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The Relationship between the Source of Protein Intake and Obesity Risk in Children

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ACCEPTANCE

This thesis, THE RELATIONSHIP BETWEEN THE SOURCE OF PROTEIN INTAKE AND OBESITY RISK IN CHILDREN, by Grace Stuhrman, was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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ABSTRACT

THE RELATIONSHIP BETWEEN THE SOURCE OF PROTEIN INTAKE AND OBESITY RISK IN CHILDREN

by

Grace Stuhrman

Background: Previous research has reported a relationship between high protein intake (>15% of energy) during early childhood and an increased risk of obesity later in life. However, few studies have investigated this relationship during middle childhood to early adolescence or examined the effects of different sources of protein.

Objective: The aim of this study was to investigate the relationship between the source of protein intake (animal vs. plant) and body mass index (BMI) in children between the ages 6-14 years.

Participants/setting: 285 healthy 6-14 year old (male *n*=154) Caucasian and African American (*n*=171) children from Pittsburgh, Pennsylvania completed a food frequency questionnaire.

Main outcome measures: Median protein intake (grams) by total, animal, and plant protein and BMI-for-age classification.

Statistical analysis: The Kruskal-Wallis test was used to evaluate differences in median protein intake (grams) by weight classification (normal weight [BMI 5^{th}%ile to <85^{th}%ile], overweight [BMI 85^{th}%tile to <95^{th}%tile], obese [BMI ≥95^{th}%tile]). Correlation statistics were also conducted to examine the relationship between protein intake and BMI.

Results: The population used in the data analysis included 285 children/early adolescents (median age 9.8 ± 2.1 years; 53% boys; 40% Caucasian). Data from 11 children were excluded due to outliers or missing data. Girls had a significantly higher BMI than boys (20.1 vs. 18.2 kg/m^2, respectively; *P*=<0.001). The majority (62%) of participants had a normal BMI while 20% of participants were overweight and 18% of participants were obese. No difference in the source of protein intake was observed by gender. However, median protein intake differed significantly by weight classification for total protein and for animal and plant protein (*P*=<0.05). Reported total protein intake was adequate for all children. Calorie intake was sufficient in children who were of normal weight or overweight but was below the requirement for age in those who were obese. The percent of total protein calories but not animal or plant protein differed by BMI weight classification (normal, 15.3%; overweight and obese, 14.6%; *P*=<0.01). Correlation statistics showed no association between the amount of total protein or animal or plant protein intake and BMI.

Conclusions: We observed a significant curvilinear versus linear trend in total protein and animal and plant protein intake by weight classification in middle-aged children that may be due to under-reporting in overweight and obese children. Total percent protein intake was significantly higher in children of normal weight. Future longitudinal studies using multiple measures of body fatness should be conducted to determine the relationship between protein intake and BMI during middle childhood to early adolescence.

THE RELATIONSHIP BETWEEN THE SOURCE OF PROTEIN INTAKE AND OBESITY RISK IN CHILDREN

by
Grace Stuhrman

A Thesis

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ABBREVIATIONS

%tile	Percentile
25(OH)D	25- hydroxyvitamin D
ANOVA	Analysis of variance
AR	Adiposity Rebound
BDHQCA	Brief Self-Administered Diet History Questionnaire for Japanese Children and Adolescents
BF%	Body Fat Percentage
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
cm	centimeter
D3	vitamin D - cholecalciferol
DBSC	Diets of British School Children
DONALD	Dortmund Nutritional and Anthropometric Longitudinally Designed
DLW	Doubly Labeled Water
DRIs	Dietary Reference Intakes
FFQ	Food Frequency Questionnaire
g	gram
HELENA-CSS	Healthy Lifestyle in Europe by Nutrition in Adolescence-Cross Sectional Study

HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
IDAA	Indispensible amino acid
IGF-1	Insulin-like growth factor 1
iRIS	Integrated Research Information System
IU	International unit
kcal	kilocalorie
kg	kilogram
LDL	Low-density lipoprotein
m	meter
NCD	Non-Communicable Diseases
NDNS	National Diet Nutrition Survey
PA	Physical Activity
PAL	Physical Activity Level
PCC	Primary Care Center
PDCAAS	Protein Digestibility-Corrected Amino Acid Score
RDA	Recommended Dietary Allowance
SES	Socioeconomic status
SDS	Standard Deviation Score
SF	skin-fold thickness
TC	Total cholesterol
YANA-C	Young Adolescents' Nutrition Assessment on Computer
YAQ	Youth Adolescent Questionnaire

YCNA-W	Young Children's Nutrient Assessment on the Web
yr	year
QAT	Quality Assessment Tool

CHAPTER I

Introduction

Protein intake during childhood has a paramount effect on growth and development.[1] Protein is an essential macronutrient necessary for building and repairing tissues, defending the immune system, coordinating cellular activity, serving as a catalyst, and providing energy. There are many factors ranging from food availability to absorptive capacity that affect the quantity and quality of a child's intake of protein. Animal sources of dietary protein include meat, eggs, cheese, fish, and dairy.[2] Protein is also found in plant products such as nuts, seeds, grains, legumes, beans, and vegetables. In general, animal protein varies from plant protein in that it has a higher saturated fat content and contains cholesterol. Plant protein is higher in fiber and carbohydrates. Vegetarian diets, which are typically high in plant proteins, are associated with health advantages such as lower blood cholesterol levels, lower risk of heart disease, lower body mass index (BMI), lower overall cancer rates, lower blood pressure levels, and lower risk of hypertension and type 2 diabetes.[3]

Scoring systems have been developed to determine the quality of a dietary protein source by quantifying the amino acids profile.[4] The protein digestibility-corrected amino acid score (PDCAAS) is a measurement of the protein quality in human nutrition.[5] A PDCAA score is determined by taking the first limiting amino acid in 1 g of the test protein over the same amino acid in 1 g of the reference protein and corrected for fecal digestibility of the test protein. The higher the PDCAA score, the higher amino acid profile and quality of protein. Animal proteins (i.e., egg) and purified plant proteins (i.e., soy) have higher scores than plant proteins (i.e., wheat) with intact cell walls. Various plant proteins consumed throughout the day can provide all indispensible amino acids and adequate nitrogen retention. However, some plant foods are low in specific amino acids, such as lysine in cereal, and can be problematic when other protein containing foods are not consumed.[6]

The effect of a diet higher in animal protein versus plant protein on BMI in children 6-14 years of age is unknown. Previous studies have primarily focused on overall protein intake during infancy and early childhood.[7,8,9,10, 11,12,13] The type of protein in regards to animal vs. plant-based protein and its relationship to BMI in children has not been thoroughly investigated. However, studies have found that a high protein intake (>15% of energy) during early childhood may increase the risk of obesity. Furthermore, a high protein intake during certain periods of childhood (12 months, 18-24 months, and 5-6 years) results in an increased BMI years later and even into adulthood. A child with a high BMI-for-age percentile has an increased risk of being overweight or obese in adulthood.[14]

Shumei and colleagues (2002), analyzing secondary weight and height data from 327 white participants at 3 to 20 years of age and again at 30 to 39 years of age, found that both male and female participants who were overweight or obese at 35 years of age had significantly higher BMI values in childhood ($P < 0.05$) than normal weight adults. Children with a higher BMI-for age and children obese at older ages had a greater risk of adult obesity. Childhood obesity also increases the risk of developing morbidities in adulthood.[15] A review by Maffeis and Tatò (2001) examined the relationship between childhood and adult obesity and the effects of childhood obesity on morbidity and mortality. Rates of diabetes, coronary heart disease, atherosclerosis, hip fracture, and gout were increased in individuals who were overweight as adolescents. Psychosocial consequences such as distorted self-image, fewer years of education completed, lower family income, higher rates of poverty, and lower rates of marriage also persisted. Lastly, cardiovascular risk factors (total blood cholesterol, blood LDL- and HDL-cholesterol, and blood pressure levels) and being overweight tracked significantly from childhood into adulthood.

High BMI-for-age values can be a useful tool in identifying children and adolescents at increased risk of becoming overweight or obese in adulthood. Furthermore, dietary factors such as protein sources may have an impact on childhood obesity and should be examined as potential contributors in the development of morbidities in adulthood. Several studies have found that a high intake of protein in infancy and early childhood is associated with increased risk of obesity later in life, which may also be true for later childhood.[7,8,9,10,11,12,13]

Body Mass Index is calculated by dividing weight in kilograms by height in meters squared (BMI = weight/height^2).[3] The BMI for a child is plotted on BMI-for-age gender-specific growth charts developed by the Centers for Disease Control and Prevention (CDC) to obtain a percentile ranking. A BMI plotted between the 5th percentile to less than the 85th percentile is considered a healthy weight. A BMI plotted between the 85th percentile and less than the 95th percentile is considered overweight. A BMI plotted at equal to or greater than the 95th percentile is considered obese.[16]

Identifying dietary intake markers for increased BMI in children could assist with the development of dietary recommendations that may decrease the risk of developing obesity as an adult. For example, if a high intake of animal protein compared to plant protein predisposes children to obesity, dietary recommendations for increasing plant protein intake and decreasing animal protein intake could be developed.

The purpose of this study was to evaluate whether animal versus plant protein intake in children ages 6-14 years is associated with an increased BMI during childhood. As seen in infancy and early childhood, a high protein intake may also predispose children to obesity. Previous studies have not evaluated animal and plant protein as isolated contributors to increased BMI during middle childhood to early adolescence. This study evaluated animal protein intake (dairy, meat, eggs, and other significant animal derived protein sources) and plant protein (vegetables, grains, legumes, beans, and plant-based products such as tofu). The sample population for this study included Caucasian and African American children between the ages of 6-14 years in Pittsburgh, Pennsylvania who participated in vitamin D clinical research protocols. This study is a secondary analysis of existing nutrient data obtained from a validated food frequency questionnaire (FFQ) that was completed prior to the start of the vitamin D studies. We hypothesized that children who consumed a higher intake of animal protein would have a significantly higher BMI than children who had a diet that consisted primarily of plant protein. In the case that high intakes of animal protein is associated with a high BMI in children, parents can intervene during early childhood by providing adequate amounts of animal and plant protein for proper growth and weight maintenance. In order to ensure that healthy children grow up into healthy adults, it is important for the public to be aware of contributing factors to obesity.

CHAPTER II

Review of Literature

Protein Requirements in Children and Young Adolescents

The abundance of protein and its functional diversity in living systems makes it essential for virtually all life processes. Dietary protein consists of amino acids that the body must have in order to synthesize its own unique set of proteins and nitrogen-containing compounds. Only twenty amino acids out of the hundreds found in nature are commonly found in human proteins. In addition, there are nine indispensable amino acids (IDAAs), meaning they are only provided by exogenous protein and cannot be synthesized by the body. Dietary protein and amino acid requirements are affected by age, body size, body composition, physiological state, and the level of energy output.[1,17] The requirement for dietary protein is based on the indispensable amino acids under all conditions and under specific physiological and pathological conditions as well as the synthesis of the dispensable amino acids and other nitrogen-containing compounds.[6] Protein requirements for children, which are based on the Recommended Dietary Allowance (RDA), increase with age (Table 1).[18] The criteria for determining protein needs in children between ages 6-14 years are based on nitrogen equilibrium plus protein deposition. Dietary protein provides the body with indispensable and dispensable amino acids and the additional nitrogen needed to synthesize nitrogen-containing compounds.

Table 1: Protein requirements for children/adolescents between 1-18 years of age

Age (yr)	Protein RDA (g/kg/day)
1-3	1.10
4-8	0.95
9-13	0.95
14 - 18	0.85

yr - years; g - grams; RDA - Recommended Dietary Allowance

Protein Intake and Obesity Risk

Current literature focuses primarily on the relationship between protein intake at infancy and early childhood and later obesity risk.[7,8,9,10,11,12,13] The 'early protein hypothesis', first proposed by Rolland-Cachera, suggests a high protein intake in early infancy increases the risk of obesity.[19] Rolland-Cachera and collegues (1995) conducted a longitudinal study to review the relationship between nutrient intake at 2 years of age and adiposity development at 8 years of age. Although the percentage of protein intake at 2 years of age was positively correlated with BMI and BF% before and after adjusting for energy intake, no significant correlation was found between the percentage of carbohydrate intake or fat intake. In addition, early protein intake had an inverse relationship with adiposity rebound which is a risk factor for later obesity.

Infancy and early childhood protein intakes are of interest because of differing macronutrient distribution found in infant formula versus human milk and the stimulating effect protein has on insulin-like growth factor (IGF-1) and insulin secretion.[20] Insulin and IGF-1 have anabolic effects that stimulate growth in children. Higher concentrations are found in infants fed formula compared to infants fed human milk. However, children breast-fed during infancy are taller and have higher IGF-1 concentrations later in life.[21] A low IGF-1 concentration later in life is associated with increased risk of non-communicable diseases (NCDs) facilitated through IGF-1. Michaelsen and colleagues (2012) conducted a literature review on inappropriate intake of dietary protein, both quantity and quality, during the first two years of life in relation to an increased risk of NCDs later in life.[7] There is a concern that high protein intakes during the first years of life will cause later obesity by altering IGF-1 concentrations. Infants experience a rapid increase of protein intake been transitioning to solid foods, typically 3-4 times greater than the physiological requirements of the average infant. Protein energy percentage (PE%) is 7-8% in infant formula, 20% in full fat cow's milk, ~5% in breastfed infants, and 15-20% in the typical family diet. In turn, breast-fed infants are shorter, weigh less, and have a lower BMI than formula-fed infants. Furthermore, studies have found an inverse relationship between IGF-1 measured in infancy and in late adolescence. The type of protein consumed could have different effects on IGF-1. For example, several studies have shown that early intakes of cow's milk increases linear growth in well-nourished populations through stimulating circulating IGF-1.[22] The risk factor for NCDs later in life is associated more with a poor protein quality intake with no animal protein than with high intakes of total protein.

A systematic literature review conducted by Hörnell and colleagues (2013) aimed to assess the health effects of different intakes and sources of protein (animal- or plant-based) in infancy and childhood in a Nordic setting.[8] Healthy children between the ages of 0-18 years in English or Nordic language-speaking populations were included in the study. Thirty-seven papers were assessed using the quality assessment tools (QAT); only 23 papers graded A or B were chosen to evaluate the association between protein intake and BMI, growth, body composition, and IGF-I. One study found that weight gain from birth to 12 months was positively related to BMI at the age of 6 years in males and females (P = 0.008). Furthermore, boys in the highest quartile of protein intake at the age of 9-12 months had a significantly higher BMI at 6 years than the lowest and second-lowest quartiles (P = 0.039 and P = 0.01). Similarly, another study found that a high protein intake (>15 % of total energy) at 12 and 18-24 months was related to a higher mean BMI at 7 years of age and a higher risk of having a BMI above the 75th percentile. These studies and others represented in the systematic review concluded that a high intake of protein in infancy and early childhood is associated with an increased risk of obesity later in life.

A review by Martorell and colleagues (2001) looked at the relationship between overall nutrition in early life and the development of adiposity later in life.[9] The researchers had three hypotheses: 1) overnutrition increases the risk of later fatness; 2) undernutrition is associated with increased risk of fatness; and 3) optimal nutrition during infancy, the gold standard being breastfeeding, is protective against future obesity. Possible mechanisms for these outcomes include disruptions in organ function, increase in the number and/or size of fat cells or alterations in adipose tissue function, and dysfunction of the central nervous system resulting in appetite regulation disturbances. The authors reviewed observational, experimental, and quasi-experimental studies. Birthweight and adult BMI are often used to indicate the influence of fetal nutrition on adult fatness. The results from these studies are conflicting. Some studies found a J-shaped relationship between birthweight and adult BMI, while others only found an association between high birthweight and increased adult BMI. A majority of the studies found that higher birthweights, particularly birthweights above 10 pounds, led to increased fatness in adulthood. A proportion of the causes of high birthweight may be attributed to early exposure to famine or gestational diabetes. The fetuses of mothers with diabetes are exposed to high concentrations of blood glucose, which leads to hyperinsulinemia and increased growth of fat, lean body, glycogen stores, and overall birthweight. Increased risk of fatness from undernutrition is not well understood and contradictory. However, one hypothesis suggests that individuals whose nutrient intake is scarce early in life move to abundance or excess in adulthood. In addition, intrauterine overnutrition is associated with a greater risk of fatness and may play a role in childhood obesity.

A longitudinal study conducted by Gunther and colleagues (2007) evaluated whether certain time points or periods of protein intake in infancy, early childhood, or the preschool years contribute to BMI at 7 years of age, and if the association is attributed to distinct protein sources (animal, vegetable, dairy, meat, or cereal protein).[10] The purpose of the study was to investigate the validity of the early protein hypothesis, which suggests that high protein intake during infancy increases the risk of fatness later in life. The early protein hypothesis is based on the thought that infant formula has significantly higher protein content than human milk and that children experience a rapid increase in protein intake when transitioning to solid foods. Previous studies have failed to investigate whether distinct protein sources are associated with the early protein hypothesis. The study population was derived from the DONALD study, an ongoing cohort study that follows subjects through young adulthood. An average of 40-50 infants from Dortmund, Germany were recruited on a yearly basis and examined at the age of 3-6 months. Up until early adulthood, investigators intermittently collected information from participants on nutrition, growth, metabolism, and health status. Information was collected at specific time points including 6 months (baseline), 12 months, 18-24 months, 3-4 years, 5-6 years and 7 years (endpoint). Inclusion criteria were term singletons infants with a birthweight of 2500 g, complete anthropometric measurements, plausible dietary records, and information on potential confounders (n = 203). Information collected included parental characteristics (educational status and employment and anthropometric measurements), birth characteristics (birthweight, birth length, and gestational age), anthropometric measurements (weight, height, and 4 skinfold thicknesses), and dietary data. Dietary data were assessed using 3-day weighed-diet records where parents weighed all foods and beverages consumed by their children and recorded food types. Breast-fed infants were weighed before and after breastfeeding to measure intake. The dietary records were analyzed using LEBTAB nutrient database and mean energy and protein intakes were derived from the 3 dietary records at each time point. Animal and vegetable protein intakes were divided into subgroups including dairy protein, meat protein, and cereal protein. Three tertiles of protein intake subgroups were constructed at each time point and their association with BMI SDS and BF% at 7 years of age was examined. Protein values as percentages of energy intake and total energy intake were reported. After following children from infancy to 7 years, the study identified median protein intake as exceeding recommendations at each age interval. In addition, the study identified two critical periods where higher percentage of protein intake from animal sources was associated with a higher BMI SDS. These periods were at 12 months (strong association, $P = 0.03$) and 5-6 years of age (weak association, $P = 0.07$). Total protein intakes at 12 months and 5-6 years of age were significantly associated with a higher BMI SDS and higher BF% at

7 years of age (P= 0.02). This relationship was not found with vegetable protein intake. In addition, a higher percentage of protein intake from dairy foods at 12 months of age was associated with a higher BMI SDS at 7 years of age (P = 0.02). Lastly, a higher vegetable protein intake at 5-6 years showed an inverse relationship to BF% at 7 years of age (P = 0.05). Previous studies identified protein intake during the first few years of life as an indicator for later fatness. This study suggests an additional time period (5-6 years of age) as a sensitive period of total and animal protein intakes for later body fatness.

A similar prospective study by Scaglioni and colleagues (2000) examined the influences of the early intake of macronutrients on the development of overweight in healthy children.[12] A large sample size of 171 Caucasian infants were randomly selected from live births at San Paolo Hospital in Milano, Italy. Inclusion criteria were singleton infants with a birthweight of ≥2500 g, gestational age of 37-42 weeks, no neonatal disease or congenital malformation, and Caucasian parents. A final total of 164 infants entered the study at 9 months of age. Information on anthropometrics and nutrition were collected at different time points: birth, 12 months, 5 years, 8 years, and 12 years of age. Mean weight and height from 6 measurements conducted by two pediatricians were used to measure BMI. A dietitian evaluated an age-adjusted food frequency questionnaire consisting of 116 items. In addition, the mothers were interviewed to analyze the content and quantity of each meal, and a 24-hour recall was conducted at the end of the interview. Dietary data were analyzed using an ad hoc PC software program. Student’s t test and the non-parametric Wilcoxon and Mann-Whitney tests were used to compare between-groups differences of continuous variables, and the Fisher’s test was used to compare discrete variables. The incidence of overweight (BMI > 90th percentile) increased from

10.9% at 1 year of age to 23.1% at 5 years of age ($P < 0.001$). Overweight children at 5 years of age had a significantly higher protein percentage of energy intake at 1 year than non-overweight children ($P = 0.024$). In addition to high protein intakes (~20% of energy intake) influencing the development of adiposity at 5 years of age, parental BMI (at least one parent with a BMI > 25 kg/m^2) was associated with children overweight at 5 years of age (father's BMI, $P = 0.003$; mother's BMI, $P = 0.05$). Daily energy intake was compared between overweight and non-overweight grouped participants and no significant difference was found. Overweight children at 5 years of age had a lower percentage of energy as carbohydrates at 1 year than non-overweight children. Fat intake was comparable in overweight and non-overweight children. As found with previous studies, this study suggests a high protein intake early in life increases the likelihood of later obesity in children.

Skinner and Colleagues (2004) examined longitudinal growth and energy intake in children ages 2 to 8 years and identified factors related to the children's BMI.[13] The study included 70 healthy Caucasian children from middle or upper socioeconomic status (SES) families. These families were selected because of their access to resources that provided children with adequate nutrition and health care for normal growth and development. Registered dietitians conducted interviews in the children's homes, measured growth (weight and height), and evaluated dietary information (24-hour recalls and 3-day food records) throughout the 8 year study. Weight, height, and BMI percentiles were determined based on the Center for Disease Control (CDC) growth charts. Dietary intake from three day food records were averaged and analyzed using Nutritionist IV, version 3.5. Each child was assessed 15-17 times by the end of the study and additional behavior information was obtained at certain time points. Variables included in the predictive models to determine BMI at 8 years of age included gender, birthweight, breastfeeding duration, age of cereal introduction, BMI at 2 years of age, estimated adiposity rebound (AR), longitudinal intakes of each macronutrient, longitudinal percentages of energy from each macronutrient, mother's perception of child's food acceptance at 6 years of age, the number of foods liked at 8 years of age, average daily screen time reported by mothers, average dietary variety score at ages 3.5-7 years, and parental BMIs. At 8 years of age, 23% of children exceeded the 85^{th} CDC BMI percentile and of these children, 9% exceeded the 95^{th} CDC BMI percentile. Average percentages of energy intake from protein, fat, and carbohydrates were 14%, 32%, and 56%, respectively. Dietary fat and dietary protein had a positive relationship to BMI while dietary carbohydrate intake was negatively related. Longitudinal dietary intake of macronutrients (2-8 years of age), BMI at 2 years of age, and age of adiposity rebound contributed to 5-8, 16, 19% of the variability in the predictive models, respectively (R2 = 0.41-0.43). These findings suggest childhood overweight is multifactorial and impacted primarily by BMI at 2 years of age and AR.

These studies propose interesting dietary predicators for increased body mass index and body fat percentage later in life. The finding that critical age periods of protein intake were associated with higher body fat mass brings into question whether other critical periods exist later in childhood. The finding that children who are obese at older ages have a greater risk of adult obesity suggests that predictive markers for later childhood obesity are needed. These studies indicate that both the quantity and quality of protein intake during childhood affects BMI and BF% later in life. Future studies are needed to compare protein intake later in childhood and BMI as predictors for adult obesity.

Dietary Intake Assessment in Children and Young Adolescents

Assessing dietary intakes in children is a challenging aspect of nutrition research. Erroneous methodology often limits dietary intake data in outcome evaluations. However, this information is essential for advancing dietary recommendations for overweight and obese children.[23] Selection of the appropriate measuring tool or method for dietary intake in children proposes a challenge in itself, whether it is a total diet assessment, a targeted diet, or related behaviors assessment. Each depends on the dietary features of interest and the characteristics of the study population.

Subjects' weight status and study design are important factors when selecting an appropriate methodology for dietary assessment. Dietary studies on children and adolescents have found a positive association between under-reporting and increased body fatness. Collectively, reports from large-scale surveys have shown a decrease in energy intake over the decades despite weight gain worldwide, suggesting energy expenditure has declined and/or measurements of dietary intake are flawed.[24] One cross-sectional study examined the extent of under-reporting of energy intake in children between the ages of 4-18 years.[24] The aim of the study was to determine if under-reporting has increased among children by comparing the National Diet Nutrition Survey (NDNS) from 1997 and the Diets of British School Children (DBSC) survey in 1983. The sample size included 2127 children ages 4-18 years from the NDNS and 2017 children 10-11 years and 1219 children 14-15 years from the DBSC survey. Both surveys measured anthropometrics and accessed dietary intake using two non-consecutive 7 day weighed diet records checked by nutritionists for completeness. Estimated energy requirements (EER) were determined by using published equations based on gender, age, weight, and height validated from collected doubly labeled water (DLW) energy expenditure data. Under-reporting was significantly higher among overweight boys and girls over the age of 7 years (except girls aged 7-10 years) compared to their lean counterparts when comparing the difference between EER and reported energy intake ($P < 0.001$ in all groups except girls aged 7-10 years). In addition, there was a significant trend of increased under-reporting with increasing age ($P < 0.001$). Median reported energy intakes were significantly lower while mean body weight was significantly higher in the NDNS compared with the DBSC survey ($P < 0.05$ in girls, $P < 0.001$ in boys). The study concluded that under-reporting was substantially higher among overweight and older children compared to their counterparts and approximately 20% of energy needs were unreported in the NDNS compared to the DBSC survey.

The target group is also a critical element when selecting a dietary intake method. Different age groups have varying literacy and numeracy skills, memory capabilities, attention spans, and cognitive abilities.[23] For example, children under 8 years of age have limited competency in providing an accurate dietary recall and children less than 10 years of age lack the conceptual skills required for reporting usual intake, serving sizes, and frequency of behaviors. Thus, assessing dietary intake in children under the age of 10 years requires parental involvement. The dietary habits and level of structured eating environment should also be considered for different age groups, as older children tend to eat more meals away from home and follow a less stringent eating schedule. Total diet methods such as food records, 24-hour recall, FFQ, and targeted diet and/or behaviors methods have advantages and disadvantages (Table 2).[23,25] This proposed study will extract animal and protein intake data from a FFQ previously used to assess dietary intake in children.

Table 2: Advantages and Disadvantages of Dietary Assessment Methods

Dietary Assessment Method	Advantages	Disadvantages
Food record	▪ Frees participants from relying on memory. ▪ Includes a specific time period ▪ Allows researchers to be trained in groups ▪ Offers absolute and relative intakes	▪ Sets a burden on participants ▪ Proposes a challenge for participants to record foods eaten away from home ▪ Alters dietary habits of participants in some cases ▪ Requires participants to have literacy and numeracy skills ▪ Proposes a high cost ▪ Challenges feasibility for large studies ▪ Proposes a burden on participants due to necessary multiple records
24-hour recall	▪ Frees participants from requiring literacy or numeracy skills ▪ Allows dietary habits of participants to be left unaltered ▪ Provides a low burden for participants ▪ Provides a quick assessment ▪ Includes a specific time period ▪ Offers automated data entry	▪ Requires participants to rely on memory for recall ▪ Proposes a challenge for estimating food quantity ▪ Requires training for researchers ▪ Proposes a high cost ▪ Challenges feasibility for large studies ▪ Proposes a burden on participants due to necessary multiple records
Food Frequency Questionnaire (FFQ)	▪ Allows dietary habits of participants to be left unaltered	▪ Requires participants to rely on memory for recall ▪ Requires participants to

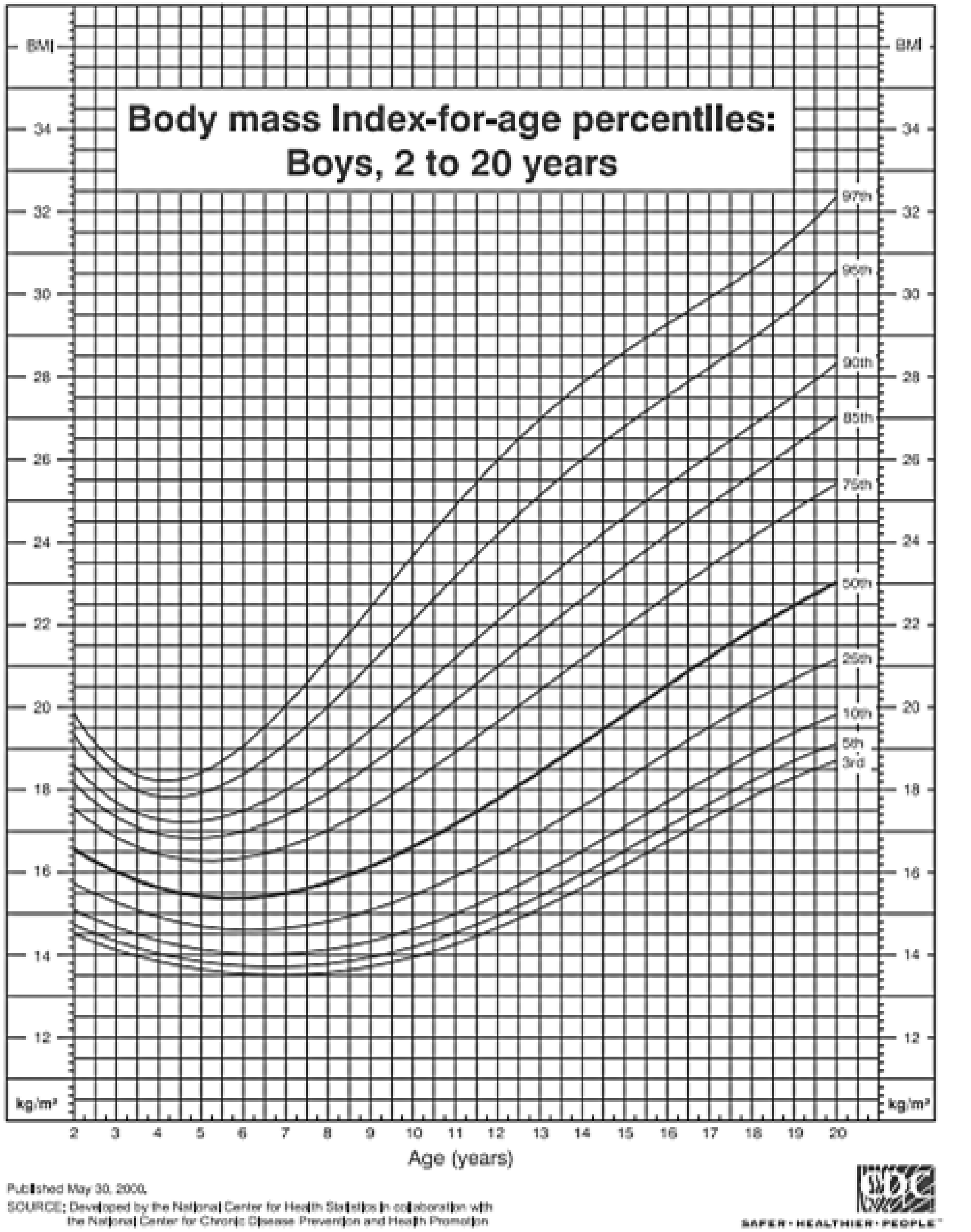
	▪ Offers a low burden for participants ▪ Frees the training of researchers ▪ Allows administration in multiple formats ▪ Offers a quick and inexpensive assessment ▪ Allows automated data entry ▪ Allows practicality for large studies ▪ Offers assessment of both total diet and select nutrients ▪ Offers assessment of current or past diet ▪ Allows participants to be divided into groups based on intake	have literacy and numeracy skills ▪ Proposes a challenge for estimating food quantity ▪ Bypasses food descriptions ▪ Bypasses assessment of meal patterns ▪ Limits data when FFQ is nutrient specific
Targeted diet and/or Behaviors Assessments	▪ Allows dietary habits of participants to be left unaltered ▪ Provides a low burden for participants ▪ Allows practicality for large studies ▪ Offers a simple and inexpensive assessment ▪ Allows automated data entry ▪ Allows trends to be monitored ▪ Allows behaviors and environmental information to be assessed	▪ Requires participants to rely on memory for recall ▪ Requires participants to have literacy and numeracy skills ▪ Requires research before developing assessment ▪ Challenges validity ▪ Bypasses the collection of nutrient intakes ▪ Limits food intake information

A review of the literature on the effectiveness of FFQs in assessing intake found that intakes of total fat, polyunsaturated fatty acids, fiber, and calcium were frequently overestimated as compared to the validation standard (multiple food records, a single 24-hour recall, multiple 24-hour recalls, or total energy expenditure).[26] Adapted FFQs are often designed using adult portion sizes, which can explain why energy and nutrients estimates in children's studies are overestimated.[26,27] In contrast, one study aimed to estimate the relative validity of a FFQ with the Young Children's Nutrient Assessment on the Web (YCNA-W), an online dietary assessment tool.[28] Overall, the FFQ was an accurate alternative for estimating energy and macronutrient intake. Another study examined the effects of age on reliability between age groups (9-12, 13-15, and 16-18 years of age) with results showing no differences.[29] A third study found that FFQs administered to parents rather than children overestimated energy intake less (21% vs. 36%, respectively).[30] Assistance from parents in completing a FFQ may be warranted in studies that assess dietary intake in young children.

Body Composition Assessment in Children and Young Adolescents

Body mass index (BMI) is commonly used in clinical practice to represent adiposity status (normal, overweight, and obese).[31] BMI, formulated from weight and height, is an inexpensive and convenient indicator of body fat mass for most children and shows the relative position of the child's BMI among children of the same sex and age. BMI in children is often reported as a percentile ranking and subsequent weight status category (Table 3). BMI is plotted on the CDC BMI-for-age gender specific growth charts (Figure 1) and is used to assess the size and growth patterns of children age 2 years and older in the United States.[32]

Figure 1: Example of the CDC BMI-for-age growth chart - boys, 2 to years



CDC - Centers for Disease Control and Prevention;
BMI - Body Mass Index; kg - kilograms; m - meters

Table 3: CDC BMI-for-age weight status categories and corresponding percentiles

Weight Status Category	Percentile Ranking
Underweight	< 5^{th} percentile
Healthy weight	5^{th} percentile - < 85^{th} percentile
Overweight	85^{th} percentile - < 95^{th} percentile
Obese	≥ 95^{th} percentile

CDC - Centers for Disease Control and Prevention; BMI - body mass index

The relationship between BMI and adiposity was demonstrated in a study on Italian children with a wide range in age.[33] Although a strong positive relationship was found between BMI and total body fat, BMI compared across different age groups can be erroneous and results must be evaluated cautiously. Total body fat was shown to increase with age while percentage of body fat decreased with age. The association between childhood BMI and adult adiposity has been supported in some studies while refuted in others.[34] A large longitudinal study aimed to compare the accuracy of childhood levels of BMI and triceps skin-fold thickness (SF) in predicting adult adiposity. The study found that childhood BMI-for-age is significantly associated with adult levels of BMI (correlation ranging from r = 0.44 to 0.64). This correlation was stronger among girls and among older children (9-17 years). Adult obesity and excessive body fat mass consistently increased as childhood BMI-for-age increased, even among young children.

CHAPTER III

Methods

The sample population for this study included Caucasian and African American children between the ages of 6-14 years who participated in Dr. Kumaravel Rajakumar's National Institutes of Health-funded (R03-K23 grants) vitamin D clinical research protocols. The study objectives were to assess the seasonal variation and racial differences in African American and Caucasian children and refine the serum 25(OH) D thresholds for defining vitamin D insufficiency in children. The study occurred in two phases: Phase I monitored the subjects for sunlight exposure and vitamin D intake and was conducted in 2006-2008; Phase II randomly assigned a vitamin D supplement (1000 IU D3) or placebo to the subjects and was conducted between 2008 and 2011. Participants were recruited from the Primary Care Center (PCC) of the Children's Hospital of Pittsburgh and from the Greater Pittsburgh area.[35]

This current study is a secondary analysis of existing nutrient data obtained from a validated FFQ that was completed prior to the start of the vitamin D studies. Study participants completed the Youth Adolescent Questionnaire (YAQ) FFQ designed and validated by Rockett et al. (2007; Harvard Medical School, © 1995 Brigham and Women's Hospital) at the baseline and 6 month follow-up visits. The YAQ is a semi-quantitative FFQ that evaluates dietary intake over the past year and consists of seven food groups and 152 questions. Total protein intake (meats, dairy, poultry, fish, nuts, seeds, legumes, beans, eggs, grains, fruits, vegetables, and compound food items) and animal protein intake (meats, poultry, cheese, milk, butter, eggs, fish, and compound food items) were included in the nutrient variables. Furthermore, animal proteins, except for compound food items, were subdivided based on preparation method and fat content. For example, chicken fried with skin versus chicken broiled without skin. Plant protein intake was determined by calculating the difference between total protein and animal protein intake. The percentages of total protein and animal and plant protein calories were determined based on kilocalorie intake and grams of protein intake. Dietary Reference Intakes (DRIs) for estimated energy requirements (EER) and protein requirements were determined using the 2005 Institute of Medicine of the National Academies equations for each subject (Table 1 and Table 4).[18]. A low physical activity (PA) coefficient was used for all subjects due to missing information on physical activity level (PAL) (Table 4). Body mass index was calculated from weight and height measures taken at the beginning of the initial study. Furthermore, BMI was categorized into normal (5^{th} to <85^{th} percentile), overweight (85^{th} to <95^{th} percentile), and obese (≥95^{th} percentile).[16] Only baseline nutrient intake was used in the proposed study. Demographic characteristics were also assessed for the purpose of evaluating the association between type of protein intake and BMI by gender and race.

Table 4: Estimated Energy Requirements for Normal and Overweight/Obese Children

Normal Weight Children	
Age	Estimated Energy Requirements (kcal)
0-3 months	(89 x wt [kg] - 100) + 175 kcal
4-6 months	(89 x wt [kg] - 100) + 56 kcal
7-12 months	(89 x wt [kg] - 100) + 22 kcal
13-36 months	(89 x wt [kg] - 100) + 20 kcal
Boys, 3-8 years	108.5 – (61.9 x age [y]) + PA x (26.7 x wt [kg] + 903 x ht [m])
Girls, 3-8 years	155.3 – (30.8 x age [y]) + PA x (10 x wt [kg] + 934 x ht [m])
Boys, 9-18	113.5 – (61.9 x age [y]) + PA x (26.7 x wt [kg] + 903 x ht [m])
Girls, 9-18	160.3 – (30.8 x age [y]) + PA x (10 x wt [kg] + 934 x ht [m])
Overweight/Obese Children	
Age (years)	Estimated Energy Requirements (kcal)
Boys, 3-18	114 - (50.9 x age [y]) + PA (19.5 x wt [kg] + 1161.4 x ht [m])
Girls, 3-18	389 - (41.2 x age [y]) + PA (15.0 x wt [kg] + 701.6 x ht [m])
Physical Activity Coefficient (low active)	
Boys, normal weight	PA = 1.13 if PAL is estimated to be ≥ 1.4 < 1.6
Girls, normal weight	PA = 1.16 if PAL is estimated to be ≥ 1.4 < 1.6
Boys, overweight/obese	PA = 1.12 if PAL is estimated to be ≥ 1.4 < 1.6
Girls, overweight/obese	PA = 1.18 if PAL is estimated to be ≥ 1.4 < 1.6

kcal - kilocalories; wt - weight; kg - kilogram; y - years; m - meters;
PA - Physical Activity; PAL - Physical Activity Level

The data for this study are currently stored on a secure server at Georgia State University and participant data have been de-identified. Secondary data without identifiers are generally not considered human subjects research. An Application for Designation of Not Human Subjects Research on the iRIS Portal at Georgia State University was developed and approved. The study was a cross-sectional examination of protein intake and BMI in pre- and early adolescents. This study design was chosen due to the lack of continuous data on dietary intake, height, and weight among participants.

We analyzed data through IBM SPSS Statistics 20. Frequency tests were performed to describe the demographic and anthropometric characteristics of the population as well as animal and plant protein intake within each BMI subgroup. Normality testing was conducted on continuous variables. Grams and percentage of total calories of animal and plant protein intakes for each BMI category were not normally distributed, therefore Kruskal-Wallis analysis of variance by ranks was used to compare variables.

CHAPTER IV

Results

Demographic Data

The Pittsburgh pediatric population in this study included 296 African American and Caucasian children with an age range from 6 to 14 years (mean age 9.8 ± 2.1 years). Data from 11 children were excluded due to outliers or missing data (n = 285). Outliers included children classified as underweight based on their BMI-for-age, with a protein intake greater than 200 grams, or with a BMI-for-age greater than 41.0 kg/m^2; several children had missing data for height and weight. The majority (>50%) of participants were male (Figure 2) and African American. Race distribution by gender is shown in Figure 3.

Figure 2. Gender distribution of the Pittsburgh Pediatric Population

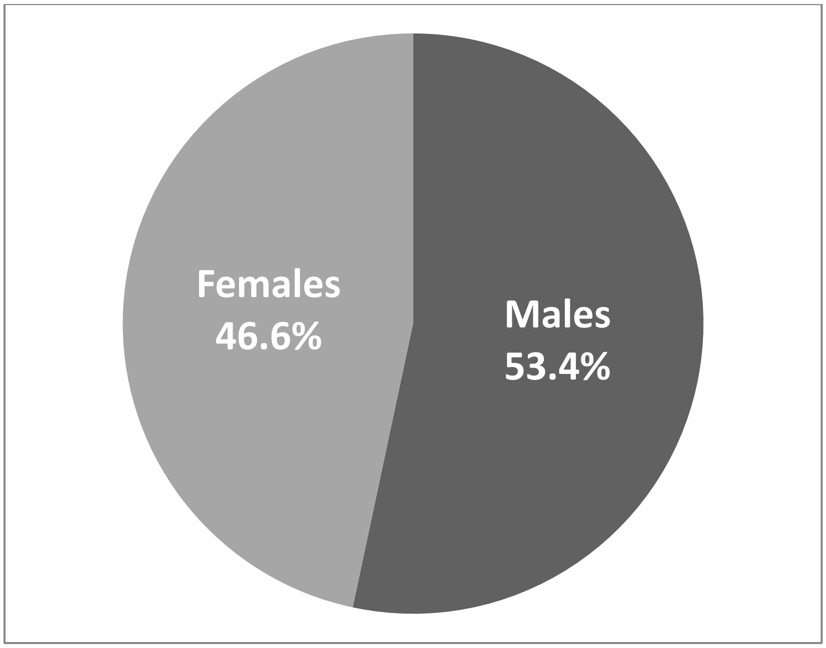
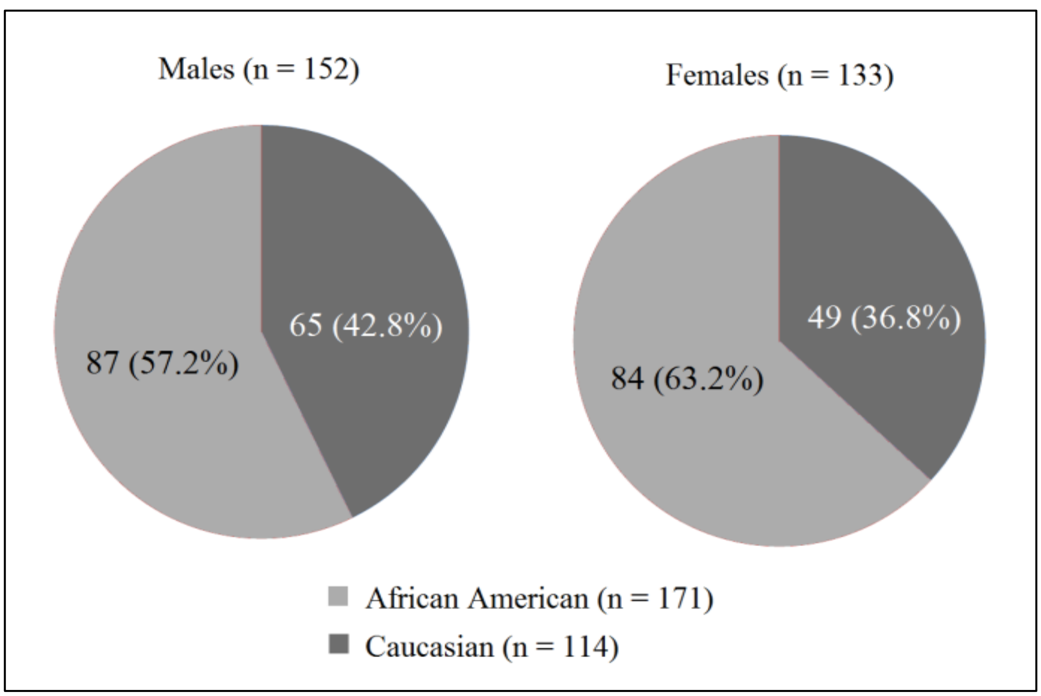


Figure 3. Race distribution by gender of the Pittsburgh Pediatric Population



Anthropometric Characteristics

Girls had a significantly higher weight and BMI than boys (Table 5). The majority (62%) of participants had a normal BMI while 20% of participants were overweight and 18% of participants were obese. The median BMI for each weight classification was 18.0 kg/m^2 for normal weight, 20.9 kg/m^2 for overweight, and 24.5 kg/m^2 for overweight (Table 6). Weight classification by gender is shown in Figure 4.

Table 5. Anthropometric characteristics of the Pittsburgh Pediatric Population for the total population and by gender

Outcome Measures	Total (n= 285)	Males (n= 152)	Females (n=133)	P-value
Weight (kg)*	38.5 (31.2, 49.7)	36.7 (30.4, 48.4)	41.7 (31.7, 55.4)	**.029**
Height (cm)*	140.7 (132.2, 152.7)	140.9 (132.6, 150.4)	140 (131.0, 154.1)	.928
BMI (kg/m^2)*	19.2 (17.0, 22.7)	18.2 (16.5, 21.1)	20.1 (17.8, 24.1)	**<.001**

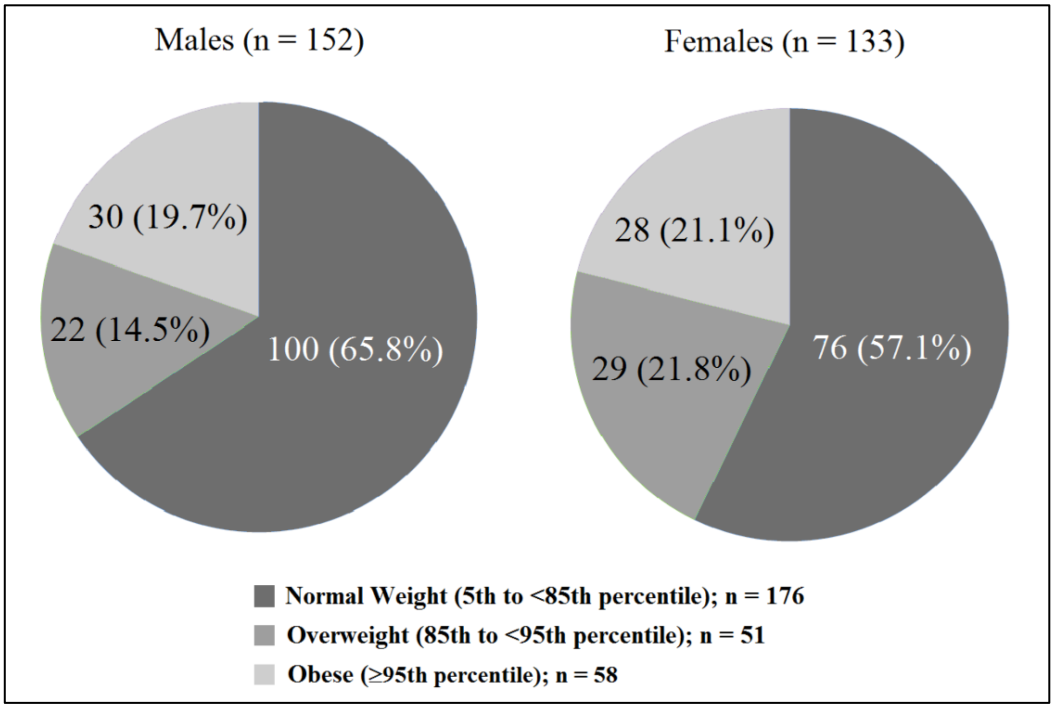
*Median (25%, 75%)
kg – kilogram, cm – centimeter, BMI – body mass index, m - meter

Table 6. Anthropometric characteristics of the Pittsburgh Pediatric Population by weight classification

Outcome Measures*	Normal Weight (n = 176)	Overweight (n = 51)	Obese (n = 58)
Weight (kg)	36.0 (30.0, 45.1)	40.3 (32.0, 56.5)	49.3 (34.8, 67.9)
Height (cm)	140.6 (130.7, 150.2)	141.0 (133.1, 153.0)	140.8 (132.8, 156.0)
BMI (kg/m^2)	18.0 (16.5, 20.1)	20.9 (18.9, 23.7)	24.5 (19.2, 30.7)

*Median (25%,75%)
kg – kilogram, cm – centimeter, BMI – body mass index, m - meter

Figure 4. Weight classification by gender of the Pittsburgh Pediatric Population



<u>Assessment of Protein and Caloric Intake</u>

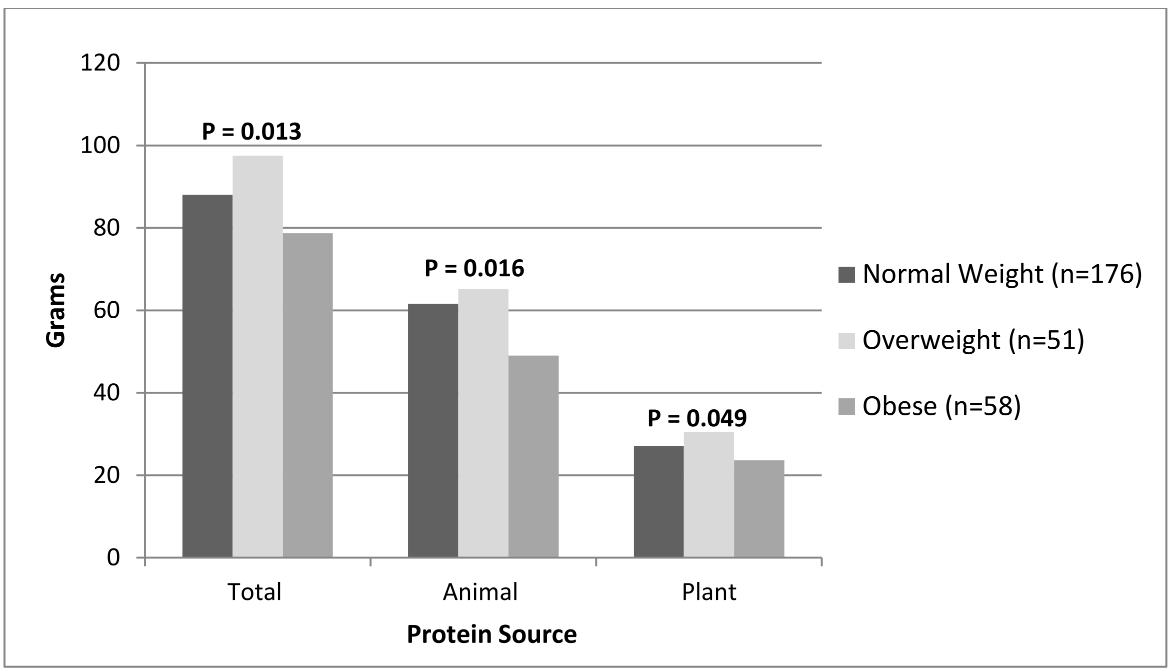
No difference in total protein and animal and plant protein intake was observed by gender (Table 7). However, median protein intake differed significantly by weight classification for total protein and animal and plant protein ($P \leq 0.05$; Figure 5). In the total population, overweight participants had the highest median intake of total protein (97.5 grams), followed by normal weight participants (88.0 grams) and overweight participants (78.7 grams). This observation was consistent when the population was subdivided by gender.

Table 7. Median protein intake of the Pittsburgh Pediatric Population for the total population and by gender

	Total (n=285)	Males (n=152)	Females (n=133)	P-value
Total Protein (g)*	87.9 (66.3, 114.5)	88.7 (65.7, 117.7)	86.0 (67.1, 109.9)	0.845
Animal Protein (g)*	61.3 (44, 79.0)	61.9 (44.1, 80.1)	59.4 (44.0, 77.3)	0.744
Plant Protein (g)*	27.5 (19.9, 36.4)	26.4 (19.5, 35.3)	28.2 (20.2, 37.3)	0.481

*Median (25%,75%)
g - grams

Figure 5. Median protein intake by weight classification of the Pittsburgh Pediatric Population



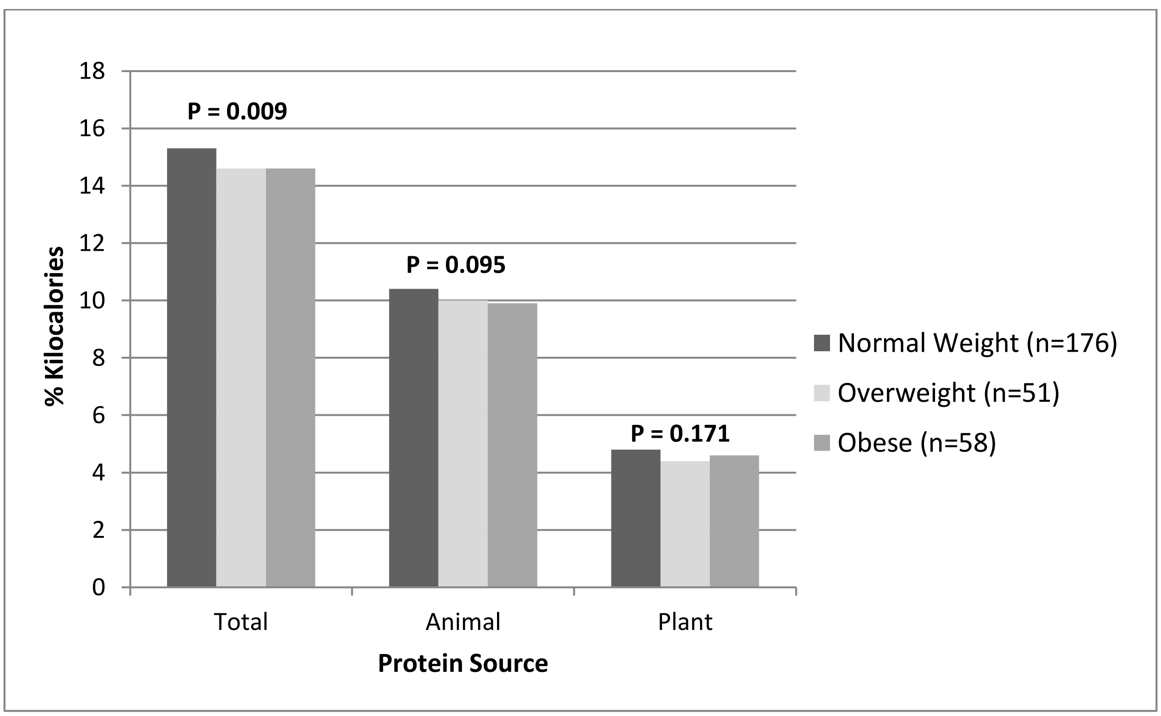
Reported total protein intake was more than adequate for total, normal weight, overweight, and obese children based on age and the RDA for protein (Table 8). Calorie intake was sufficient in children who were of normal weight (110%) or overweight (120%) but was below the energy requirement for age in those who were obese (80%).

Table 8. Ratio of median protein and calorie intake to requirements for age in the Pittsburgh Pediatric Population

	N	Total	Normal Weight	Overweight	Obese
Protein Intake	285	2.4 (1.6, 3.3)	2.6 (1.9, 3.5)	2.4 (1.7, 3.4)	1.7 (1.0, 2.5)
Calorie Intake	285	1.1 (0.8, 1.5)	1.2 (0.9, 1.5)	1.2 (0.9, 1.7)	0.8 (0.7, 1.3)

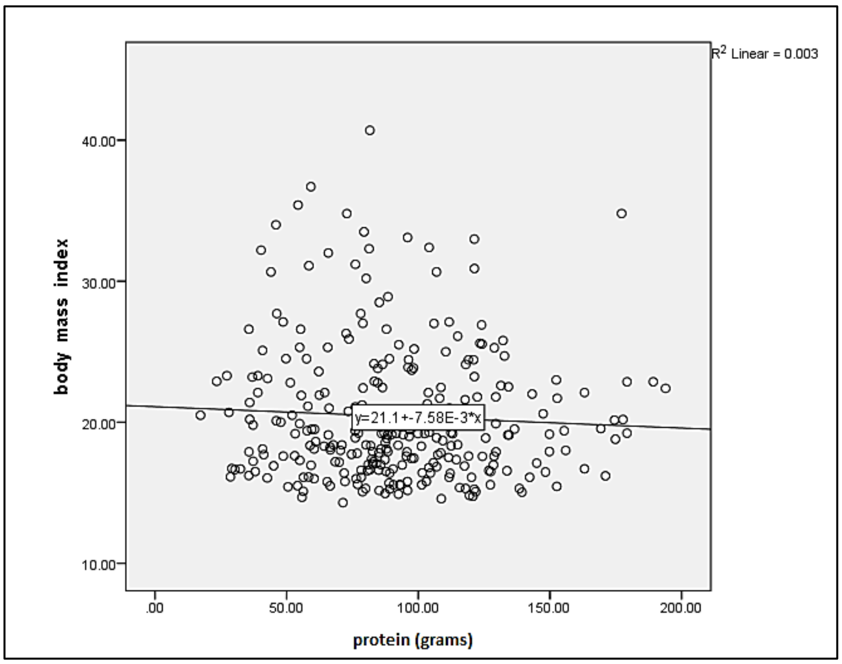
We determined the percentage of protein calories from total protein and animal and plant protein by weight classification (Figure 6). The percentage of total protein calories differed by BMI weight classification (normal, 15.3%; overweight and obese, 14.6%; *P*≤0.01) but did not differ by protein source.

Figure 6. Percentage of calories from protein by weight classification of the Pittsburgh Pediatric Population



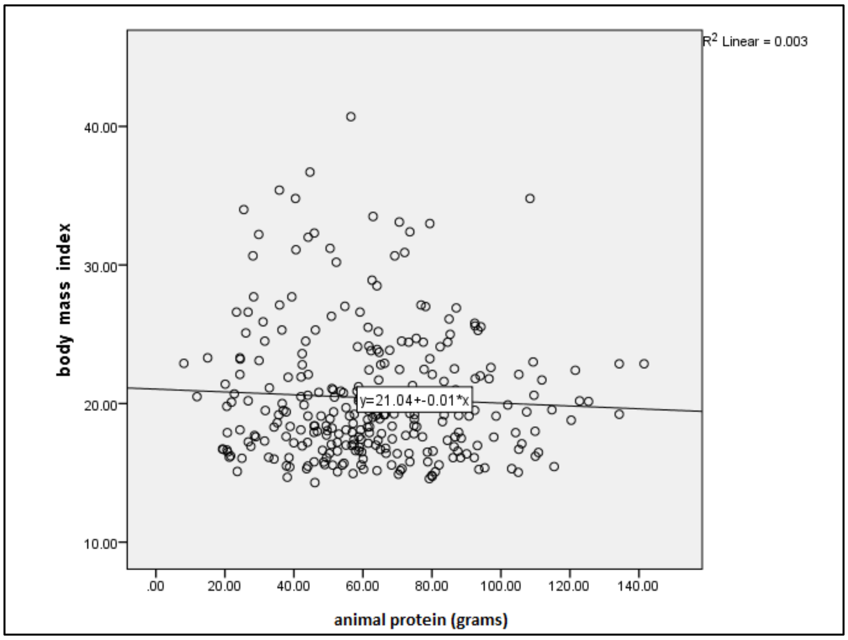
Correlation statistics were used to determine the association between grams of protein intake and BMI (Figures 7-9) and the percentage of protein calories and BMI (Figures 10-12). There were no associations between the amount of total, animal, or plant protein intakes and BMI.

Figure 7. Association between grams of total protein intake and BMI of the Pittsburgh Pediatric Population



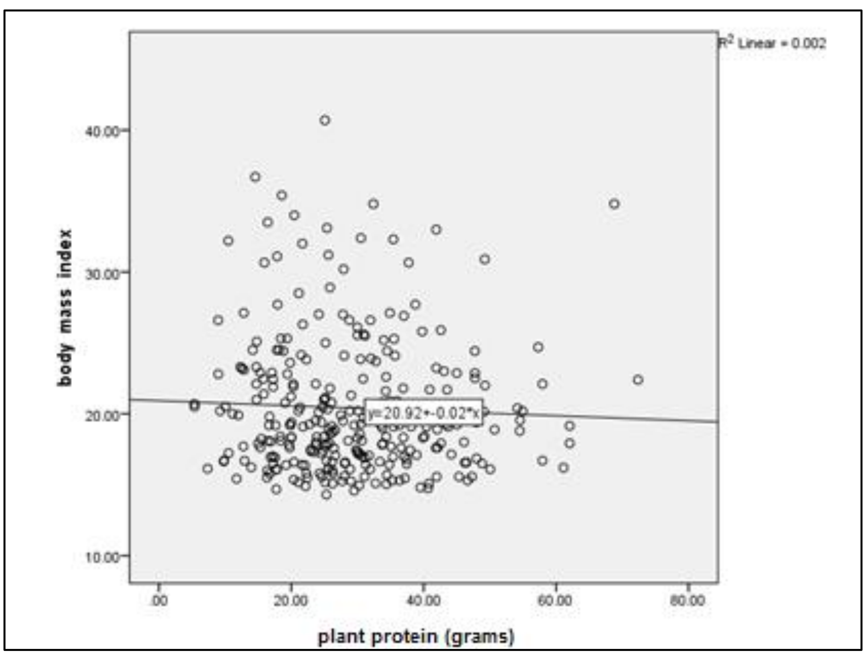
P-value: 0.432, Correlation: -0.047

Figure 8. Association between grams of animal protein intake and BMI of the Pittsburgh Pediatric Population



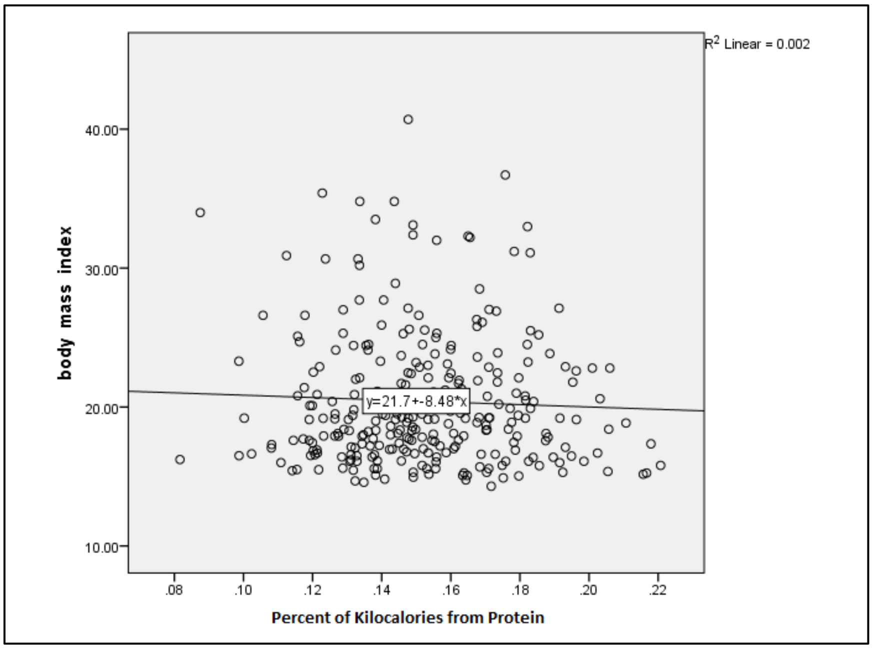
P-value: 0.664, Correlation: -0.025

Figure 9. Association between grams of plant protein intake and BMI of the Pittsburgh Pediatric Population



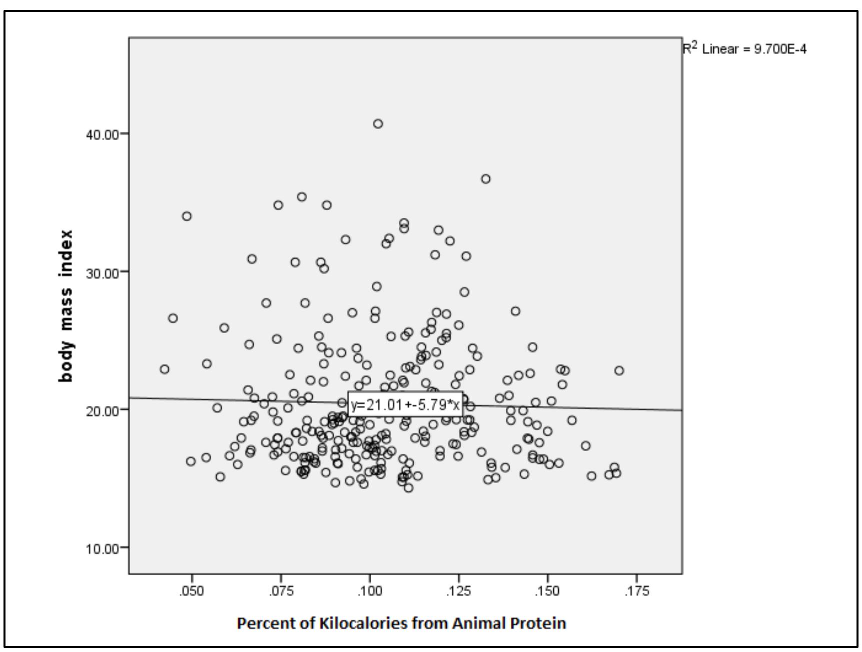
P-value: 0.296, Correlation: -0.062

Figure 10. Association between percentage of total protein calories and BMI of the Pittsburgh Pediatric Population



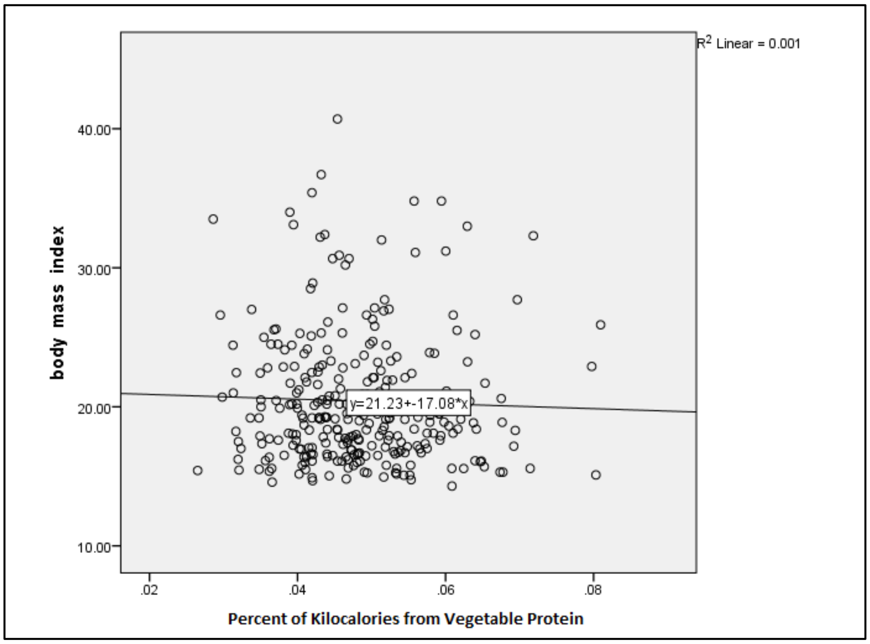
P-value: 0.979, Correlation: -0.002

Figure 11. Association between percentage of animal protein calories and BMI of the Pittsburgh Pediatric Population



P-value: 0.516, Correlation: 0.39

Figure 12. Association between percentage of plant protein calories and BMI of the Pittsburgh Pediatric Population



P-value: 0.283, Correlation: -0.064

CHAPTER V

Discussion and Conclusions

We observed a significant curvilinear trend in total protein intake, animal protein intake, and plant protein intake by weight classification. We speculate that this observation may be due to under-reporting in overweight and obese children, which is a common occurrence.[24,23,26,36] The total protein intake of the Pittsburgh Pediatric Population exceeded the recommended protein requirements; animal protein was the main source of protein consumed. In addition, total percent protein intake was significantly higher in children of normal weight and total energy intake was insufficient in obese children. We did not find a correlation between the amount of total, animal, or plant protein intake and BMI in children and early adolescents in contrast to findings from previous studies.[37,38] A study by Hermanussen and colleagues (2008) analyzed the dietary intake of 1028 children aged 2-18 years and found a significant positive correlation between total and animal protein intake and body mass index ($P < 0.0001$). The percentage of protein calories in the Western diet is on the rise with more protein rich-foods being advertised as weight-loss therapies. However, few studies have investigated the relationship between protein intake and prevalence of obesity among youth.

The curvilinear trend observed in our findings brings into question the accuracy of reported dietary intake among participants. Although this study did not validate reported intake to actual intake, we theorize that under-reporting and possibly over-reporting were present in our study. Previous dietary studies of children and adolescents have found a positive association between under-reporting and increased body fatness, especially among older children.[24] Furthermore, similar results among children worldwide have been reported in several studies.[39,40]

An Australian study by Rangan and colleagues (2014) aimed to describe the differences in reported food consumption among children classified as plausible, under-, and over-reporters.[39] A total of 4826 randomly selected children aged 2-16 years participated in a 24-h recall led by trained interviewers. Primary caregivers provided dietary information for children less than 9 years of age. Under and over-reporting was determined by applying the 95% Goldberg cut-offs, which calculates the lower and higher 95% confidence limit of the ratio of energy intake to basal metabolic rate assuming a given PAL requirement. Younger children (parental report) were less likely to under-report snack foods (potato chips, pastries, cheese, soda, etc.) compared to older children (self-report). Interestingly, children classified as under-reporters had significantly higher protein and starch intakes and less total fat, saturated fat, and sugar intakes than plausible reporters ($P < 0.05$).

A study by Murakami and colleagues (2012) conducted a cross-sectional study on factors associated with under, acceptable, and over-reporters of energy intake in Japanese children and adolescents.[40] A total of 28,885 students, ages 6 to 15 years, from schools in Naha City and Nago City completed a brief self-administered diet history questionnaire for Japanese children and adolescents (BDHQCA). Parents answered questions for elementary school children and assisted junior high school children. Self-reported body weight, age, gender, and energy requirements were used to calculate energy requirements through equations from the Expert Consultation Report on Human Energy Requirements. Participants were grouped into under, acceptable, or over-reporters of energy intake based on the ratio of reported energy intake to estimated energy requirement (within, below, or above the 95% confidence limits of the expected ratio of 1.0). Under-reporters had the highest intake of protein, fat, and dietary fiber and the lowest intake of carbohydrates ($P<0.0001$). Over-reporters had the highest intake of carbohydrates and lowest intake of protein and fat ($P<0.0001$). Under-reporting was associated with females, older age, overweight and obese participants, and those who independently completed the dietary questionnaire ($P\leq0.0001$). Over-reporters were associated with younger age, normal weight, and independent completion of the dietary questionnaire ($P<0.0001$).

Collectively, these studies suggest children with a higher BMI report a higher protein intake than children with a lower BMI. In the current study, we observed a downward trend of reported protein intake to estimated energy requirement among normal weight, overweight, and obese participants. Obese children reported the lowest intake of protein, which we speculate was due to under-reporting. Conversely, the ratio of reported protein intake to estimated protein requirement was highest among normal weight participants (2.6) and closely followed by overweight participants (2.4), suggesting that over-reporting may also have been at play. In addition, total percent protein calories intake was significantly higher among normal weight participants, contradicting the finding from previous studies that over-reporters consume less protein. This type of error in dietary data is considered as bias, which is the over- or underestimation of intake, either intentionally or unintentionally.[36] The direction and magnitude of bias varies between-subjects; results may include a combination of under-reporters and over-reporters. Avoiding error all together is an impossible task when estimating dietary intake, but attempting to understand the data is important when interpreting results.

The current study had several limitations including study design, dietary assessment tool, and limited data. The cross-sectional design of this study cannot assess causality between protein intake and obesity risk in children. This study evaluates dietary intake using a FFQ at one time point and may have provided different results if multiple FFQs were administered over time. Furthermore, current weight status of participants may reflect dietary intake at a younger period. For example, the early protein hypothesis suggests high protein intake may increase body fatness later in children, therefore assessing body mass index would require a longitudinal study design.[10] Measuring body mass index late in adolescents and early adulthood may be more relevant than measuring at the time of dietary assessment. This study design was chosen due to the lack of continuous data on dietary intake, height, and weight among participants. More longitudinal studies are needed to clarify the independent role of protein intake and sources of protein intake on the development of overweight and obesity in children and early adolescents.

Body mass index is a simple, low-cost assessment method of body fatness for routine clinical evaluations of children and adolescents.[31] Body mass index as a measure of fatness in children has a large prediction error (5-7% standard error of estimate). Furthermore, BMI as a predictor for excess fatness among children between the 85th and

95th percentile is less effective. The current study did not use anthropometric measures such as body fat percentage or waist circumference in addition to BMI. Body mass index in accordance with skinfold thickness and waist circumference measures may be a better predictor of overweight and obesity in children.[41] A study by Lin and Colleagues (2015) released earlier this year evaluated total, animal, and plant protein intakes in European adolescents and their associations with cardio-metabolic indicators.[37] This comparative cross-sectional study assessed BMI of adolescents using various anthropometrics measurements such as body mass index and percent body fat.

The prevalence of overweight and obesity in European children and adolescents has risen to 38%, increasing their risk for later coronary events. There is an ongoing debate on the benefits of high protein diets (≥20% of total energy) as a treatment method for improving weight status and satiety among adolescents. In addition to protein quantity, the nutritional quality may contribute to the development of overweight and obesity in adolescents. A high percentage of high-protein foods from animal sources such as meat, meat products, cheese, and dairy products are significantly high in fat and/or simple carbohydrates. However, reports from several studies found an association between total protein intake and/or animal protein intake and BMI, but did not find an association carbohydrates or fat and BMI.[12,38] In addition, evidence has shown that soy protein and dietary phytoestrogens improve glycemic control and lipid blood profile.[42]

Lin et al. (2015) used a population derived from the Healthy Lifestyle in Europe by Nutrition in Adolescence-Cross Sectional Study (HELENA-CSS), a study on lifestyle and nutrition among adolescents throughout Europe that was conducted from October 2006 - December 2007. The study included 1804 randomly selected adolescents aged

12.5-17.5 years (47% male). Participants included in the study were not simultaneously participating in a clinical trial, had no acute infections, and provided two 24-h recall interviews (HELENA-DIAT) and anthropometric measurements. Dietary recalls were conducted by dietitians and completed twice within 2 weeks during the school period. HELENA-DIAT is a validated, self-administered computer program based on the Young Adolescents' Nutrition Assessment on Computer (YANA-C) that included 800 food items, 25 food groups, and 300 photographs of food portion sizes. Dietary intakes were analyzed using the German Food Code and Nutrient DataBase (version II.3.1, 2011) and the percentage of animal and plant protein intakes were calculated using food composition databases. Under-reporters (ratio of energy intake over basal metabolic rate = <0.96) were excluded in the study. Trained researchers measured participants' weight and height to determine their BMI and divided them into categories: underweight, normal weight, overweight, and obese. In addition, physical maturations were based on Tanner stages and classified as pre-pubertal, pubertal, and post-pubertal. Blood samples were collected on a randomly selected subsample to determine cardio-metabolic indicators. Physical activity (PA) was measured by a uniaxial accelerometer and participants were categorized by 1 hour of PA per day, less than 1 hour of PA per day, or no PA per day. Statistical differences for total energy and total, animal, and plant protein intakes between gender and age were evaluated using the Student T-test and ANOVA. General linear Model multivariate analysis was used to assess the associations between animal and plant protein intake and anthropometry and serum biomarkers. Females had a higher BF% and serum lipid profiles and leptin levels but lower BMI z-score compared to males. Average total protein intakes were greater than the World Health Organization recommendations

(10.0 - 15.0%; median percent of total protein calories = 15.5%). Mean/median animal protein intakes were greater than plant protein intakes (59% versus 41%, respectively). Total, animal, and plant protein intakes were significantly lower in females than in males and total and plant protein intakes were lower younger participants (12.5-14.9 years). The inverse association of plant protein intakes was stronger with BMI z-score and BF% compared to animal protein intakes. BMI and BF% were positively associated with energy percentage of animal protein. Leptin was inversely associated with animal protein intake. BF%, TC, and HDL-C were inversely associated with plant protein intake. The researchers' findings suggest that plant protein intakes may help prevent overweight and obesity in European adolescents.

Findings from the current and the study by Lin et al. (2015) provide insight to future study designs. As discussed earlier, under-reporters and over-reporters may result in biased results. Under-reporters and over-reporters should be excluded from the study to decrease inaccurate findings. This will provide more plausible dietary intake across participants and draw stronger conclusions. Furthermore, physical activity was evaluated in the study by Lin et al., to provide a more accurate energy requirement estimate for participants. The database used for the current study did not have information on participants' physical activity level. Therefore, we used a constant PA to determine estimated energy requirements for each participant. An individualized estimated energy requirement would provide a more accurate ratio of kilocalorie intake to kilocalorie requirement if PA was reported. The previous study by Lin et al. also used pictures of food portions to provide participants with a visual reference to portion sizes. This is especially helpful among younger children because of their lack of conceptual skills.[23]

When conducting a study across multiple age groups, the evaluation tool should be designed for the lowest grade level completing the assessment. The previous study, however, did not use a FFQ; instead it used two non-consecutive 24-hr dietary recalls. Dietary recalls may be less reliable than FFQ due to the possibility of an abnormal dietary intake upon examination.[25] Another important aspect to consider when assessing protein intake in future studies designs is the various levels of fat content in animal proteins. For example, FFQ questionnaires should divide cheese products into fat-free, low-fat, or full-fat cheese in order to limit confounding results and to better understand the affect protein has on BMI.

In conclusion, we observed a significant curvilinear versus linear trend in total, animal, and plant protein intakes by weight classification in pre- and early adolescents. We speculate that this trend was due to under-reporting in overweight and obese children, which is a common finding reported in literature. The intake of total percent protein calories was significantly higher among normal weight participants, contradicting the finding from previous studies that over-reporters consume less protein. This finding in addition to the ratio of reported protein intake to estimated protein requirement highest among normal weight participants, suggests that over-reporting may also have occurred. We did not find a correlation between the amount of total, animal, or plant protein intake and BMI in pre- and early adolescents. Future longitudinal studies using multiple measures of body fatness need to be conducted to determine the relationship between protein intake and BMI during middle childhood to early adolescence.

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