse in later life, i.e. adolescence or adulthood. We also hypothesized that associations for animal protein would be larger in magnitude than those observed for plant protein.

This estimation of how protein relates to contemporaneous BMI was a logical first step in understanding how protein may be modified to promote optimal nutritional status in the short term.

2) Is history or trajectory of protein intake associated with body composition in young adulthood?
   a) First, discrete trajectories or patterns of protein intake from 2-22y were classified using latent growth curve analyses.
b) Next, multivariable linear regression models estimated the association of trajectories of protein intake with BMI, lean mass and fat mass at age 22y, with special attention paid to modification of these associations by gender.

We hypothesized that the trajectories would capture biologically important aspects of protein intake and that these trajectories would facilitate the estimation of the cumulative effects of these dietary patterns on BMI, lean mass and fat mass.

This aim is central to understanding how chronically low or high protein intake patterns, changing levels of intake, or perhaps markedly high protein intakes at certain periods of the life course may modify later nutritional status. This is especially relevant in those populations currently undergoing the nutrition transition where individuals may experience both malnourishment and overnutrition in a single lifetime.

3) Is protein intake at certain periods of the life course differentially associated with young adult body composition? Multivariable linear regression models were employed to estimate the association of protein consumed at age 2, 11, 15, 19 and 22y with body mass index, lean mass and fat mass at age 22y, with special attention paid to modification of these associations by gender.

We hypothesized that protein intake would be associated with body composition at age 22y, and that these associations would vary in magnitude depending on the age of protein intake; for example, early life may be a particularly vulnerable period compared to mid-adolescence.

By fulfilling this aim, we hope to identify vulnerable windows in which nutritional or educational interventions may be most beneficial for lifelong health.
CHAPTER 2: LITERATURE REVIEW

Overview

Dietary protein is an integral part of the human diet; it provides nitrogen which can be incorporated into a wide array of compounds necessary for healthy metabolism.\(^{(17,18)}\) Adequate protein intake is therefore critical to growth and development. Deficiencies in protein and/or other macronutrients result in failure to attain age-appropriate height (stunting), weight (underweight) or weight-for-length (wasting).\(^{(19)}\) These forms of undernutrition account for nearly half of all child deaths each year.\(^{(4)}\) In resource-poor settings, diets characterized by cereals and limited access to animal protein exacerbate risk of undernutrition.\(^{(20)}\) This is because plant proteins have limited amounts of essential amino acids such as lysine, threonine, cysteine and methionine relative to animal proteins.\(^{(8)}\) The role of protein in reducing undernutrition is well-established, however, recent literature suggests that in higher-income countries, intakes of high quality protein in excess of needs may result in infant overnutrition, potentially laying the foundation for later chronic disease risk.\(^{(20,21)}\) What is more, adult diets that are higher in protein may also mitigate adult obesity. What follows is a brief review of the nuanced literature that links protein to body size/composition from infancy to adulthood.

How is protein related to overnutrition in infancy?

Much of the research which links high infant protein intake to overnutrition in infancy and childhood emerged from the European Childhood Obesity project which explored associations of protein intake with weight in a cohort of 1150 infants randomized at birth in a
double-blind trial.\(^{(22)}\) Infants was assigned to receive formula containing ‘high’ (7.1%) or ‘low’ (11.7%) of energy from protein, with varying compositions of dietary fat used to keep intakes isocaloric.\(^{(23)}\) At 6-months, it was evident that the high protein group of infants had experienced higher weight gain (daily intakes of 807.8 g ±93.8 vs 724.2g ±110.10 in high vs low protein treatment arms respectively).\(^{(24)}\) By 24 months, the infants in the low protein composition group had similar length as their high protein counterparts, but the high protein group had significantly greater standardized relative weight at 24 months (beta and 95% CI: 0.20SD (0.06, 0.34).\(^{(23)}\)

Since infant protein has been associated with rapid early growth, this might provide another potential pathway for increasing risk of adulthood overweight, even in low- and middle-income settings. This study of five birth cohorts from low and middle-income countries, including the Cebu Longitudinal Health and Nutrition survey, found that faster increases in relative weight in the first two years were associated with elevated risk of adult overweight (1·51, (1·43,1·60)) respectively.\(^{(25)}\)

**How might breastfeeding affect infant nutritional status?**

Milk is the primary source of protein in early life. Indeed, breast milk is widely established as the ideal staple of infancy and dietary recommendations for this period of life are heavily based on the nutrient composition of human breast milk.\(^{(17)}\) Both human breast-milk and infant formula provide protein to meet the infant’s nutritional needs, however, the two sources vary in macronutrient profiles.\(^{(22)}\) A critical difference between these two sources is that formula-fed infants are estimated to receive 66-70% more protein (g/kg of body weight) than breastfed infants in the first 6 months of life.\(^{(26)}\) Importantly, breastfed infants still receive sufficient protein quantities, even though they receive less than their formula-fed peers do. Breastfeeding is associated with slower growth velocity from 3-18 months\(^{(26-28)}\) but this does not appear to
adversely affect infant sleep, activity or developmental milestones such as age of first walk.\(^{(29)}\)

With that in mind, the excess protein in infant formula may not be necessary and may actually be disadvantageous.

**How does infant protein intake relate to childhood overnutrition?**

There is also evidence of infant protein intake being related to adiposity in childhood.\(^{(30)-(34)}\) A follow-up study conducted in the European Childhood Obesity Project found that at 6y, children who were assigned to the high protein treatment arm were heavier (0.67 kg (-0.04, 1.39)) than the low-treatment control group, although there were no significant differences in height.\(^{(35)}\) More evidence comes from a study of a German cohort which showed that participants who consumed more than the median protein at 12 and 18-24 months had significantly higher BMI (0.37 SDs 95%CI (0.12, 0.61) and were more likely to be in the top quartile of percentage body fat at age 7 (OR 2.28 (1.06, 4.88)) compared to those who had below the median protein intake in that period.\(^{(32)}\) When they examined how protein intakes affected body composition, they found that total protein as well as dairy protein consumed at 12 months was positively associated with body fat percentage at age 7y.\(^{(32)}\) Another study showed that protein intake at 17-18 months was positively related to BMI of 4-year-old Swedish children (a 1 gram increase was associated with a 0.042 SD (±0.025) increase in BMI z score).\(^{(30)}\) Similar findings were reported from a study which found that Italian overweight (>90\(^{th}\) centile) 5-year-olds had received higher energy from protein (22%) at 12 months, compared to normal weight peers (who received 20% energy from protein).\(^{(36)}\)

One mechanism by which early protein intake may influence overnutrition in childhood is via its effects on the adiposity rebound. The adiposity rebound is the increase in BMI following the minimal point or nadir seen in the BMI growth charts of children. During infancy,
BMI tends to increase rapidly during the first year of life, then declines until age 3-5 years, after which it ‘rebounds’. Early adiposity rebound is associated with elevated waist circumference at age 7\(^{37}\) as well as BMI between 21-29y.\(^{38,39}\) High protein intake in early life seems to hasten timing of the adiposity rebound and subsequently promote obesity risk.\(^{34}\) For example, a study of 1330 infants from Australia followed from infancy to age ~14 showed that infants introduced to formula before 4 months had earlier age of adiposity rebound, higher BMI at the age of adiposity rebound and were more likely to be obese or overweight at 14 years, relative to their counterparts who did not receive other formula until after 4 months.\(^{40}\) Furthermore, those breastfed for longer than 4 months experienced a later adiposity rebound, lower BMI at the age of the adiposity rebound and were less likely to be obese at 14 years.\(^{40}\) These results suggest that longer exposure to non-human milk and lower breastfeeding duration are risk factors for both earlier age of adiposity rebound and overweight/obesity in later life.

Protein also promotes linear growth in childhood, an association potentially mediated by serum insulin-like growth factor-1 (IGF-1),\(^{41-43}\) though recent studies suggests that other factors underly this relationship.\(^{43}\) One study examined the association of total, animal- and plant-sourced PI with linear growth and serum IGF1 in 30-month-old Danish toddlers.\(^{41}\) They found that both animal PI and milk PI were positively associated with IGF-1 and height in childhood; and that IGF-1 was significantly associated with height.\(^{41}\)

Overall, a systematic review found that the evidence linking high infant protein intake to childhood adiposity was ‘convincing’, and that this early protein intake may also affect adult risk of overweight but that the threshold for these potentially detrimental effects is not yet known.\(^{33}\) It is plausible that infant protein intake may affect adulthood obesity risk via its effects on childhood adiposity or the adiposity rebound. Few studies have tested this association of early
protein intake with adulthood obesity, since data is often limited to childhood or adolescent years. Furthermore, much of this research has emerged from higher income settings where protein quality and quantity are presumably high enough to be detrimental. While this is a reasonable presumption, it is also important to interrogate these associations in other socioeconomic settings where problems of adult overnutrition have only recently become a major public health concern. Though protein intakes in these settings are likely lower in low-income settings, clarifying the contributions of protein to nutritional status across the life course is important for anticipating malnutrition risk in populations in diverse socioeconomic contexts.

**How does post-infancy protein intake affect overnutrition?**

Protein may exert beneficial effects on metabolic risk by promoting satiety (thus reducing overeating), increasing thermogenesis (which promotes fat metabolism) and improving glucose metabolism.\(^{(44,45)}\) In fact, proponents of the ‘protein leveraging’ hypothesis stress that the satiating effects of protein strongly regulate total energy intake and so modifying dietary protein may be one approach to curbing the obesity epidemic.\(^{(46,47)}\) The nutrition transition, specifically the increased consumption of refined carbohydrates and fats, has diversified diets in several low- and middle-income countries.\(^{(48-51)}\) Thus it is plausible that the reduced protein density of diets higher in refined foods may induce overeating to meet daily protein requirements.\(^{(46,47)}\)

There is epidemiologic evidence that diets higher in protein may mitigate obesity risk. An American cross-sectional study in the National Health and Nutrition Examination Survey (NHANES) found that being in a higher decile of protein intake was associated with lower BMI (-0.47kg/m\(^2\)) and waist circumference (-0.53cm).\(^{(52)}\) These associations may also vary by protein source. In a study of European adolescents, daily plant protein intake (g) was strongly and inversely associated with cross-sectional body fat percentage although modest associations also
existed for animal protein intake. Another study in Belgian adults found a positive association of animal protein with BMI and waist circumference, whereas plant protein intakes were inversely associated with these outcomes.

**How does breastfeeding history affect long-term health?**

The protein content of breast milk might partly explain why breastfeeding has been favorably (inversely) linked to several non-communicable disease risk factors. For example, a study of 962 members of the Framingham Third Generation Offspring study found that breastfed infants had lower adult BMI and a recent meta-analysis which compiled data from 25 studies determined that breastfeeding was associated with lower risk of obesity in childhood (OR 0.78 (0.74, 0.81)). The literature on this topic is quite varied: different evidence from a very large, randomized control trial in which 17046 Belarussian infants were assigned to receive a breastfeeding promotion intervention through their hospitals, relative to a standard-practice control, found no significant association with exclusive or prolonged breastfeeding adiposity at 6.5 years. These behavioural, nutritional and biological explanations for why breast feeding might be linked to reduced adiposity were nicely summarized in a review which still regards these hypothesized associations as plausible but controversial. Breastfeeding may be a health behaviour that tracks through infancy- where breastfed infants are likely to be exposed to healthful nutritional and social conditions which predispose them to lower lifestyle-disease risk in adulthood. Secondly, the lower protein content of breast milk is also implicated in this association. Since the protein content of infant formula far exceeds that of breast milk, perhaps the protein to which non-breastfed infants are exposed is what programs greater adiposity. Thirdly, formula-fed infants are known to grow more rapidly (presumably because of the higher protein content relative to breastmilk). Specifically, early growth rate, both in infancy and
childhood, is positively associated with greater adiposity in later life. Therefore, complementary protein intake may lead to faster growth rate in infancy which then predisposes infants to obesity risk in later life.

Why is it important to employ a life course perspective?

The developmental origins of health and disease or DOHaD hypothesis emerged from the observation that nutrition and environmental conditions experienced in utero or during infancy were associated with later chronic disease risk. Thus protein intake in the vulnerable period of infancy may have important consequences for long-term health, beyond its more short-term impact on growth and development during the early years of life. The literature offers many examples of how breastfeeding and protein intake may be related to adiposity. It is important to analyze these associations across the life course for several reasons.

Firstly, infancy may not be the only vulnerable window that is sensitive to levels of protein intake. Others have suggested that adolescence could also grant important insights into later chronic disease risk, particularly because this period is also characterized by rapid growth and many developmental changes. Few studies have investigated these other potential vulnerable windows.

Secondly, although some studies have reported the effects of protein intake on short-term body size, these have never been fully explored in an LMIC setting thus the question of whether protein programs long-term adiposity in socioeconomic settings where protein intakes tend to be lower is still unanswered.

Thirdly, the DOHaD literature has appropriately appreciated the role of infant protein intake in programming long-term overnutrition. However, possibly because of limited follow-up periods, only childhood or adolescent outcomes are typically estimated. Ours will be the first
attempt to characterize the health effects of protein intake from infancy to adulthood. Altogether, these findings should reveal important insights regarding the role of diets at distinct periods of the life course in mitigating or promoting contemporaneous as well as future health risk. Findings may thus inform public health efforts to target those vulnerable windows in which nutritional or educational interventions may be most beneficial.
CHAPTER 3: ASSOCIATION OF PROTEIN INTAKE WITH BMI FROM 2MO TO 22Y: INSIGHTS FROM A FILIPINO BIRTH COHORT

Overview

Background: Protein intake is an important determinant of growth and development that may also alter adiposity. However, few studies have explored the nuances of age-specific associations of protein with BMI.

Objective: To analyze how protein intake and breastfeeding relate to BMI from age 2mo to 22y.

Methods: Random-effects longitudinal regression models were used to estimate the joint association of daily breastfeeding (BF) frequency and energy-adjusted protein residuals with concurrent BMI Z-scores (zBMI) measured bi-monthly from 2 to 24 months (n=2899), and the association of breastfeeding history (BH) and protein residuals with concurrent BMI using 5 surveys from 2 to 22 years (n=2435). Models included statistical interactions between protein intake, breastfeeding, age and energy and adjusted for socio-demographics, early life anthropometry and other potential confounders.

Results: Breastfeeding was associated with higher zBMI, though this effect decreased with age (Beta and 95% CI=0.491SD (0.422, 0.560) at 6mo and 0.114SD (0.032, 0.197) at 18mo). An additional 5 BF episodes per day contributed to higher predicted zBMI in younger infants (0.036 SD (0.012, 0.060) at 6mo) and lower zBMI in later infancy (-0.079SD (-0.109, -0.049) at 18mo). Those with longer BF history (19mo) were significantly smaller at age 11 (-0.220 kg/m² (-0.342, -0.097)) than those with shorter (4mo) duration. Total complementary protein was positively associated with zBMI while complementary animal protein was positively associated with zBMI in non-breastfed infants. Plant protein intake was inversely associated with zBMI at 6mo, though
this reversed modestly by 18mo. In post-infancy analyses, at 22y, contrasts between high (75th percentile) and low (25th percentile) protein intake showed that animal protein was associated with higher BMI (0.187 kg/m² (0.045, 0.329) and total protein was inversely related to BMI - 0.008 kg/m² (-0.015, -0.001).

Implications: Breastfeeding frequency, breastfeeding history and protein intake significantly contribute to concurrent BMI across the life course.

Keywords: BMI, early protein, protein, longitudinal data, life course epidemiology

Introduction

In recent years, some literature has revealed significant but nuanced associations of protein intake with body size. During infancy, metabolic demand for protein is high to support its indispensible structural and functional roles in promoting healthy growth and development.\(^\text{(17)}\) Poor nutritional status (such as stunting, underweight or wasting) can occur when protein or other macronutrient intake is low.\(^\text{(19)}\) In low- and middle-income countries, reliance on plant-based proteins and limited intakes of animal protein can also contribute to malnutrition.\(^\text{(20)}\) The role of protein in reducing undernutrition is well-established, however, studies from the developmental origins of health and disease (DOHaD) perspective add more complexity to this protein-adiposity discourse. The DOHaD literature suggests that, at least in settings with relatively high average intake, higher protein intake in infancy may accelerate infant weight gain and thus promote obesity in childhood and later life,\(^\text{(20,21,60)}\) perhaps by promoting adipocyte differentiation and adipogenesis in infancy.\(^\text{(22,63)}\) For example, epidemiologic evidence suggests that infants fed high-protein formula weigh more in the first two years of life\(^\text{(23)}\) and have higher risk of childhood obesity.\(^\text{(35)}\) Protein also promotes linear growth in childhood, an association potentially mediated by serum insulin-like growth factor-1,\(^\text{(41-43)}\) though recent studies suggests
that other factors underlie this relationship. Intriguingly, formula-fed infants are estimated to receive 66-70% more protein (g/kg of body weight) than breastfed infants in the first 6 months of life. Despite, or perhaps because of its lower protein content, breastfeeding has been associated with more favorable growth outcomes: a recent meta-analysis which compiled data from 25 studies determined that breastfeeding was associated with lower risk of obesity in childhood. Since protein is necessary but potentially detrimental in high quantities, it is important to clarify how protein intake affects affect concurrent weight gain in less industrialized settings, where breastfeeding—and not infant formula, is a more common source of protein in early life.

Clearly these developmental perspectives from the DOHaD literature have far-reaching implications: if infant protein intake and breastfeeding have consequences that persist into adulthood, then optimizing nutrition during infancy may mitigate both current and future morbidity. Nevertheless, the question of how breastfeeding and complementary protein intake jointly are associated with body size in the short term in low-income settings is worthy of further investigation.

In addition to epidemiologic literature on infant protein intake and obesity, other research has identified inverse associations of adulthood protein intake with concurrent obesity. Protein may reduce body fat by improving satiety and promoting the metabolism of fat and glucose among other mechanisms. Moreover, proponents of the protein leveraging hypothesis remind us the protein intake is strongly regulated and the reduced protein density of modern diets might increase total energy intake to meet protein demands and thus promote obesity risk. The complexity of these age-specific associations of protein intake with body size demand further attention.
Understandably, few cohorts have the anthropometric and dietary intake necessary to elucidate the nuances of the protein intake-adiposity hypothesis across the life course. Furthermore, few studies have explored this protein-adiposity association in rapidly industrializing low- and middle-income countries where both undernourishment and overnourishment may be experienced within a single lifetime. The life course epidemiologic perspective employed in this study acknowledges that protein may differentially affect BMI at different ages, so detailed dietary histories should be carefully considered when exploring the etiology of health and disease. To further clarify the protein-adiposity hypothesis, we present the study of protein intake and body mass index (BMI) from 2 months to 22 years. Random-effects, longitudinal regression models are used to fully exploit 12 bimonthly surveys available for the vulnerable developmental period of infancy, as well as five surveys from 2 years to 22 years in this large prospective cohort. Since protein promotes adipocyte differentiation in infancy but also promotes satiety in post-infancy years, we hypothesized that protein intake would be positively associated with infant BMI but inversely associated with post-infancy BMI. Plants typically have limited amounts of indispensable amino acids such as lysine, threonine, cysteine and methionine, so we anticipated that since protein quality varies by source then the protein-BMI association would also vary by source. Additionally, we expected that breastfeeding would be positively associated with BMI during infancy, but breastfeeding history would be inversely associated with later BMI.

Methods

Study population

The Cebu Longitudinal Health and Nutrition Survey follows a one-year birth cohort of infants born in Cebu, Philippines. The survey provides detailed dietary and anthropometric

Primary exposure: protein intake

Twenty-four hour dietary recalls provide dietary data, from one recall for each infant survey and two recalls from 11-22y. In 1991, a food frequency questionnaire (FFQ) provided dietary information. Due to systematic differences in estimated nutrient intakes from that survey, it was excluded from this study. Protein intake was estimated using the Filipino food composition tables. Nutrients from breast milk were not quantified, thus dietary data during infancy only account for estimated nutrient intake from complementary foods rather than total nutrient intake in breastfed infants. To assess whether the origin or source of protein modified the association between protein intake and BMI, protein was divided into animal and plant sources. Animal protein came from dairy, eggs, fish, shellfish, meat and poultry. Plant protein came from vegetables, tubers, legumes, seeds, grains and fruits. Absolute protein intake is significantly correlated with energy intake; therefore protein was expressed as residuals using the Willett method. For each survey, grams of protein consumed were regressed upon total energy intake (excluding energy from human breast milk) to obtain energy-independent residuals of protein intake. Using an absolute specification of protein intake would have been misleading since heavier individuals generally eat more and thus have larger energy intakes. Protein residuals represent that quantity of protein consumed that is not explained by energy intake; therefore residuals were used as the primary exposure in all regression analyses.

Breastfeeding was analyzed as a separate variable since breast milk is the primary source of energy for these infants, and is a key source of protein throughout the first two years of life.
Breastfeeding frequency was based on the mother’s report of number of times the infant was breastfed in the previous day (this coincided with the period covered by the 24-hour recall).

Breastfeeding frequency was inversely associated with complementary energy intake and so was expected to be positively correlated with and a reasonable approximation of breast milk intake. A dummy variable for no-breastfeeding (1=non-breastfed, 0=breastfed) was also included since we hypothesized that the estimated effect of any-versus-no breastfeeding was much larger in magnitude than the effect of an additional episode of breast feeding among breastfed infants. The breastfeeding frequency variable was also truncated at the 95th percentile to diminish the impact of implausibly large values and potential outliers. In the post-infancy analyses, breastfeeding history was represented by the duration of breastfeeding in months.

Primary outcome: Body mass index (BMI)

Body mass index (kg/m$^2$) served as the outcome since it is a reasonable indicator of adiposity. In infancy, the WHO growth standards were used to create BMI z-scores (zBMI) which were then used as the outcome to avoid the challenges of modelling the complex BMI-age association in infancy. BMI was used for post-infancy analyses as it is a more interpretable characterization of body mass in children and adults, and participants in this birth cohort were approximately the same age at any given survey. Women were excluded from any given survey in which they were pregnant.

Confounders and key predictors

Models were adjusted for socio-demographic and anthropometric variables related to the offspring, mother and household. These included maternal education, maternal height and maternal age at baseline, offspring sex, birth weight, time-varying offspring age and education, and time-varying household assets and urbanicity and for the child-adult models, age-2 BMI.
The composite score of assets ranged from 0 to 11 and was included as a measure of socioeconomic status; assets used to construct the score were: electricity, house, material of house, air conditioner, television, tape recorder, refrigerator, electric fan, jeepney, car and clothing iron. The urbanicity score was included to capture differences related to the urban- or rural-dwelling. (15) All these were included as potential confounders since they have been shown in this and other studies to confound diet-BMI associations.

Statistical methodology

To estimate the association of breastfeeding and protein intake with BMI, we used random-effects longitudinal regression models with a robust variance estimator. The primary exposures were either residuals from protein, plant protein or animal protein. In appreciation of the hypothesized dynamic associations of breastfeeding with infant BMI, separate models were run for the early life period from 2 to 24 months, and the post-infancy period of 2 to 22 years. All models were first specified to match hypothesized synergies among dietary factors and elucidate associations between diet and BMI that were modified by age. Thus, we modelled BMI using two-way product terms or statistical interactions for protein residuals, energy intake, current breastfeeding frequency or historic breastfeeding and age. All models were adjusted for the above-described confounders. Post-infancy models were additionally adjusted for residuals of carbohydrate intake since they were found to significantly confound the protein-BMI association.

In the first 2 years, 2899 infants provided data from an average of 10.4 of 12 surveys. From age 2-22y, 2435 offspring contributed data from an average of 4.1 surveys out of a possible 5 surveys. Analyses were executed using Stata 14 (College Station, TX).
**Predictions**

To elucidate the implications of significant age interactions, coefficients from each model were used to estimate the difference in predicted BMI for those with high versus low protein intake. Survey-specific values for the 75th or 25th percentile were designated as high or low protein intake respectively. Wald tests were executed to assess whether the predicted differences in BMI for these high vs low protein contrasts were statistically significantly different from 0. Coefficients from infant regression analyses were used to predict these differences at ages 6 and 18 months while coefficients from the post-infancy analyses were used to predict differences at ages 11 and 22 years. These ages were selected to represent earlier and later periods of the infancy and post-infancy analyses.

**Results**

Mothers in the CLHNS had received ~8 years of formal education at the time of the offspring’s birth (Table 1). Urbanicity score was low at birth (31 out of a possible range of 0-70) but the sample became more urban by age 22 (41). Infants were breastfed for a mean of 12.4 months.

*Macronutrient intakes and food groups contributing to protein intake*

Figure 1 shows the top five contributors to infant complementary protein intake (Figure 1A) and total protein intake in post-infancy surveys (Figure 1B). In each survey, the five food groups shown contributed to at least 70% of the total protein intake consumed (excluding protein from breast milk). In the infant surveys (Figure 1A), infant foods and dairy products (Cerelac, powdered and condensed milks, etc.) were the primary non-breast milk contributors to infant protein intake in the earliest months of life. As the infants grew, grains (gruels of rice, corn,
oatmeal, etc.) and vegetables were added to the complementary diet, and by 24 months, meat, poultry and seafood also became top contributors to complementary protein intake. In post-infancy surveys (Figure 1B), meat, poultry, seafood and grains were consistently in the top 5 contributors of total protein intake for all four surveys from 11 to 22 years. Mean macronutrient intakes from complementary foods increased from 2 to 24 months (Table 2). As expected, the results of the t-tests indicate that there were statistically significant differences in the complementary macronutrient intakes for breastfed and non-breastfed infants. For example, at any given survey between 2 and 24 months, mean intakes of fat, carbohydrates and protein were significantly higher in non-breastfed infants. The same was true for the nutrient densities of fat and protein from 2 to 20 months, but the complementary diets of breastfed infants were characterized by higher carbohydrate density from 2 to 24 months. Mean absolute intakes of protein, fat, carbohydrates and energy tended to increase as the cohort grew older (Table 3).

Results of the longitudinal regression of body mass index on protein intake

The longitudinal regression analyses revealed many statistically significant associations of protein intake, breastfeeding with BMI from age 2 months to 22 years (Table 4). The magnitude of these associations varied for total, animal and plant protein intake. Due to the many significant statistical interactions, post-estimation tests were used to estimate differences in BMI for those with high vs. low breast milk and protein intakes. The following are the results of these post-estimation tests.

Breastfeeding frequency and any-breastfeeding during infancy

Breastfeeding showed significant, positive, age-dependent associations with infant zBMI (Figure 2). Compared to non-breastfed infants, breastfed infants had a zBMI ~0.5 SD higher than their breastfed counterparts at 6 months (Beta and 95%CI= 0.491 (0.560, 0.422)), but this
positive association was mitigated with age: by 18 months, BMI of breastfed infants was only 0.114 (0.197, 0.032) higher than non-breastfed infants. Additionally, among breastfed infants, an additional 5 feedings per day was associated with higher zBMI at 6 months (0.036 (0.012, 0.060)). However, by 18 months, an additional 5 feedings was associated with lower zBMI (-0.079 (-0.109, -0.049)).

Complementary protein intake in infancy

Total complementary protein intake was also significantly associated with zBMI (Figure 3). Among 6-month-olds, those receiving high quantities of complementary protein (at the 75th percentile of protein residuals) had a greater predicted zBMI than those who received low quantities (at the 25th percentile) (Beta=0.015 (0.007, 0.022) and 0.062 (0.038, 0.085) for breastfed and non-breastfed infants respectively). Trends were similar for both breastfed (0.051 (0.024, 0.078)) and non-breastfed infants (0.030 (-0.001, 0.061), p<0.10) at 18 months.

Source-specific associations were only significant in contrasts among non-breastfed infants. While animal protein was positively associated with BMI at 6 and 18 months (0.067 (0.027, 0.107) and 0.034 (0.003, 0.066) respectively), plant protein was inversely associated with BMI at 6 months (-0.049 (-0.080, -0.018)) and showed modest, positive associations with BMI at 18 months (0.008 (0.001, 0.015)).

Breastfeeding duration in post-infancy analyses

As shown in Figure 2, at age 11y, offspring with a long breastfeeding duration (at the 75th percentile, or 19 months) had a significantly lower BMI (-0.220 (-0.342, -0.097)) than those with a short breastfeeding duration (at the 25th percentile or 4 months). However, there was no significant association of breastfeeding history with BMI by age 22y.
Protein intake and BMI in post-infancy analyses

There was a modest, inverse association between total protein intake and BMI at age 22 (-0.008 (-0.015, -0.001)) (Figure 3). Plant protein intake was not significantly associated with BMI in post-infancy analyses, while animal protein was positively associated with BMI at age 22 (0.187 (0.045, 0.329)).

Discussion

This study estimated the association of protein intake with BMI from infancy to early adulthood in a low-income country that has seen rapid industrialization in recent times. These life course analyses showed important modifications of the protein-adiposity association by protein source and age of protein intake in a large Filipino birth cohort. Our analyses also revealed important time-varying contributions of breastfeeding frequency and breastfeeding history to growth and BMI from infancy to the mid-childhood years.

There are age-dependent associations between breastfeeding frequency and duration with BMI during infancy

Due to its nutritional and immunological properties, breast milk is an ideal food during the first months of life. Consistent with other studies that showed that changes in weight and / or weight-for-length in breastfed infants are most pronounced in first trimester of life then change rapidly thereafter, (73-75) we found the strongest contributions of breastfeeding to zBMI occurred in the earlier months. We reported similar findings in this Cebu cohort: breastfed infants had superior weight-for-age z scores at 6 months but the benefits were no longer significant by 24 months. (76) This may be because socioeconomically disadvantaged mothers were more likely to breastfeed, but may have fed their infants lower quality complementary foods. (77) As we showed
here, the macronutrient composition of the diets varied significantly by breastfeeding status. Furthermore, only 18% of breastfed infants consumed non-human milks at 2 months, and this consumption prevalence was 5% at 24 months while, in contrast, 83% of nonbreastfed infants consumed these milks at 2 months and this fell to 23% at age 24 months. In the second year of life when the infants’ needs exceed that which breast milk provides, such differences in quality of complementary foods matter. We included relevant socioeconomic variables to control for confounding, but cannot rule out remaining residual confounding.

*Duration of breastfeeding was inversely associated with BMI though these effects were diminished with age*

Like other studies that examined the association between breastfeeding history and later BMI, ours also found a significant, inverse association in adolescence (at age 11) although this inverse association was not significant by age 22. These findings suggest some protection against obesity risk, however, at age 11, fewer than 1% of participants were obese (i.e. had a standardized BMI > 2). With this negligible obesity prevalence, the inverse association with BMI in this cohort is more appropriately interpreted as a tendency for those who were breastfed longest to be thinner. Since breastfeeding histories in this cohort were inversely associated with markers of socioeconomic status, it is possible that these socioeconomic differences also drove divergent dietary patterns in childhood and thus led to thinness or lower BMI. A second possibility is that those who were breastfed were thinner adolescents since they received less complementary protein during infancy, and so did not experience the kind of obesogenic programming that underpins the early protein-adiposity DOHaD hypothesis. Breastfeeding may also improve the infants’ ability to regulate food intake, potentially programming improved food intake patterns in later life.
Dietary protein intake was differentially associated with BMI in an age- and source-dependent manner

We showed that protein intake was positively associated with infant BMI, regardless of breastfeeding status. Evidence of this association also comes from randomized clinical trials conducted in the European Childhood Obesity Trial study cohort where the high protein group received 2.9 or 4.4g/100kcal and the low protein group received 1.77 and 2.2g/100kcal for infant and follow-on formula respectively. Relative to the low-protein control group, infants randomized to the high protein treatment arm gained significantly more weight during the first 6 months of life and this weight gain was significantly correlated with fat mass and fat mass index. The ‘high protein’ group also had significantly greater weight-for-length z scores though these infants did not differ significantly in length. While figures are not directly translatable to the protein residuals used in our observational cohort, similar contrasts were made in our cohort since, for example, 6-month-old Cebu infants in the highest quintile of protein residuals received a median of 3.6g of complementary protein per 100kcal compared to a median of 1.6g per 100kcal in the lowest quintile. Furthermore, a recent study in this Filipino cohort found that the protein composition of the diet (in g/kcal) was positively associated with infant weight, but not length. Although this prior study did not consider the important role of breastfeeding in our cohort, its findings suggest that protein may be exerting its effects on infant BMI by primarily driving weight gain in this population, rather than changes in linear growth.

As hypothesized, total protein intake was inversely associated with post-infancy BMI, but only in contrasts conducted for the age-22 survey. An American cross-sectional study using National Health and Nutrition Examination Survey (NHANES) data found that being in a higher decile of protein intake was associated with lower BMI (-0.47kg/m²) and waist circumference (-
The magnitude of our association was relatively small compared to that from the NHANES, however it may be because protein has been shown to be associated with both length and weight in these Filipino participants from age 8-22y.\(^{(82)}\) If protein simultaneously affects these two anthropometric indicators of growth, this may explain why its effects on BMI were so modest.

We found evidence that the protein-BMI association also varied by protein source. In non-breastfed infants at 6 months, animal protein intake was positively associated with zBMI while plant protein intake was inversely associated with zBMI. The rice and corn gruels that characterized these infants’ diet did not support their growth needs.\(^{(76,83)}\) This reinforces the importance of recommending animal-source foods during the complementary feeding period to stave off undernutrition. In fact, interventions that specifically recommend including animal-source foods in the complementary diet work better to promote diet quality and infant nutritional status compared to vague recommendations to increase protein.\(^{(84,85)}\) This finding thus underscores the WHO recommendations on appropriate complementary feeding, where animal-source foods are promoted for higher protein quality and micronutrients.\(^{(86)}\) Similar positive associations were evident for animal protein and BMI while plant protein intake was non-significant in our post-infancy analyses. These results concord somewhat with other studies: a study in Belgian adults found a positive association of animal protein with BMI and waist circumference, whereas plant protein intakes were inversely associated with these outcomes.\(^{(54)}\) Additionally, while no significant associations were observed in our 11y contrasts, a study in European adolescents found daily plant protein intake (g) was strongly and inversely associated with cross-sectional body fat percentage although modest inverse associations also existed for
animal protein intake. Our null findings for plant protein may be due to differences in the quality of overall diets being different in the context of this region.

The merits of this study must be taken within context of important substantive and technical limitations. The first has to do with inadequacies of the dietary data. Despite having data from birth to adulthood from 16 surveys (12 in infancy and 4 in post-infancy), we are missing the 2y-11y period. This time period is when the adiposity rebound occurs, and is potentially a vulnerable window during which protein intake may program risk for later obesity. Dietary data did not include nutrients from breastmilk and thus underestimated total intake. However, to circumvent this problem we used breastfeeding frequency, a binary any-breastfeeding variable and product terms between breastmilk intake and complementary protein and energy intakes so we could best estimate the effects of protein effects in breastfed and non-breastfed infants. Additionally, the protein-BMI effects estimated here do not necessarily reflect enhanced obesity risk. In this context, these associations may indicate reduced risk of stunting or wasting, or even enhanced growth in lean tissue.

Despite the limitations of these analyses, there are key, noteworthy strengths of this study. This is the first exploration of the age- and source-specific associations of protein with BMI that was conducted in a low- or middle-income country. The longitudinal richness of this study and the careful attention paid to dietary details make this a significant contributor to the literature on this topic. We included product terms in order to elucidate any age-specific associations of protein intake and modifications by breastfeeding status. We further analyzed animal- and plant-specific protein intake to detect any modifications by source. The longitudinal richness of this study and the careful attention paid to dietary details make this a significant contributor to the literature on this topic.
Table 1 Mean* of select demographic characteristics at birth in 3080 offspring of the CLHNS (1983-2005)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal education (years)</td>
<td>7.56 ± 3.72</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>150.56 ± 5.00</td>
</tr>
<tr>
<td>Household Assets (score ranging 1-11)</td>
<td>2.51 ± 1.94</td>
</tr>
<tr>
<td>Household Urbanicity</td>
<td>30.58 ± 12.61</td>
</tr>
<tr>
<td>(score ranging from 0-70)</td>
<td></td>
</tr>
<tr>
<td>Offspring weight (kg)</td>
<td>3.00 ± 0.42</td>
</tr>
<tr>
<td>Offspring length/height (cm)</td>
<td>49.07±2.0 7</td>
</tr>
<tr>
<td>Offspring BMI (kg/m²)</td>
<td>15.56 ± 1.23</td>
</tr>
<tr>
<td>Total months of breastfeeding</td>
<td>12.39 ± 8.32</td>
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</tbody>
</table>
Table 2 Differences in mean macronutrient intake from non-human milk for breastfed and non-breastfed infants

<table>
<thead>
<tr>
<th>Age /months</th>
<th>Not-Breastfed</th>
<th>Breastfed</th>
<th>Difference (non-breastfed - breastfed intake)</th>
<th>Full sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fat</td>
<td>Protein</td>
<td>Carbs</td>
<td>n</td>
</tr>
<tr>
<td>2</td>
<td>15.97 ± 7.60</td>
<td>9.92 ± 4.03</td>
<td>44.99 ± 19.00</td>
<td>453</td>
</tr>
<tr>
<td>4</td>
<td>18.12 ± 9.01</td>
<td>13.54 ± 5.95</td>
<td>59.97 ± 27.07</td>
<td>571</td>
</tr>
<tr>
<td>8</td>
<td>16.77 ± 10.71</td>
<td>17.41 ± 8.95</td>
<td>91.61 ± 39.96</td>
<td>734</td>
</tr>
<tr>
<td>10</td>
<td>15.84 ± 11.24</td>
<td>17.66 ± 9.14</td>
<td>99.65 ± 42.83</td>
<td>834</td>
</tr>
<tr>
<td>12</td>
<td>14.96 ± 11.05</td>
<td>18.76 ± 10.36</td>
<td>104.38 ± 46.73</td>
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</tr>
<tr>
<td>14</td>
<td>13.35 ± 10.97</td>
<td>20.03 ± 11.37</td>
<td>117.20 ± 48.89</td>
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<tr>
<td>16</td>
<td>11.92 ± 10.53</td>
<td>19.47 ± 11.30</td>
<td>120.12 ± 52.00</td>
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</tr>
<tr>
<td>18</td>
<td>11.25 ± 10.68</td>
<td>19.76 ± 11.42</td>
<td>124.18 ± 54.02</td>
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<tr>
<td>20</td>
<td>11.47 ± 11.12</td>
<td>20.63 ± 12.04</td>
<td>129.75 ± 56.28</td>
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</tr>
<tr>
<td>22</td>
<td>11.12 ± 11.06</td>
<td>20.97 ± 12.04</td>
<td>132.35 ± 57.00</td>
<td>2014</td>
</tr>
<tr>
<td>Age /months</td>
<td>Not-Breastfed</td>
<td>Breastfed</td>
<td>Difference (non-breastfed - breastfed intake)</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat ±</td>
<td>Protein ±</td>
<td>Carbs ±</td>
<td>n</td>
</tr>
<tr>
<td>2</td>
<td>39.16 ± 12.87</td>
<td>11.41 ± 5.84</td>
<td>49.43 ± 15.05</td>
<td>453</td>
</tr>
<tr>
<td>4</td>
<td>35.49 ± 14.05</td>
<td>12.31 ± 5.59</td>
<td>52.20 ± 16.61</td>
<td>571</td>
</tr>
<tr>
<td>6</td>
<td>29.86 ± 13.54</td>
<td>12.27 ± 5.15</td>
<td>57.87 ± 16.36</td>
<td>652</td>
</tr>
</tbody>
</table>

*Survey-specific difference between the absolute or relative intakes of indicated nutrient of non-breastfed and breastfed infants

*p=0.20; ** p=0.87; All other p values<0.001
Table 3 Association of protein residuals and A) standardized BMI from 2 to 24mo and B) BMI from 2 to 22y

<table>
<thead>
<tr>
<th>A) zBMI for infancy analyses 2-24 months</th>
<th>B (95% CI) Total</th>
<th>B (95% CI) Animal</th>
<th>B (95% CI) Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-breastfed (relative to any breastfeeding)</td>
<td>-0.81* (-0.92, -0.70)</td>
<td>-0.81* (-0.92, -0.70)</td>
<td>-0.82* (-0.94, -0.71)</td>
</tr>
<tr>
<td>Protein residuals per day</td>
<td>0.01* (0.00, 0.02)</td>
<td>0 (0.00, 0.01)</td>
<td>-0.01* (-0.03, -0.00)</td>
</tr>
<tr>
<td>Non-breastfed*Protein residuals</td>
<td>0.01* (-0.00, 0.02)</td>
<td>0.01* (0.00, 0.02)</td>
<td>0 (-0.02, 0.01)</td>
</tr>
<tr>
<td>Calories (1000kcal units)</td>
<td>-0.08 (-0.25, 0.09)</td>
<td>-0.1 (-0.27, 0.06)</td>
<td>-0.08 (-0.25, 0.09)</td>
</tr>
<tr>
<td>Non-breastfed*Calories</td>
<td>0.28* (0.13, 0.43)</td>
<td>0.30* (0.15, 0.45)</td>
<td>0.27* (0.11, 0.43)</td>
</tr>
<tr>
<td>Protein residuals * calories</td>
<td>0 (-0.01, 0.00)</td>
<td>0 (-0.01, 0.00)</td>
<td>-0.01 (-0.02, 0.00)</td>
</tr>
<tr>
<td>age (months)</td>
<td>0.01* (0.00, 0.01)</td>
<td>0.01* (0.00, 0.01)</td>
<td>0.01* (0.00, 0.01)</td>
</tr>
<tr>
<td>Non-breastfed*age</td>
<td>0.03* (0.03, 0.04)</td>
<td>0.03* (0.03, 0.04)</td>
<td>0.03* (0.03, 0.04)</td>
</tr>
<tr>
<td>Calories * age</td>
<td>0.01* (0.00, 0.01)</td>
<td>0.01* (0.00, 0.02)</td>
<td>0.01* (0.00, 0.01)</td>
</tr>
<tr>
<td>Breastfeeding frequency</td>
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<td>0.02* (0.01, 0.03)</td>
<td>0.02* (0.01, 0.03)</td>
</tr>
<tr>
<td>Breastfeeding frequency* protein residuals</td>
<td>0.00 (-0.00, 0.00)</td>
<td>0.00 (-0.00, 0.00)</td>
<td>0.00+ (-0.00, 0.00)</td>
</tr>
<tr>
<td>Breastfeeding frequency* calories</td>
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<td>-0.01 (-0.03, 0.01)</td>
<td>-0.02+ (-0.04, 0.00)</td>
</tr>
<tr>
<td>Breastfeeding frequency* age</td>
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<td>-0.00* (-0.00, -0.00)</td>
<td>-0.00* (-0.00, -0.00)</td>
</tr>
<tr>
<td>Protein residuals * age</td>
<td>-0.00* (-0.00, -0.00)</td>
<td>-0.00* (-0.00, -0.00)</td>
<td>0.00* (0.00, 0.00)</td>
</tr>
</tbody>
</table>

B) BMI for post-infancy analyses 2-22 years

<table>
<thead>
<tr>
<th></th>
<th>B (95% CI) Total</th>
<th>B (95% CI) Animal</th>
<th>B (95% CI) Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein residuals per day</td>
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<td>-0.01* (-0.02, -0.00)</td>
<td>0.01 (-0.01, 0.03)</td>
</tr>
<tr>
<td>Calories (1000kcal units) per day</td>
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<td>-2.13* (-2.27, -1.99)</td>
<td>-2.14* (-2.28, -2.00)</td>
</tr>
<tr>
<td>age (years)</td>
<td>0.05* (0.02, 0.07)</td>
<td>0.05* (0.03, 0.07)</td>
<td>0.05* (0.03, 0.07)</td>
</tr>
<tr>
<td>Breastfeeding duration</td>
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<td>-0.04* (-0.05, -0.03)</td>
<td>-0.04* (-0.05, -0.03)</td>
</tr>
<tr>
<td>Protein residuals * calories</td>
<td>0.01* (0.00, 0.01)</td>
<td>0.01* (0.00, 0.01)</td>
<td>-0.01* (-0.02, -0.00)</td>
</tr>
<tr>
<td>Protein residuals * age</td>
<td>0.00 (-0.00, 0.00)</td>
<td>0.00 (-0.00, 0.00)</td>
<td>0.00 (-0.00, 0.00)</td>
</tr>
<tr>
<td>Calories * age</td>
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<td>0.13* (0.12, 0.14)</td>
<td>0.13* (0.12, 0.14)</td>
</tr>
<tr>
<td>Breastfeeding duration * age</td>
<td>0.00* (0.00, 0.00)</td>
<td>0.00* (0.00, 0.00)</td>
<td>0.00* (0.00, 0.00)</td>
</tr>
</tbody>
</table>

plant protein in animal protein models and animal protein in plant models

0.01* (0.00, 0.01) 0.00* (0.00, 0.00) 0.01* (0.00, 0.01)
Models 1, 2 and 3 had total protein intake, animal protein intake and plant protein intake as the primary protein intake residual measures respectively. Models 2 and 3 were additionally adjusted for plant and animal protein intake residuals respectively. A) Row A models were random-effects longitudinal regression models for 2899 infants 2-24 months of age, adjusted for maternal education, age and height at baseline, infant birth weight, male sex and time-varying neighborhood urbanicity and household assets scores. Infants contributed an average of 10.4 of a possible 12 surveys. B) Row B models were random-effects longitudinal regression models for 2435 offspring 2 to 22 years old, adjusted for male sex, birth weight, BMI at age 2 and time-varying offspring education, household assets and neighborhood urbanicity. Offspring contributed an average of 4.1 surveys of 5 possible surveys. Asterisks (*) indicate beta was statistically significant, p < 0.05. + indicates p for beta<0.10.
Figure 1 Top 5 contributors to A) total complementary protein intake in infant surveys from 2-24 months and B) total protein intake in post-infancy surveys from 11-22 years
Breastfeeding frequency and breastfeeding duration are associated with body size across the life course.

The beta estimates show the difference in predicted body size for an additional 5 breastfeeding episodes, or 15 months of breastfeeding duration. These breastfeeding values represent the difference between being at the 75th and 25th percentiles. * indicates that the p value for Beta < 0.05. Estimates for months 6 and 18 months were derived from random-effects longitudinal regression models adjusted for maternal education, age and height at baseline, infant birth weight, male sex and time-varying neighborhood urbanicity, household assets scores and complementary energy intake. Estimates for 11 and 22 years came from random-effects longitudinal regression models adjusted for male sex, birth weight, BMI at age 2 and time-varying offspring education, household assets, neighborhood urbanicity, energy intake and carbohydrate residuals.
Figure 3 Protein intake (PI) residuals are significantly associated with body size across the life course.

The beta estimates show the difference in predicted body size between those with high (at the 75th percentile) or low (at the 25th percentile) of protein intake from A) all non-human milk sources, B) all animal protein sources and C) all plant sources. A ‘0’ on the y-axis or confidence
intervals that include ‘0’ indicate no significant differences in size between those with high and low intakes of the protein source indicated. * p for Beta <0.05; + p for Beta <0.10. Estimates for months 6 and 18 months were derived from random-effects longitudinal regression models adjusted for maternal education, age and height at baseline, infant birth weight, male sex and time-varying neighborhood urbanicity, household assets scores and complementary energy intake. Estimates for 2, 11 and 22 years came from random-effects longitudinal regression models adjusted for male sex, birth weight, BMI at age 2 and time-varying offspring education, household assets, neighborhood urbanicity, energy intake and carbohydrate residuals.
CHAPTER 4: HOW DO TRAJECTORIES OF PROTEIN INTAKE AND AGE-SPECIFIC PROTEIN INTAKES FROM 2-22Y RELATE TO BODY COMPOSITION IN EARLY ADULTHOOD?

Overview

Background/Objectives: Although protein intake (PI) has been shown to be associated with body composition, no study has analyzed how PI in early childhood, pubescence, mid-adolescence and young adulthood relate to adult BMI in a single cohort and it is unclear whether trajectories of PI affect later BMI. We sought to estimate whether PI at ages 2, 11, 15, 19 and 22y were differentially associated with age- and sex-standardized BMI at age 22 (early adulthood) and estimate the association of trajectories of PI from 2-22y with BMI in early adulthood.

Subjects/Methods: Dietary and anthropometric data were obtained from 2586 individuals from a Filipino birth cohort (1985-2005). Excess PI was defined as PI relative to age-specific recommended daily allowance (g/kg body weight). Confounder-adjusted linear regression models were used to relate excess PI to early adulthood BMI. Latent growth curve analysis was used to identify groups according to their excess PI, and then confounder-adjusted linear regression models were used to relate trajectory membership to early adulthood BMI. Lean mass and fat mass at age 22 served as secondary outcomes. Confounders included socioeconomic, dietary and anthropometric covariates from early life and adulthood.

Results: a) Excess PI at age 2 was positively associated with BMI and lean mass at age 22y, while excess PI at ages 11, 15 and 22 were inversely associated with early adulthood BMI. b) Individuals were classified into 4 mutually exclusive trajectories of excess PI characterized by i) normal consumers (referent trajectory, 58% of cohort), ii) high consumers in infancy (20%), iii)
usually high consumers (18%) and iv) always high consumers (5%). Compared to the normal consumers, ‘always high’ and ‘usually high’ consumers had lower predicted BMI, lean mass and fat mass at age 22.

Conclusion: Our findings suggest that adolescent and adult PI are stronger, more important contributors to adult body composition when compared to PI in early childhood.

Introduction

Conventionally, proximal behaviors are thought to strongly modify disease risk but examining early life factors may yield intriguing insights into the origins of later disease. (5) Clarifying how early protein intake affects body size is important for designing interventions that promote healthy nutritional status across the life course. Adequate protein intake is critical to infant growth and development. (17) Deficiencies in protein and/or other macronutrients result in failure to attain age-appropriate height and weight. (19) In resource-poor settings, diets limited in high quality or animal protein exacerbate risk of undernutrition. (20) The role of protein in reducing undernutrition is well established, however, studies from the developmental origins of health and disease (DOHaD) perspective suggest that infant protein intake may promote later obesity via its effects on adipocyte differentiation and adipogenesis (22) and the timing of the adiposity rebound. (34,38) In fact, studies from higher-income settings have found independent associations of infant protein intake and obesity risk in later life. (20) A systematic review found convincing evidence regarding the positive association of protein with childhood obesity risk. (33)

Both fat and carbohydrates have been implicated as dietary contributors to obesity risk but recent literature proposed that protein may deserve further attention. (47) Protein may exert beneficial effects on adult metabolic risk by promoting satiety (thus reducing overeating), increasing thermogenesis (which promotes fat metabolism) and improving glucose metabolism.
In fact, proponents of the ‘protein leveraging’ hypothesis stress that the satiating effects of protein strongly regulate total energy intake and so modifying dietary protein may be one approach to curbing the obesity epidemic. The nutrition transition, specifically the increased consumption of refined carbohydrates and fats, has diversified diets in several low- and middle-income countries. Thus it is plausible that the reduced protein density of diets higher in refined foods may induce overeating to meet daily protein requirements.

Although a review of the literature yields these important examples of how protein intake may modify obesity risk in an age-dependent manner, no study has fully appreciated the role of dietary protein patterns in modifying obesity risk. When studies focus on protein intakes in distinct time periods, this approach neglects the potential roles of cumulative protein intake patterns across the life course. In light of the rapid westernization of traditional diets worldwide, an individual may experience periods of excess, adequate and insufficient protein intake in a single lifespan. It is unclear how combinations of such exposures across the life course may influence later BMI. Moreover, outside of infancy, other sensitive periods may exist but are yet to be identified, so studying pubescence or adolescence could also grant important insights. Thus we employed a life course approach to analyze how protein intake affects later body composition. We asked two main questions: i) are there vulnerable periods of the life course during which protein intake may modify later adiposity? And ii) how does life history of protein intake differentially relate to later BMI? To this end, we first estimated how intakes of protein in early childhood, pubescence, adolescence and young adulthood related to later body composition. We expected that high protein intake in early childhood would be positively associated with later adiposity. (This is because even in this low-income cohort, protein promotes infant/young child nutritional status and childhood BMI is positively related to later
BMI.) We also hypothesized that high protein intakes at later ages would be inversely associated with later body composition. Next, our study explored how histories of protein intake related to adulthood body composition. We hypothesized that distinct patterns of protein intake would emerge in this cohort, and that these trajectories of protein intake would be differentially associated with BMI in young adulthood. The Cebu Longitudinal Health and Nutrition Survey has multiple survey waves of dietary data, providing a stellar opportunity to examine patterns of protein intake from infancy to young adulthood.\(^{(14)}\)

**Methods**

*Study population*

The Cebu Longitudinal Health and Nutrition Survey (CLHNS) follows a one-year birth cohort of infants born in Cebu, Philippines.\(^{(14)}\) Detailed dietary and anthropometric data were collected at bimonthly intervals between birth and 24 months (1983-1986), and in 5 subsequent post-infancy surveys: 1991-92 (~age 8y), 1994-95 (~11y), 1998-99 (~15y), 2002 (~19y) and 2005 (~22y).\(^{(14)}\) There were 3080 singleton infants at birth and 1885 individuals in 2005 (~22y).

*Diet*

Diet was assessed using twenty-four hour dietary recalls, one recall for each bimonthly survey from birth to 24 months and two recalls for surveys at 11+ years. The means of the dietary intakes reported at 22 and 24 months were used to estimate usual intake at age 2. Protein intake was estimated using the Filipino food composition tables.\(^{(67)}\) Nutrients from breast milk were not quantified, thus dietary data at 22 and 24 months only account for estimated nutrient intake from complementary foods and underestimate total nutrient intake in the 14% infants that were still being breastfed. Analyses presented here include surveys that collected recall data from age 22 and 24 months, 11y, 15y, 19y and 22y. The 1991 survey (administered at ~8y) employed
a food frequency questionnaire that systematically overestimated macronutrient intake data and so it was omitted from these analyses.

**Primary exposure**

We defined excess protein intake as the difference between estimated protein intake and the age-specific recommended daily allowance (RDA) of protein in grams/kg body weight. An absolute specification of protein intake would have been inappropriate and misleading since heavier people have greater energy needs and so tend to consume more macronutrients. Our specification of excess protein intake in grams/kg body weight more appropriately conveys whether a surplus (if positive) or deficit (if negative) of protein intake was consumed relative to individual needs. RDAs for years 2, 11, 15, 19 and 22 were 1.05, 0.95, 0.85, and 0.80, 0.80g/kg body weight respectively. (17)

**Confounders and key covariates**

Statistical models were adjusted for variables that might potentially confound the association of protein intake with later adiposity, given their hypothesized associations with both protein intake and body composition. Models also included variables that were strongly associated with body composition at age 22 to clarify whether observed associations between protein intake trajectories and body composition were independent of these variables. We included sex, early life factors (birth weight (kg), maternal education (years), maternal height (cm), duration of breastfeeding (months), household assets at age 2 and offspring BMI (kg/m$^2$) at age 2) and variables related to the offspring at age 22 (household assets, education (years), energy intake and residuals of carbohydrate and fat intake. The composite score of household assets ranged from 0 to 11 and was based on individual possession of the following: electricity,
house, material of house, air conditioner, television, tape recorder, refrigerator, electric fan, jeepneys (customized taxi jeeps), car and clothing iron.

**Primary Outcome**

Body mass index (kg/m$^2$) at age 22 ($\text{BMI}_{\text{age22}}$) was the primary outcome. Lean mass and fat mass at age 22 were secondary outcomes. Weight, height and skinfold thicknesses were measured by trained personnel. Body fat percentage was calculated from skinfold thicknesses using equations validated in Asian populations.$^{(87)}$ Fat mass (kg) was then calculated as % body fat*weight (kg); lean mass (kg) was the difference between body weight and fat mass. All three outcomes were stratified by sex and then standardized to aid in interpretability across estimates. The kg or kg/m$^2$ equivalents are provided in-text for context. Pregnant women were omitted from all analyses of body composition.

**Statistical Analyses**

**Body composition at age 22 as a function of age-specific protein intake**

To assess the relationship of age-specific excess protein intakes with body composition at 22y, multivariable linear regression analyses were fit with excess protein intakes (%) at each age simultaneously included as the primary predictors. Three models were executed with BMI, lean mass and fat mass as the outcomes. Product terms between sex and excess protein intake were included to assess sex-specific associations. Analyses were adjusted for the above-described anthropometric, dietary and socioeconomic from early life factors.

**Derivation of protein intake trajectories**

The second research question in this study relies on finite mixture models which classified heterogeneous excess protein intake (g/kg body weight) patterns from 2-22y into a finite number
of latent growth curves or trajectories. This approach assumes homogenous protein intake within each trajectory and that individuals assigned to the same trajectory are different from individuals assigned to other trajectories.\(^{(88,89)}\)

An a priori criterion was established to select the optimal number of trajectories. The main considerations were i) Parsimony: the simplest number of trajectories to describe meaningful patterns in the data so no more than 5 groups would be tested, ii) Reasonable subset sizes: trajectories would have at least 3% of the cohort to reduce the possibility of capturing idiosyncratic or anomalous protein intake patterns\(^{(90)}\) and iii) Bayesian information Criteria (BIC), which consider sample size. For a given model with \(n\) groups, third-order (cubic) trajectories of excess protein intake in grams/kg body weight were first specified and this order was then reduced if p values indicated that the higher order term was unnecessary (p>0.10).\(^{(91,92)}\) A similar process was used to select the optimal orders for a model with \(n+1\) trajectories. Next, the a priori criteria were used to select the final model. Trajectories were derived using the traj command in Stata 14.\(^{(88)}\)

**Characteristics of each trajectory**

One-way ANOVA and chi-squared tests were employed to assess whether the distribution of covariate characteristics in early life varied significantly by trajectory membership.

**Estimation of BMI at age 22 as a function of trajectory membership**

Multivariable linear regression models were also used to estimate the association of trajectory membership with lean mass\(_{age22}\), fat mass\(_{age22}\) and BMI\(_{age22}\). Product terms between sex and protein intake trajectories were included to elucidate sex-specific associations. Analyses were similarly adjusted for anthropometric, dietary and socioeconomic factors from early life.
and adulthood. Post-estimation Wald tests were then conducted to compare outcomes between trajectories for each sex. In each case, the null hypothesis was that the outcomes for that sex did not vary by trajectory membership, for example, Girls’ BMI trajectory A - Girls’ BMI referent trajectory = 0.

Results

Descriptive demographic and dietary details in the CLHNS

Mothers in the CLHNS had received ~8 years of formal education at the time of the offspring’s birth (Table 4). The sample had a relatively low urbanicity score in early life (urbanicity ~31 and 28 at birth and 2y respectively out of a possible range of 0-70) but, due to migration to more urban areas as well as urbanization of all communities, the sample became more urban by age 22 (urbanicity ~41). Mean duration of breastfeeding was 12.4 months. At age 22y, offspring had spent an average of 11 years in formal education, and had an average BMI of 21kg/m², lean mass of 39.4kg and fat mass of 12.3kg. Mean protein intake exceeded the RDAs at every survey: RDAs used for years 2, 11, 15, 19 and 22 were 1.05, 0.95, 0.85, 0.80, 0.80g/kg body weight respectively (17) while mean intakes ± SD were 2.04± 1.05, 1.96± 0.96, 1.10± 0.49, 1.36± 0.63 and 1.44± 0.67 g/kg body weight at those ages respectively. Across all surveys, the top three food groups of contributing to protein intake were meat and poultry, seafood and grains (Figure 1).

Age-specific intakes of protein were differentially associated with body composition at age 22

Protein intake was differentially associated with body composition in an age-dependent manner (Panel A, Figure 4. As displayed in Figure 4, a 20% higher intake of protein (relative to age-specific recommended daily allowance in g/kg body weight) consumed at age 2 was modestly but positively associated with girls’ BMI (Beta (95%CI) was 0.020 (0.006, 0.033), the
equivalent to 0.06kg/m² in girls’ BMI) and lean mass (0.014 (0.001, 0.028) or 0.06kg in girls’ BMI) at 22y.

At all other ages, protein intake in excess of needs was inversely associated with body composition at age 22 and the strength and magnitude of these associations varied with age (Figure 4). The largest of these estimated associations were for protein consumed at age 22. A 20% higher intake of protein relative to needs was associated with lower lean mass (-0.147 (-0.165, -0.129) or -0.31kg in girls and -0.175 (-0.197, -0.153) or -0.51kg in boys), fat mass (-0.156 (-0.175, -0.136) or 0.36kg in girls and -0.161 (-0.184, -0.137) or 0.36kg in boys) and BMI at 22y (-0.164 (-0.184, -0.145) or 0.27kg/m² and -0.177 (-0.201, -0.153) or -0.26kg/m² in girls and boys respectively).

**Derivation of protein intake trajectories using latent class growth curve analyses**

The 4-trajectory model was selected because it had optimal parsimony, interpretability and fit statistics (BIC=-12006.93). Spaghetti plots from random subsets of each trajectory are displayed (Figure 5A-2D). All latent trajectories of mean protein intake for the 2586 individuals in the CLHNS are shown in Figure 5F. The referent trajectory (58% of the sample) was characterized by ‘normal consumers’ whose protein intake was just slightly higher than recommended (<1g/kg above RDA) (Figure 5A). The second trajectory (20%) was characterized by ‘high consumers during infancy’ who an average of 2g/kg above RDA at age 2y and relatively normal intakes thereafter (Figure 5B). The ‘usually high consumers’ (18%) had excess protein intakes at ages 2, 15 and 22y (~1g/kg above RDA) and markedly high intakes at age 11 (2g/kg above RDA at age 2y) (Figure 5C). The smallest trajectory (5%) was characterized by ‘always high consumers’ who had extremely high protein intake at age 2 (3.5g/kg above RDA) and high protein intakes (1-2g/kg above RDA) thereafter (Figure 5D). All mean intakes for
derived trajectories were in excess needs (Figure 5E) but though trends were similar for trajectories of absolute protein intake (with normal consumers generally consuming the lowest grams of protein in each survey) the distinctions were not as marked (Figure 5F).

*Early life characteristics vary by trajectory*

   Early life characteristics varied significantly by trajectory (Table 6). ‘Normal consumers’ lived in households with fewer assets at age 2, lived in more rural communities, and had the longest average duration of breastfeeding. Maternal education at baseline was highest for the groups of ‘high consumers during infancy’ and ‘always high’ consumers. The ‘usually high’ consumers had a disproportionately more males while members of ‘always high’ consumers had the lowest mean birth weight and age-2 BMI.

*Trajectories of excess protein intake are differentially associated with BMI at age 22*

   Trajectory membership was differentially associated with body composition at age 22 (Figure 6). Female or male normal consumers served as the referent category in the sex-specific contrasts. ‘Usually high’ consumption was associated with lower BMI (Beta (95% CI)= -0.241 (-0.008, -0.403) equivalent to -1.08kg/m² and -0.337 (-0.154, -0.553) or -0.77kg/m² in males and females respectively) (Figure 6). ‘Usually high’ consumption was also associated with lower fat mass (-0.167 (-0.326, -0.008) or -1.72kg and -0.373 (-0.592, -0.154) or -0.77kg) and lower lean mass (-0.386 (-0.403, -0.080) or -1.71kg and -0.295 (-0.553, -0.121) or -2.24kg) in boys and girls respectively. ‘Always high’ consumption was associated with lower lean mass in boys (-0.442 (-0.629, 0.092) or -2.56kg) and lower lean mass (-0.368 (-0.622, 0.167) or -2.14kg) and fat mass (-0.336 (-0.732, 0.059) or -1.55kg) in girls although these girls’ estimates were not statistically significant (p<0.10). In both sexes, high consumers during infancy were similar to normal consumers when age 22 BMI, lean mass and fat mass were compared. Similar inferences were
drawn when the analytical sample was restricted to participants with data from at least 3 of 5 protein survey rounds.

Discussion

In this study, protein intake from age 2 to 22y was associated with body composition at age 22. Early childhood protein intakes were positively associated with later BMI and lean mass. However, protein intake from age 11-22 was inversely associated with later BMI, lean mass and fat mass. Of the four latent trajectories identified, trajectories marked by high adolescent and adult intakes of protein were associated with reduced BMI, lean mass and fat mass at age 22. These life course investigations suggest that compared to the modest effects of early life protein intakes, adolescent and adult protein intakes are more strongly associated with later body composition.

A growing body of literature has associated protein intake in the vulnerable period of infancy (<12 months) with risk of later obesity in childhood or even adulthood.\cite{20,22,23,33,35} For example, protein intake (g/kcal) from 9-12mo was associated with higher BMI in 6-y-old Icelandic boys\cite{93} while German boys with high intakes of protein (g/kcal) at 12 months had higher BMI at age 7.\cite{32} Our current findings lend credence to the early protein- later BMI hypothesis—even in a low-income population marked by high rates of stunting and undernutrition.

There are noteworthy caveats pertaining to our analysis of the early protein hypothesis in this cohort. Firstly, we identified this modest but positive association of protein consumed at a later time point of age 2y; thus the window during which protein programs long-term effects on body size may be wider than previously thought. Secondly, published studies conducted associations with BMI (not body composition); it is unclear whether their findings showcase true
effects of early protein on adiposity. Our results indicate that the higher BMI was due to increases in lean mass, but not fat mass so individuals were larger but not necessarily more adipose. In this low-income cohort, this might also translate to individuals who were better nourished in early life being less stunted and underweight as adults. Infant protein intake also programs linear growth, an association potentially mediated by the insulin-like growth factor. A recent study in this Cebu cohort did find that childhood protein intakes (grams) were positively associated with attained height at age 22. Since protein is associated with both lean and fat mass, it is important to explicitly investigate whether associations with BMI are due to lean mass, fat mass or both, as we did here.

A third caveat is that instead of expressing protein intake in absolute grams or percentiles we deliberately chose to express protein in grams/kg body weight relative to the RDA. This is a strength of our analyses since this specification more appropriately captures whether intakes were truly high or low relative to needs. However, this difference may also explain why the results were not fully concordant with prior literature, which mostly expressed protein intake in absolute grams or percentiles. Finally, this Filipino cohort experienced high wasting and stunting prevalence and diets much lower in high-quality animal protein and rich in energy from grain-based porridges. Differences in protein source and/ or quality presumably drive the associations of protein intake with height and body size. So although protein intakes in this cohort typically exceeded the RDA, they may not truly be in excess of needs; higher early childhood intakes may simply signify better nourishment, rather than over-nourishment as it does in high-income settings. The dietary differences in our cohort may have further contributed to the null association of early protein intake with later fat mass and the significant association with BMI and lean mass.
Though our findings do support long-term positive associations of early protein intake with later body composition, our analyses show that protein intakes (from ages 11-22) were strongly and inversely associated with lean mass, fat mass and BMI. In contrast, a study from a German cohort showed that protein intake post-infancy was positively related to later lean mass: women’s fat free mass at 18-25y tended to increase by tertile of pubertal (between 9-15 years) energy-adjusted animal protein intake. (94) They reported similar findings for animal protein intake around the adiposity rebound and men’s adulthood fat free mass. (94) Though we could not include the period of the adiposity rebound period, our analyses suggested an inverse association of intakes after 2y with lean mass. The discrepancies may result from their focus on energy-adjusted animal protein intakes whereas we only reported on total protein intakes relative to needs. However, both results do suggest that adolescent protein does play a role in explaining body composition, and future studies should clarify how protein quality plays into these associations.

Our trajectory analyses also suggest that adolescent and adult intakes drove differences in body composition: trajectories characterized by higher protein intakes at age 22y were associated with lower BMI, fat mass and lean mass but body composition of the high infant trajectory was similar to the normal consumption category, presumably because they had low protein intakes at age 22y. Future studies that explore long-term effects of early life experiences should seek to explicitly interrogate and compare these to the effects of later exposures. The magnitude of these early life exposures may be less relevant in terms of their magnitude, and are certainly less actionable compared to adolescent or adult diet as it pertains to adult health.

These findings must be contextualized within the greater limitations of this study. Firstly, since this study was conducted in an observational cohort, the findings presented here cannot be
deemed to be causal—we attempted to control for factors that were related to both the outcomes and predictors but it is possible that omitted factors (such as the quality or source of protein) or poorly measured confounders may leave our estimates residually confounded. Potential for bias also comes from attrition in this 22-year longitudinal study. In this rapidly urbanizing society, the study sample decreased from 3080 at birth, to 2456 individuals at 24 months and 1885 participants at 22 years. However, other work which included the CLHNS found little potential for attrition bias when they compared findings from Heckman-weighted selection models to their unweighted analyses of the association of early growth rates with later markers of metabolic risk (such as BMI). A third limitation is that we only used dietary data from the 2 to 22y period. Ideally, we would have included infant intakes to be consistent with other DOHaD studies of the early protein hypothesis. Since breast milk nutrients were not quantified, the use of infant nutrient data would have seriously underestimated total protein intake. More complete data for infancy and more surveys between 2y and 11y data may have improved trajectory derivation and strengthened these life course analyses. Finally, the latent class trajectories derived here are highly driven by the unique socioeconomic and dietary characteristics of this population. While the insights are valuable, they may not be generalizable to other populations.

Despite its limitations, this is an important methodological contribution to the life course epidemiology literature. Ours is one of few studies to innovatively classify longitudinal dietary exposures and relate them to later health. Only one other study has analyzed trajectories of protein; in this German cohort, researchers classified individuals into trajectories of below/above the median protein intakes at ages 12 and 18-24 months. They did find that those who with consistently high protein at those two time points had significantly greater standardized BMI and percentage body fat at age 7y. This German study did capture more of early life exposures
than we covered here, however, the crude derivation of trajectories, short follow-up period and childhood outcome ascertainment clearly limits its generalizability. To our knowledge, only one other study has analyzed a macronutrient across such a wide breadth of the life course: researchers used cluster analysis to isolate fat intake patterns from 2-18 years in a German cohort\(^{(95)}\); they later found that these patterns did not explain later BMI.\(^{(96)}\) Another study of Chinese adults used latent class trajectory analysis to group individuals according to a dietary pattern score, they found that those with healthier dietary patterns had lower hemoglobin A1c, and when trajectories with recent healthy dietary patterns were compared, those who had prolonged recent exposure to a healthy dietary pattern had lower hemoglobin A1c compared to those who whose trajectory showed recent improvements in dietary pattern.\(^{(97)}\) These longitudinal perspectives add meaningful insights into the cumulative role of diet in programming chronic disease risk. Moreover, a novelty of our study is that we were able to demonstrate how both varied patterns of protein intake and period-specific exposures explain later body composition within a single cohort.

In the future, similar studies conducted with more repeated measures, more complete dietary data, in different socioeconomic contexts and across longer follow-up periods may help us to isolate additional vulnerable windows and nutrient intake patterns that are significantly associated with later health and disease.
Table 4 Mean* of select demographic and dietary characteristics from birth to 22y CLHNS (1983-2002)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Birth</th>
<th>Age 2y</th>
<th>Age 11y</th>
<th>Age 15y</th>
<th>Age 19y</th>
<th>Age 22y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>3080</td>
<td>2456</td>
<td>2181</td>
<td>2089</td>
<td>2023</td>
</tr>
<tr>
<td>Maternal education (years)</td>
<td>7.56 ± 3.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>150.56 ± 5.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household Assets (score ranging 1-11)</td>
<td>2.51 ± 1.94</td>
<td>2.65 ± 1.96</td>
<td></td>
<td></td>
<td>5.23 ± 2.03</td>
<td></td>
</tr>
<tr>
<td>Household Urbanicity (score ranging from 0-70)</td>
<td>30.58 ± 12.61</td>
<td>28.41 ± 13.69</td>
<td></td>
<td></td>
<td>41.04 ± 13.35</td>
<td></td>
</tr>
<tr>
<td>Offspring weight (kg)</td>
<td>3.00 ± 0.42</td>
<td>9.78 ± 1.23</td>
<td></td>
<td></td>
<td>51.62 ± 9.99</td>
<td></td>
</tr>
<tr>
<td>Offspring length/height (cm)</td>
<td>49.07 ± 2.07</td>
<td>79.15 ± 3.68</td>
<td></td>
<td></td>
<td>157.44 ± 8.21</td>
<td></td>
</tr>
<tr>
<td>Offspring BMI (kg/m²)</td>
<td>12.39 ± 1.38</td>
<td>15.56 ± 1.23</td>
<td>15.75 ± 2.06</td>
<td>18.69 ± 2.48</td>
<td>20.11 ± 2.76</td>
<td>20.74 ± 3.15</td>
</tr>
<tr>
<td>Offspring lean mass (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.36 ± 9.22</td>
<td></td>
</tr>
<tr>
<td>Offspring fat mass (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.27 ± 5.36</td>
<td></td>
</tr>
<tr>
<td>Offspring education (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.84 ± 3.65</td>
<td></td>
</tr>
<tr>
<td>Total months of breastfeeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.39 ± 8.32</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52.99</td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>713.58 ± 359.34</td>
<td>1719.72 ± 821.95</td>
<td>1587.99 ± 672.83</td>
<td>1871.96 ± 820.84</td>
<td>1940.12 ± 832.18</td>
<td></td>
</tr>
<tr>
<td>Protein (g/kcal body weight)</td>
<td>2.04 ± 1.05</td>
<td>1.96 ± 0.96</td>
<td>1.10 ± 0.49</td>
<td>1.36 ± 0.63</td>
<td>1.44 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>16.94 ± 11.80</td>
<td>54.58 ± 14.60</td>
<td>26.36</td>
<td>48.46 ± 20.67</td>
<td>67.01 ± 32.30</td>
<td>73.23 ± 34.66</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>11.80 ± 127.36</td>
<td>24.32 ± 200.27</td>
<td>20.67</td>
<td>41.43 ± 25.23</td>
<td>61.11 ± 32.50</td>
<td>51.68 ± 39.87</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>51.69 ± 14.60</td>
<td>79.47 ± 25.23</td>
<td>10.58</td>
<td>101.58 ± 259.95</td>
<td>111.12 ± 263.62</td>
<td>109.31</td>
</tr>
</tbody>
</table>

*Mean ± SD unless otherwise indicated
Table 5 Results of linear regression of protein intake on standardized BMI, lean mass and fat mass at age 22y

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Standardized BMI</th>
<th>Standardized lean mass</th>
<th>Standardized fat mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>95% CI</td>
<td>Beta</td>
</tr>
<tr>
<td>A</td>
<td>Linear regression of age-specific protein intake on body composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI at Age 2y</td>
<td>0.010*</td>
<td>[0.003,0.017]</td>
<td>0.007*</td>
</tr>
<tr>
<td>PI at Age 2y* male</td>
<td>-0.007</td>
<td>[-0.016,0.002]</td>
<td>-0.006</td>
</tr>
<tr>
<td>PI at Age 11y</td>
<td>-0.010*</td>
<td>[-0.017,-0.003]</td>
<td>-0.009*</td>
</tr>
<tr>
<td>PI at Age 11y* male</td>
<td>0.009*</td>
<td>[0.001,0.018]</td>
<td>0.003</td>
</tr>
<tr>
<td>PI at Age 15y</td>
<td>-0.014*</td>
<td>[-0.026,-0.001]</td>
<td>-0.020*</td>
</tr>
<tr>
<td>PI at Age 15y* male</td>
<td>-0.006</td>
<td>[-0.022,0.011]</td>
<td>-0.009</td>
</tr>
<tr>
<td>PI at Age 19y</td>
<td>-0.010*</td>
<td>[-0.019,-0.002]</td>
<td>-0.015*</td>
</tr>
<tr>
<td>PI at Age 19y* male</td>
<td>0.010+</td>
<td>[-0.002,0.022]</td>
<td>0.011*</td>
</tr>
<tr>
<td>PI at Age 22y</td>
<td>-0.082*</td>
<td>[-0.092,-0.072]</td>
<td>-0.074*</td>
</tr>
<tr>
<td>PI at Age 22y* male</td>
<td>-0.006</td>
<td>[-0.018,0.005]</td>
<td>-0.014*</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.241+</td>
<td>[-0.500,0.019]</td>
<td>0.154</td>
</tr>
<tr>
<td>B</td>
<td>Linear regression of trajectories of protein intake on body composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female normal consumers</td>
<td>referent</td>
<td>n/a</td>
<td>referent</td>
</tr>
<tr>
<td>Female usually high consumers</td>
<td>0.337*</td>
<td>[-0.553,-0.121]</td>
<td>0.295*</td>
</tr>
<tr>
<td>Female high consumers in infancy</td>
<td>-0.083</td>
<td>[-0.264,0.098]</td>
<td>-0.021</td>
</tr>
<tr>
<td>Female always high consumers</td>
<td>-0.228</td>
<td>[-0.622,0.167]</td>
<td>0.368+</td>
</tr>
<tr>
<td>Male normal consumers</td>
<td>0.103</td>
<td>[-0.129,0.335]</td>
<td>0.075</td>
</tr>
<tr>
<td>Male usually High consumers</td>
<td>-0.139</td>
<td>[-0.388,0.111]</td>
<td>-0.311*</td>
</tr>
<tr>
<td>Male high consumers in infancy</td>
<td>0.138</td>
<td>[-0.109,0.385]</td>
<td>0.093</td>
</tr>
<tr>
<td>Male always high consumers</td>
<td>-0.166</td>
<td>[-0.566,0.235]</td>
<td>0.367+</td>
</tr>
<tr>
<td>N</td>
<td>1635</td>
<td>1607</td>
<td>1607</td>
</tr>
</tbody>
</table>
Panel A shows the linear regression of age-specific protein intakes on body composition; all age-specific intakes were simultaneously entered into the linear regression model. Panel B shows the regression of trajectories of protein intake on body composition. *p<0.05 + p<0.10. PI= excess protein intake as a percentage of RDA in 10% units, i.e. 10*(Protein intake at indicated age– age-specific RDA in grams/kg body weight)/(age-specific RDA); Adjusted for characteristics at birth (offspring weight, maternal education and maternal height), characteristics at age 2 (offspring BMI and household assets), characteristics from age 22 (offspring education and assets, carbohydrate residuals, fat residuals and energy intake)
<table>
<thead>
<tr>
<th>Trajectory of excess PI</th>
<th>Maternal education at birth (years)</th>
<th>Maternal height at birth (cm)</th>
<th>Infant weight at birth (kg)</th>
<th>Male</th>
<th>BMI at age 2y (kg/m²)</th>
<th>Assets at age 2y</th>
<th>Urbanicity at age 2</th>
<th>Breastfeeding duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>7.51</td>
<td>150.63</td>
<td>3.00</td>
<td>0.52</td>
<td>15.56</td>
<td>2.65</td>
<td>28.42</td>
<td>13.53</td>
</tr>
<tr>
<td>Normal consumers</td>
<td>6.61</td>
<td>150.46</td>
<td>3.01</td>
<td>0.50</td>
<td>15.59</td>
<td>2.23</td>
<td>25.47</td>
<td>15.06</td>
</tr>
<tr>
<td>High consumers in infancy</td>
<td>9.26</td>
<td>151.17</td>
<td>3.01</td>
<td>0.50</td>
<td>15.59</td>
<td>3.46</td>
<td>33.27</td>
<td>10.6</td>
</tr>
<tr>
<td>Usually high consumers</td>
<td>8.38</td>
<td>150.64</td>
<td>2.98</td>
<td>0.65</td>
<td>15.55</td>
<td>3.00</td>
<td>32.74</td>
<td>12.12</td>
</tr>
<tr>
<td>Always high consumers</td>
<td>10.11</td>
<td>150.68</td>
<td>2.85</td>
<td>0.50</td>
<td>15.03</td>
<td>3.92</td>
<td>34.66</td>
<td>8.5</td>
</tr>
<tr>
<td>F statistic*</td>
<td>102.99</td>
<td>2.56</td>
<td>5.01</td>
<td>28.01</td>
<td>6.35</td>
<td>75.18</td>
<td>67.17</td>
<td>61.01</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>0.053</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*ANOVA for continuous variables or chi-squared test for categorical variables (sex)
Figure 4 Excess protein intakes were differentially associated with fat mass, lean mass and BMI at age 22y in an age-dependent manner.

*p<0.05  +p<0.10. Each bar represents the change associated with a 20% increase in protein intake relative to needs at the indicated age. Coefficients were derived from the linear regression of age-specific protein intakes on body composition, adjusted for characteristics at birth (offspring weight, maternal education and maternal height), characteristics at age 2 (offspring BMI and household assets), characteristics from age 22 (offspring education and assets, carbohydrate residuals, fat residuals and energy intake).
Figure 5 Trajectories of excess protein intake from 2 to 22 years in the CLHNS (n=2586).
Panels A to D each show spaghetti plots of excess protein intake (g/kg body weight) for randomly selected individuals for each of the 4 trajectories derived from the latent class growth
curve analyses. Panel A shows a subset of the ‘normal consumers’ who constituted 58% of the sample. Panel B are the ‘high consumers during infancy’ (20%). Panel C shows the ‘usually high consumers’ (18%). Panel D shows the ‘always high consumers’ (5%). Panel E shows mean excess protein intake (g/kg body weight) by trajectory with 95% CI. Panel F shows mean protein intake in grams with standard deviations.
Figure 6 Trajectories of protein intake are differentially associated with BMI, lean mass and fat mass at age 22y

*p<0.05, +p<0.10. Coefficients are derived from the regression of trajectories of protein intake on body composition, adjusted for characteristics at birth (offspring weight, maternal education and maternal height), characteristics at age 2 (offspring BMI and household assets), characteristics from age 22 (offspring education and assets, carbohydrate residuals, fat residuals and energy intake). Standardized outcomes for one sex in a given trajectory were compared to outcomes for the normal consumer trajectory for that sex.
CHAPTER 5. SYNTHESIS

Overview of Findings
The association of dietary protein intake with body size from birth to 22y was examined using multiple repeated measures of diet and anthropometry. There were three specific aims: i) to elucidate the association of protein with contemporaneous BMI from birth to 22y with random-effects longitudinal regression models, ii) to investigate the cumulative effects of dietary protein intake on later body composition using latent growth curve analyses and linear multivariable regression models, and iii) to estimate the differential effects of age-specific protein intake from 2-22y with body composition at age 22 using multivariable linear regression models. It was hypothesized that protein would promote contemporaneous higher BMI in infancy but would be inversely associated with contemporaneous BMI thereafter, that breastfeeding would modify these associations, and that associations would vary for protein from plant and animal sources. It was also expected that protein intake trajectories from age 2-22y would be differentially associated with body composition at 22y, and that age-specific protein intake would be independently associated with later body composition. This synthesis summarizes the gaps filled by these research findings, the general limitations these studies, exciting directions for future research as well as the public health significance and policy implications of this work.

Protein intake was associated with body mass index in an age-, and source-specific manner: results of random-effects longitudinal regression analyses from age 2 months to 22 years
Nearly one-third of the human population suffers from some form of malnutrition.\(^{(1)}\)

However, the literature provides exciting evidence of how protein intake across the life course
may help to modify risk of malnutrition. As it relates to undernutrition, protein is a positive contributor to infant nutritional status but the complementary feeding diets of children in vulnerable societies are often limiting in high-quality sources of this nutrient.\(^{(20)}\) In higher-income societies, this positive association between protein and nutritional status may become detrimental, potentially predisposing infants with high protein diets to obesity in childhood and later life.\(^{(27)}\) Furthermore, protein intake in adulthood has been associated with reduced risk of obesity and related illnesses.\(^{(52-54)}\) Disentangling these complex associations of protein with body size across different socioeconomic settings and in different age groups is therefore necessary for informing evidence-based interventions for mitigating malnutrition in both these forms.

An outstanding gap in the literature relates to the joint role of complementary protein and breastfeeding status in influencing growth in early life. The nuance is important--breastfeeding rates tend to be higher in lower socioeconomic settings and so it is important to analyze how breastfeeding and complementary protein diets influence BMI in infancy and beyond. As it relates to protein intake after infancy, there were few longitudinal studies of free-living participants that quantified the effect of protein on BMI and none of these were from LMICs. The aim 1 analyses were designed to elucidate these nuances of protein intake and body size in this Filipino birth cohort that suffered high prevalence of infant undernutrition but has shown signs of obesogenic diets in young adulthood.\(^{(16)}\)

Some of these findings were concordant with the extant literature from higher-income cohorts. Both breastfed and non-breastfed infants benefited from increased complementary protein, especially animal protein intake, in the first 24 months of life. In this population where 60% of infants were underweight or stunted by 24 months, the modest associations potentially mark the difference between being malnourished and having adequate weight or length-for age.
This research also revealed that while total protein intake was inversely related to BMI in adulthood, animal protein was actually a positive contributor to contemporaneous adult BMI. It is unclear whether this increase in BMI represented an increase tendency towards obesity in this relatively young and lean cohort. Other analyses in this cohort showed that protein contributed significantly to both weight and height from 8-22y,\(^{(82)}\) and protein-BMI associations were driven by changes in both lean and fat mass. Thus the findings beg for further clarifications on whether protein source and/or quality may affect BMI in ways that promote or reduce health risk.

**Trajectories of protein intake were differentially associated with later body mass index, lean mass and fat mass: results from the linear regression of body composition at 22y onto latent class-derived trajectories of protein intake from 2-22y**

The life course epidemiology posits that experiences and exposures across the life course may affect later health by exerting independent and/or cumulative effects on disease risk.\(^{(6)}\) The early protein hypothesis from the DOHaD literature posits that high protein diets in early life are detrimentally associated with later BMI.\(^{(20)}\) However, studies in adulthood suggest that adulthood protein intakes are inversely related to body composition,\(^{(52-54)}\) and the protein leveraging hypothesis provides plausible mechanisms by which this may occur.\(^{(47,98)}\)

Because there is documented evidence of how protein affects later BMI in an age-related manner, protein is an exceptional candidate for testing hypotheses about independent and cumulative effects of diet across the life course and later health. However, only one other study has analyzed trajectories of protein intake, and while they had infant protein intake patterns, they classified individuals into crude trajectories of below/above the median intakes and only childhood outcomes were measured.\(^{(31)}\) Thus the second aim was geared towards filling this gap in the literature using longer follow-up periods, adulthood anthropometry and a sophisticated statistical approach for classifying multiple repeated measures of protein intake.
Our aim 2 findings make three major contributions to the literature. Firstly, although there were heterogeneous patterns of protein intake throughout the entire period, average intakes for all the trajectories exceeded the recommended daily allowances for total protein. This total protein intake measure did not account for quality of protein consumed. For example, individuals in a given trajectory may have varying proportions of animal and plant protein intake. Aim 1 findings showed that protein source also drive differences in BMI so this finding must be cautiously interpreted. Secondly, while historic protein intake patterns were differentially associated with later body composition, these differences tended to be driven by more recent intake patterns, rather than the overall trajectory. This is an important contribution since it implies that while earlier experiences may have lasting effects, dietary modifications in later life (which are also more practical and feasible) potentially have more profound ramifications. With regards to testing the cumulative effects of distinct protein intake patterns, the data-driven LGCA approach gave no control over the resulting shapes and forms of the trajectories. Only one trajectory had consistently low protein intake throughout the 2-22y period, and this group had the highest body composition measures. Were this cohort generalizable to other populations, the results would imply that compared to relatively higher protein intakes, consuming protein at or just slightly above the RDAs was associated with higher BMI, lean mass and fat mass. Since lean mass is also positively associated with this trajectory, it is unclear whether the overall relation with chronic disease risk would be pathological.

The third contribution relates to the methodological approach, which is quite transferrable to answering other important public health questions. For example, there are implications for estimating risks in individuals who were chronically undernourished or chronically exposed to poor diets, or examining the effects of an undernourished childhood coupled with a westernized
diet pattern in later life. Such analyses can help to predict the obesity-related disease burden in transitional societies where such scenarios are increasingly common. This question may also provide evidence that clarify whether and how adherence to dietary or fitness guidelines across the life course ultimately relate to later chronic diseases.

Age-specific protein intakes are differentially associated with later body mass index, lean mass and fat mass: results from a linear regression of body composition at 22y onto age-specific protein intakes from 2-22y

Pursuant to the life course hypotheses, it was also prudent to test whether protein intakes at certain ages had differential associations with later body composition. This is because such an analysis facilitates the identification of sensitive periods, or vulnerable windows of the life course during which protein intake may program later body composition. Indeed, it is important to understand the independent and relative importance of protein from different periods of the life course, and thus potentially devise interventions that optimize health in the short and long term.

The DOHaD literature sheds a spotlight on the vulnerable period of infancy, but other vulnerable window may also exist. However, few studies examined how intakes in other potentially sensitive windows of the life course i.e. childhood, pubescence, and adolescence, may relate to later body composition. One study from a German cohort did investigate this question and found that protein intake in both infancy (12mo) and childhood (5-6y) were positively related to childhood BMI and fat mass (measured at 7y) but this analysis was obviously limited by the age of final follow-up, it is unclear whether the associations of those childhood-specific protein intakes persist past childhood and into adulthood. Only one other study suggested that pubertal intakes were related to adult body composition but it was from a higher-income socioeconomic context and it did not provide much detail on how distinct age-specific protein
intake relate to later body composition.\textsuperscript{(94)} The third aim was intended to fill these gaps by simultaneously estimating the associations of age-specific protein intakes from infancy to adulthood with adulthood body composition in an LMIC cohort.

The relatively long follow-up period and the richness of repeated dietary measures in the survey make our aim 3 a unique contribution to the literature. These findings suggested that within the Cebu cohort, protein intakes from age 2y were independently and positively associated with BMI at age 22y—this contribution is unique since most studies report these associations for earlier periods of infancy but this work suggests that in some populations this vulnerable period for the early protein hypothesis may actually be longer. Furthermore, it was evident that more recent (adolescent and adult) intakes made much larger contributions to body composition at age 22y. This finding implies that intervening upon adulthood protein intakes may be effective at mitigating obesity risk. Importantly, the decomposition of body mass into both lean and fat mass strongly underscored the importance of analyzing body composition rather than just BMI—results indicated that high protein intakes were inversely associated with both lean and fat mass, thus it is unlikely that these associations necessarily indicate reduced obesity-related chronic disease risk.

**Limitations**

Though these were interesting and novel findings, there are important limitations that must be considered. These limitations mainly relate to diet, the lack of generalizability due to the socioeconomic context and the observational nature of this cohort.

The CLHNS provided multiple repeated measures of dietary data across a relatively long follow-up period. However, having additional measures of the childhood diet may have added to the robustness of these estimations of protein with contemporaneous body mass (aim 1), may
have improved the accuracy in the derivation of protein intake trajectories (aim 3) and would have potentially granted insights around whether intakes around the time of the adiposity rebound differentially programmed long term body composition (aim 3).

A second but related limitation stems from the fact that nutrients from breastmilk were not quantified. Thus analyses were limited to the estimation of the effects of complementary protein intake. Although this limitation was somewhat circumvented by interacting complementary protein intake with breastfeeding frequency (aim 1), we cannot definitively compare this exposure or these estimates to those studies that investigated these associations in non-breastfed infants with more accurate measures of total protein intake. If these trajectories of protein intake included infancy then more heterogeneous and insightful patterns may have emerged (aim 2). It was also not feasible to explicitly test for the effects of high total protein intakes in infancy on later body composition (aim 3), which is unfortunate since we would may have been able to provide an answer for whether protein intakes from the first year of life were associated with body composition as late as 22y, in a socio-economic setting that is not well represented in the DOHaD studies on this topic.

A third shortfall of this work is one that is typical to all studies that rely on dietary data. Though 24-hour recalls are very useful dietary data collection tools, they are still subject to misreporting. Urinary nitrogen might provide a more accurate and objective measure of protein intake, but with biomarkers it is assumed individuals are in nitrogen balance—an assumption which is not well suited for life course analyses during which periods of rapid growth such as infancy or pubescence are of interest. Furthermore biomarkers are prohibitively expensive in such a large longitudinal study. When compared to urinary nitrogen, 24-hour recalls do tend to underestimate protein intakes, but the use of 2 recalls may still be reasonably
accurate for ranking individuals. (101)

The fourth limitation relates to the somewhat reductionist perspective employed here, by focusing on a single nutrient. The aim 1 analyses did attempt to crudely capture protein quality by investigating differential impacts of protein intake from plant and animal sources, however, to truly capture the degree to which protein excesses or deficits program long term risk, a better characterization of the exposure is crucial. Such protein quality scoring systems have been devised but are not readily available for the diversity of foods captured by the 24-hour recalls. (102,103) Moreover, although the focus on protein may have been appropriate given the complexities of modeling diet, these analyses ignored the potentially interactive roles of the myriad of other dietary constituents, dietary patterns and lifestyle behaviors that may also modify these relationships.

There are important socio-demographic characteristics in this cohort that limit its generalizability. Strong biological associations may exist for early protein and later adiposity—however these may only be evident in regions with greater variation in the quantity and quality of infant protein intake. This population was quite young. Obesity was rare. To more comprehensively estimate how early protein affects body composition in the long term, similar associations should be estimated other cohorts. It is likewise important to quantify these early protein associations in a population with higher rates of obesity or even obesity-related illnesses.

Additionally, these studies were prone to the analytical shortfalls inherent in all observational studies. Like most longitudinal studies, this cohort did suffer from attrition over the 22-year follow-up period. If the factors predisposing attrition are also related to protein intake and anthropometry, then this may bias the estimated associations. Hypothetical confounders were included, however, in any observational study there is still potential for residual confounding.
Altogether, though these estimates are only associational and are not causal, they are of potential public health significance and should be explored further in the literature.

**Strengths and Public health significance**

There are noteworthy strengths in this body of work. With the exception of the developmental origins literature, there is a paucity of studies that employ a life course framework to better clarify how nutritional exposures across the life course relate to health. The main research questions were designed to analyze the protein-body composition relationship from three life course perspectives. By exploiting the longitudinal richness of multiple repeated measures in a large observational birth cohort it was possible to provide insights into the effects of protein on body size in the short- and long-term, as well as methodological approaches for tackling similarly nuanced and complicated questions that help promote public health across the life course.

In addition to these contributions to the DOHaD and life course epidemiology literature, other strengths of this work lie in the careful attention paid to interactions and effect modification. By estimating interactions by sex, protein source and age, it was possible to identify the conditions under which the findings may be most relevant. Analyzing both lean mass and fat mass further clarified whether associations with BMI were truly driven by increases in adiposity and thus potentially detrimental for chronic disease risk.

Another strength of this work is related to its unique socio-economic context. While this characteristic limits its generalizability to high-income settings, it provides some evidence for the kinds of associations that may exist in LMICs. Examining the protein-adiposity hypothesis in a setting undergoing the nutrition transition provides clarity for which associations are likely the result of biological phenomenon (such as complementary protein and breastfeeding promoting...
infant growth) or whether they are also strongly guided by socio-economically driven differences in diet quality (such as protein fostering long-term positive effects on later obesity risk).

Altogether, the methodologies employed and the substantive research questions answered contribute significantly to the literature and have practical relevance to public health. The findings for each aim have practical implications for the refinement of dietary recommendations.

In aim 1, the positive effects of animal protein were clearly demonstrated for BMI throughout the study period. This reinforces the importance of recommending animal-source foods during the complementary feeding period to stave off undernutrition. In fact, interventions that specifically recommend including animal-source foods in the complementary diet work better to promote diet quality and infant nutritional status compared to vague recommendations to increase protein.\(^{84,85}\) This finding thus underscores the WHO recommendations on appropriate complementary feeding, where animal-source foods are promoted for higher protein quality and micronutrients.\(^{86}\) The post-infancy findings seem concordant with other research which shows positive effects of animal protein intake with BMI and protective or inverse effects of plant protein in adolescence and thereafter.\(^{53,54}\) Further investigations are needed to see whether these nuances for plant and animal protein are independent of overall dietary pattern, and whether specific guidelines should be devised to guide consumers on choosing the optimal source of protein which promotes normal BMI.

In aim 2, the latent growth curves protein intakes were well in excess of theoretical needs but the findings suggested that higher-protein trajectories were associated with lower BMI. Public health recommendations should not be based solely on findings within this special socio-demographic context so future studies should focus on clarifying how both protein quality \textit{and} quantity influence body composition. Should future studies concord with the work reported here
then refined recommendations on protein intake might be in order, especially for populations prone to obesity.

Aim 3 provides a framework for defining actionable windows. As it relates to early life, the evidence presented here may inform guidelines that increase protein intakes just enough to foster growth and development but not so excessive that they program biological processes that may promote later obesity risk. The analysis of age-specific intakes showed that while early life protein was independently and positively associated with later body composition, more recent protein intakes exerted a much stronger and inverse effect on later body composition. This is important as it supports conventional approaches to modifying disease risk, i.e., that optimizing protein intake in the adult diet might protect against adult obesity.

**Conclusion**

This is the most comprehensive attempt to characterize the effects of protein intake on body composition from infancy to adulthood. These results should yield valuable insights regarding the role of dietary protein at particular periods of the life course in mitigating contemporaneous as well as future health risk. The work is especially relevant since protein is one macronutrient which may potentially be manipulated to mitigate risk of both undernutrition and overnutrition- both forms of malnutrition that plague all modern societies.
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