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Relationship between protein intake in the first year of life and body mass index and IGF-1 concentration at six years

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HÁSKÓLI ÍSLANDS

Tengsl próteinneyslu á fyrsta aldursári við líkamsþyngdarstuðul og styrk IGF-1 í blóði sex ára barna

Birna Þórisdóttir

Leiðbeinendur: Ingibjörg Gunnarsdóttir og
Inga Þórsdóttir prófessorar í næringarfræði

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TABLE OF CONTENTS

ÁGRIP

Bakgrunnur og markmið: Mikil próteinneysla ungbarna hefur verið tengd við aukinn vöxt og hærri líkamsþyngdarstuðul (LÞS) á barnsaldri. Rannsóknir benda til þess að prótein frá dýraafurðum, og þá sérstaklega mjólkurafurðum, hafi meiri áhrif á vöxt en prótein frá jurtaafurðum og að áhrifum sé miðlað í gegnum insúlín-líkan vaxtarþátt 1 (IGF-1). Íslenskar ráðleggingar um mataræði ungbarna voru endurskoðaðar árið 2003. Áhersla var lögð á brjóstagjöf og takmarkaða neyslu kúamjólkur. Þessi ritgerð byggir á gögnum úr tveimur framvirkum ferilrannsóknum á íslenskum ungbörnum, fæddum 1995-6 og 2005, fyrir og eftir endurskoðun ráðlegginganna. Eftirfylgni var framkvæmd við 6 ára aldur. Markmið voru: 1) Að kanna áhrif endurskoðaðra ráðlegginga á próteinneyslu ungbarna og möguleg áhrif á LÞS og tíðni ofþyngdar við 6 ára aldur og 2) Að rannsaka tengsl milli próteinneyslu ungbarna og LÞS auk IGF-1 styrks í blóði barna við 6 ára aldur með áherslu á uppruna próteina.

Aðferðir: Þátttakendur voru 90 börn fædd 1995-6 og 170 börn fædd 2005. Fæðuneysla við 9 og 12 mánaða aldur var metin með vigtaðri fæðuskráningu. Upplýsinga um hæð og þyngd á fyrsta aldursári, við 18 mánaða aldur (bara 2005 rannsóknin) og við 6 ára aldur var aflað. Blóðprufa var tekin og styrkur IGF-1 mældur við 6 ára aldurinn (bara 2005 rannsóknin).

Niðurstöður: Helsta breyting í fæðuvali barna milli 1995-6 og 2005 rannsóknanna var minni neysla á kúamjólk (sem inniheldur 3,4 g prótein/100 g) og hennar í stað fengu börn almennt stoðmjólk til drykkjar (1,8 g prótein/100 g). Þetta leiddi til marktækt lægri próteinneyslu í síðari rannsókninni samanborið við þá fyrri, 11,9% af heildarorku (E%) samanborið við 14,4 E% (P<0,0001) við 9 mánaða aldur og 14,6 E% samanborið við 15,6 E% (P=0,016) við 12 mánaða aldur. Neysla kúamjólkur og próteina, og þá sérstaklega próteina frá dýraafurðum, við 12 mánaða aldur var jákvætt tengd LÞS við 6 ára aldur. Niðurstöðurnar spáðu því að fyrir hver auka 100 g sem 12 mánaða gömul börn neyttu af kúamjólk myndi LÞS við 6 ára aldur hækka um 0,2 (0,0; 0,3) kg/m^2. Börn í hæsta fjórðungi dýrapróteinneyslu (≥ 11,9 E%) við 12 mánaða aldur voru með marktækt hærri LÞS við 12 mánaða (0,7 (0,0; 1,3) kg/m^2), 18 mánaða (0,7 (0,1; 1,3) kg/m^2) og 6 ára aldur (0,8 (0,2; 1,4) kg/m^2) en börn í lægsta fjórðungi dýrapróteinneyslu (<7,7 E%). Börn sem enn voru á brjósti við 12 mánaða aldur (19% barna í 2005 rannsókninni) voru með lægri LÞS við 18 mánaða aldur (-0,7 (-1,2; -0,1) kg/m^2) en börn sem voru skemur á brjósti. Jákvætt línulegt samband fannst milli neyslu mjólkurpróteina 12 mánaða stúlkna og IGF-1 styrks þeirra í blóði við 6 ára aldur (5,4 (2,5; 8,2) µg/l), óháð hæð og þyngd við 6 ára aldur. Hlutfallslega færri börn voru greind sem of þung (og of feit) í 2005 rannsókninni samanborið við 1995-6 rannsóknina, 12% samanborið við 21% (P=0,045).

Ályktanir: Niðurstöðurnar benda til þess að endurskoðun ráðlegginga og tilkoma stoðmjólkur á markað hafi dregið úr próteinneyslu meðal íslenskra ungbarna. Hugsanlega á þessi breyting þátt í því að LÞS 6 ára barna virðist fara lækkandi og færri börn greinast of þung í síðari rannsókninni samanborið við þá fyrri. Niðurstöðurnar styðja áherslur um brjóstagjöf auk þess sem leita þarf leiða til að draga úr mikilli neyslu próteina úr dýraafurðum. Ástæða er til að kanna nánar þýðingu hækkaðs IGF-1 styrks meðal 6 ára stúlkna sem neyttu mikils magns mjólkurpróteina við 12 mánaða aldur. Hátt IGF-1 gæti verið vísbending um snemmbúinn kynþroska sem talinn er geta haft slæm áhrif á heilsu síðar á ævinni.

ABSTRACT

Background and objective: High protein intake in infancy has been associated with increased growth and higher body mass index (BMI) in childhood. It has been suggested that animal protein, in particular dairy protein, has a stronger association with growth than vegetable protein has and that the effects might be mediated via insulin-like growth factor 1 (IGF-1). Icelandic infant dietary recommendations were revised in 2003, emphasising prolonged breastfeeding and limited consumption of cow's milk. This thesis is based on data from two prospective cohort studies on Icelandic infants, born in 1995-6 and 2005, previous to and after the revision of recommendations. Participants were followed-up at 6 years of age. The objectives of this thesis were: 1) To study the effects of revised recommendations on protein intake among infants and possible effects on BMI and prevalence of overweight at 6 years, and 2) To study the relationship between protein intake in infancy and BMI in addition to IGF-1 concentration at 6 years, with focus on sources of dietary protein.

Methods: Subjects were 90 children born in 1995-6 and 170 children born in 2005. Dietary intake at 9 and 12 months was assessed by weighed food records. Information about height and weight during the first year of life, at 18 months (only 2005 cohort) and at 6 years was gathered. Blood samples were taken and IGF-1 measured at 6 years of age (only 2005 cohort).

Results: The main alteration in the diet of children between the 1995-6 and 2005 cohorts was a shift from cow's milk (containing 3.4 g protein/100 g) towards a general consumption of follow-on formula (1.8 g protein/100 g). This resulted in a significantly lower intake of protein in the latter cohort compared to the former, 11.9 percent of energy (E%) vs. 14.4 E% ($P<0.0001$) at 9 months and 14.6 E% vs. 15.6 E% ($P=0.016$) at 12 months of age. Consumption of cow's milk and protein, especially animal protein, at 12 months was positively associated with BMI at 6 years. The results indicate that each 100 g increase in cow's milk at 12 months of age would result in a 0.2 (0.0, 0.3) kg/m^2 higher BMI at 6 years. Children in the highest quartile of animal protein intake at 12 months (≥11.9 E%) had higher BMI at 12 months (0.7 (0.0, 1.3) kg/m^2), 18 months (0.7 (0.1, 1.3) kg/m^2) and 6 years (0.8 (0.2, 1.4) kg/m^2) than children in the lowest quartile of animal protein intake (<7.7 E%). Children still being breastfed at 12 months (19% in the 2005 cohort) had lower BMI at 18 months (-0.7 (-1.2, -0.1) kg/m^2) than children breastfed for a shorter duration. Dairy protein intake at 12 months was a positive predictor of IGF-1 at 6 years for girls (5.4 (2.5, 8.2) µg/l), independent of height or weight at 6 years. Relatively fewer children were classified as being overweight (including obese) in the 2005 cohort compared to the 1995-6 cohort, 12% vs. 21% (P=0.045).

Conclusions: The results presented in this thesis suggest that the revised recommendations and the general use of follow-on formula decreased protein intake among Icelandic infants. It is possible that this alteration is partly responsible for a downwards trend in BMI at 6 years and lower overweight prevalence in the 2005 cohort than the 1995-6 cohort. The results support the emphasis on breastfeeding and display a requirement for approaches aimed at decreasing intake of animal protein. The relevance of higher IGF-1 concentration among 6 year old girls with high intake of dairy protein at 12 months should be examined. High IGF-1 may be a suggestion of early puberty that may have negative effects on health later in life.

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ABBREVIATIONS

AR	adiposity rebound
BF%	percentage body fat
BF	breastfeeding
BMI	body mass index (kg/m^2)
E%	percent of energy
EBF	exclusive breastfeeding
GH	growth hormone
HP	high protein
ICEFOOD	a nutrient calculation program
IGF-1	insulin-like growth factor 1
IGFBP	insulin-like growth factor 1 binding protein
IOTF	International Obesity Task Force
IQR	interquartile range
ISGEM	the National Food Composition Database
KG BW	kilogram bodyweight
LP	low protein
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
RCT	randomized controlled trial
SAS	an integrated system of software products
SFA	saturated fatty acid
SD	standard deviation
WHO	World Health Organization

1. INTRODUCTION

There is growing evidence that high protein intake in infancy is associated with increased growth and higher body mass index (BMI) later in childhood (Koletzko et al., 2009a). Less is known about whether only certain protein qualities or protein from specific food groups could be responsible for the possible associations between early protein intake and later BMI and about the role of insulin-like growth factor 1 (IGF-1) as a mediator in this pathway.

A study on 90 Icelandic 6 year old children who were participants in an infant study in 1995-7 showed higher BMI of boys consuming a high protein diet in infancy (Gunnarsdottir & Thorsdottir, 2003). The high protein intake of the children in the study was mainly attributable to the practice of introducing cow's milk in the latter half of the first year when breastfeeding diminished or ceased, and the infrequent use of infant formula (Atladottir & Thorsdottir, 2000). Among other emerging issues from the study was high prevalence of iron deficiency at 12 months, also associated with high intake of cow's milk (Thorsdottir et al., 2003).

Following these results, in 2003 revised infant dietary recommendations were published in Iceland (The Icelandic Nutrition Council & Centre for Child Health Services, 2003). Icelandic follow-on formula (Stoðmjólk) with less protein (1.8 g vs. 3.4 g protein/100 g) and higher iron content than regular cow's milk was developed and recommended during weaning from 6 months to 2 years of age (Thorisdottir et al., 2011). The formula was made available at a fair price in every grocery shop ready-made in 500 ml cartons, which was set as the upper recommended daily intake of milk consumption. Breastfeeding was emphasised more than before by adopting the World Health Organization (WHO) recommendation for exclusive breastfeeding until 6 months of age (World Health Organization, 2003) instead of 4-6 months as previously recommended and also by encouraging partial breastfeeding until the age of 1 year or older if suiting mother and child. The revised recommendations are introduced to all parents of newborns by healthcare professionals at healthcare centres.

In 2005-7 a new Icelandic infant study was conducted to assess the impact of the revised recommendations in the infant population. The study showed that dietary intake in infancy had moved towards the revised recommendations. Use of follow-on formula had replaced regular cow's milk to a great extent in the latter half of the first year compared to the 1995-7 study and an enormous improvement of iron status among 12 month olds was evident

(Thorisdottir et al., 2011). A follow-up study on the 2005-7 cohort was conducted in 2011-12, when the children were 6 years old.

This thesis is based on data from the two prospective cohort studies on Icelandic infants, born 10 years apart (1995-6 and 2005), followed up at six years of age (in 2001-2 and 2011-12). Several scientific papers have been published presenting results both from the original studies on infant diet and from the follow-up studies.

The aims of the thesis were to:

1) Study the effects of revised recommendations on protein intake among infants and possible effects on BMI and prevalence of overweight at 6 years.
2) Study the relationship between protein intake in infancy and BMI in addition to IGF-1 concentration at 6 years for the 2005 cohort exclusively with focus on sources of dietary protein.

The thesis is based on a review of the literature with respect to the aims of the thesis and the following manuscripts:

1) Infant dietary predictors of BMI at 6 years: Two population based studies conducted 10 years apart.
2) Protein sources in infancy as predictors for body mass index and IGF-1 concentration at the age of 6 years.

2. REVIEW OF LITERATURE

Overweight and obesity are considered major public health threats with serious consequences, including premature mortality and long-term morbidity due to noncommunicable diseases, e.g. cardiovascular disease, type 2 diabetes and many cancers. Overweight and obesity are not only serious challenges in adults but also in children (World Health Organization, 2012).

2.1 Childhood overweight and obesity

The ideal way of defining childhood overweight and obesity is unclear, making monitoring and comparison of prevalence between studies and countries complicated. Childhood overweight and obesity are known to have both immediate and longer term health effects in addition to reductions in quality of life and a greater risk of teasing, bullying and social isolation (World Health Organization, 2012). Further, childhood overweight and obesity are known to track into adolescence and adulthood (Johannsson et al., 2006; Singh et al., 2008). Population-based approaches to prevent childhood overweight and obesity are therefore important. In this thesis the focus is on primary prevention of early childhood overweight and obesity from a public health perspective.

2.1.1 Definition of childhood overweight and obesity

Although percentage body fat (BF%) and waist circumference might be the best suited measurements to indentify children with adverse cardiometabolic risk factor profiles associated with overweight and obesity, BMI is widely accepted as a valid indirect measure of adiposity in children. BMI is associated with body composition and risk factors of overweight and obesity and, especially important in epidemiological studies, is based on widely available measurements (Reilly et al., 2010; Rolland-Cachera, 2011). BMI is calculated as weight (kg) divided by the square of height (m) and in children expressed as a function of age and gender. There are three sets of growth references commonly used to assess a child's BMI: national definitions of excessive BMI for age, the International Obesity Task Force (IOTF) cut-off points (Cole et al., 2000), and those published by WHO (WHO Multicentre Growth Reference Study Group, 2006). The most widely used definition of childhood obesity is the IOTF approach that gives age- and gender-specific cut-off points for BMI for overweight and obesity defined to pass through BMI of 25 and 30 kg/m^2 at 18 years of age (Cole et al., 2000).

2.12 Prevalence of childhood overweight and obesity

Worldwide, it is estimated that 170 million children under the age of 18 years are overweight or obese and in some countries the number of overweight and obese children has increased threefold in the last three decades (World Health Organization, 2012). While the prevalence of childhood overweight and obesity is still increasing in many countries, in particular developing countries (Gupta et al., 2012), recent studies from several areas in Europe and US report that since the early 2000s this trend seems to be levelling off or even decreasing (Bluher et al., 2011; Lazzeri et al., 2008; Rokholm et al., 2010).

There is suggestive evidence that the childhood overweight and obesity pattern in Iceland is similar to the areas in Europe and US reporting stability in the last decade. Since tracking of height and weight of 9 year old schoolchildren in Reykjavik area was initiated a steady and alarming increase in prevalence of overweight and obesity was seen, from below 7% in 1958 to 24% in 1998 (S. Jonsson et al., 2011). The previous growth then seems to be followed by stabilization from 1998 to the last published measurements in 2010, with around 20% of 9 year old children being overweight or obese. The prevalence of 6 year old schoolchildren in Reykjavik area born between 1998 and 2004 also seems stable (S. Jonsson et al., 2011).

2.1.3 The importance of primary prevention

In general, prevention of a problem is preferable to treatment of the problem, and this is certainly true in the case of childhood overweight and obesity. Firstly, by preventing overweight and obesity, children do not suffer from any of the negative physical and psychological effects associated with overweight and obesity. Secondly, treatment is often not universally effective or even available and therefore many overweight or obese children may not successfully overcome the problem. Thirdly, prevention can be less expensive than treatment. Primary prevention has been considered desirable with regard to childhood overweight and obesity since it confers benefit to the widest scope of children possible and because it may reduce labels associated with programmes targeted at specific high-risk populations (Haynos & O'Donohue, 2012).

Although the obesity epidemic is at last assumed to be a consequence of changes in cultural, behavioural and lifestyle factors that promote a positive energy balance (Singhal et al., 2010), dietary factors during the sensitive period of infancy and early childhood are increasingly recognized as being potentially critical for later predisposition to obesity (Koletzko et al., 2009a). In this context, protein has received particular attention.

2.2 Dietary protein

Protein is a component of all organic material in cells and membranes of animals and plants and performs specific functions within the body, e.g. enzyme, hormone and antibody action. The protein molecules are built of 20 amino acids. Dietary protein is needed by the human body for growth and maintenance and has two roles: a specific role as a source of nitrogen and amino acids and a non-specific role as an energy source. Thus the requirement of protein is actually a requirement of amino acids and nitrogen. The energy needs of an individual must be fully met so that dietary protein can be used for essential functions and growth. Due to the large requirements of growth, protein requirements of infants and young children are higher than that of older children and adults when expressed per kilogram bodyweight (kg BW), but the question of optimal protein intake during early childhood has generated substantial controversy (Nordic Nutrition Recommendations, 2004).

2.2.1 Protein quantity

The Nordic nutrition recommendations from 2004 present no adequate intake of protein for infants up to 6 months of age. During this period protein content of breast milk, that is estimated to be about 1.2 g/100 kcal or 5-7 percent of total energy (E%) is considered adequate in term infants (Agostoni et al., 2005; Michaelsen, 2000; Nordic Nutrition Recommendations, 2004). Infant formulas often provide infants with 55-80% higher protein per kg BW than breast milk (Koletzko et al., 2009b) as the protein content must be in the range of 1.8 g/100 kcal and 3 g/100 kcal (European Commission, 1991). For infants 6-11 months, adequate intake is estimated to be 1.1 g protein/kg BW and recommended protein intake as percentage of total energy 7-15 E%. Equivalent numbers in the 12-23 month period are 1.0 g protein/kg BW and 10-15 E% (Nordic Nutrition Recommendations, 2004). In the complementary feeding period, when infants are introduced to a diet based on family food, it is estimated that the protein energy ratio will increase from 5-7 E% in breast milk to 12-15 E%, the typical protein content of a family diet (Michaelsen, 2000).

2.2.2 Protein quality and food sources

Amino acids are classified as either essential amino acids that cannot be synthesised in the human body and must be obtained from foods or nonessential amino acids that can be synthesised within the body from other amino acids. Protein quality is an indicator of how well a protein from food matches the body's requirements and is determined from the presence and ratio of essential amino acids in the protein. As a general rule, protein from animal origin is of high quality, being rich in essential amino acids and almost fully absorbed, while protein from plant origin is of lower quality, with reduced values of essential amino acids and higher values of non-essential amino acids. The Nordic nutrition recommendations base their recommendations on average protein requirement on intake of high-quality protein. Dietary proteins of plant origin complement each other and protein quality is usually not a nutritional problem in complex meals in the typical Nordic setting (Nordic Nutrition Recommendations, 2004).

Dietary protein is found in almost all foods of animal and plant origin. Dairy, meat and cereals have been found to be the main protein sources in the complementary feeding period in Icelandic infants (Thorsdottir et al., 2008) and US infants (Fox et al., 2006). Fish also provides Icelandic infants with a significant amount of dietary protein (Thorsdottir et al., 2008).

2.3 Growth in infancy

Infancy is a period of rapid growth. In infancy, growth is highly sensitive to energy and nutrients. Studies showing that formula-fed term infants gain weight faster than breastfed infants (Atladottir & Thorsdottir, 2000; Dewey, 1998; Ziegler, 2006), gave rise to the idea that high protein intake accelerates infant growth. Today, energy and certain micronutrients are thought to stimulate growth in infancy along with dietary protein (Larnkjaer et al., 2012). Rate of growth has metabolic and hormonal effects, which have the potential to trigger lifelong consequences.

Rapid weight gain in infancy is accounted for largely by rapid accumulation of fat mass and is associated with obesity in childhood (Baird et al., 2005; Monteiro & Victora, 2005; Ong & Loos, 2006; Weng et al., 2012). In fact, high weight gain during the first 24 months of life has been considered the best overall predictor of overweight and obesity at school entry, compared to other anthropometric markers and time intervals (Koletzko et al., 2009a).

Overweight and obesity are among the major risk factors of certain types of cancer. Results from a systematic literature review, published in 2007 (World Cancer Research Fund/American Institute for Cancer Research, 2007), suggested that a growth pattern (i.e. weight and height gain) through childhood and adolescence that would result in BMI at the lower end of the normal range at 21 years should be recommended in order to reduce the risk of cancer. The main argument for this recommendation was that being at the lower end of the normal range in the beginning of adulthood would allow natural weight gain during adulthood within the defined normal weight of BMI 18.5-24.9 kg/m^2.

2.4 Protein hypothesis

The "early protein" hypothesis, that postulates that high protein intake early in life stimulates growth and concurrently increases the risk of overweight and obesity later in life was first proposed in 1995 (Rolland-Cachera et al., 1995). In this study of 112 French children, nutritional intakes at the age of 2 years and weight, length and skinfold thickness at 10 months, 2, 4, 6 and 8 years were measured. Significant correlations were found between the percentage of protein at 2 years of age and both BMI and subscapular skinfold thickness at 8 years after adjusting for energy intake at 2 years and parental BMI.

Since 1995, several cohort studies and one randomized controlled trial (RCT) have found an association between higher protein intake in infancy and increased growth during infancy or higher BMI in childhood, supporting the protein hypothesis.

2.4.1 Cohort studies

In addition to finding a relationship between childhood overweight and overweight status of their parents, an Italian study from 2000 found that children that were overweight at 5 years of age had higher intake of protein at the age of 1 year than non-overweight children (Scaglioni et al., 2000). The authors concluded that while parental overweight seemed a major risk factor for childhood overweight in the first years of life, an early high protein intake might also influence the development of obesity.

As presented in the introduction chapter of this thesis, the Icelandic study on children born in 1995 found an association between high intake of protein at 9-12 months of age and higher BMI at 6 years, only among boys. Rapid growth during the first year of life was however associated with increased BMI at 6 years in both genders (Gunnarsdottir & Thorsdottir, 2003). The authors discussed that the gender difference observed in the study might be due to different growth patterns of boys and girls or could be caused by endocrinological differences.

By contrast, results from the DONALD study in Germany found a higher relative protein intake between 12 and 24 months to be associated with higher BMI at adiposity rebound (AR), only among girls (Gunther et al., 2006). AR is the turning point, normally between 4 and 6 years of age, when a child’s BMI begins to increase again after having reached the nadir. The mean age of AR among the girls in the study was 5.4 years.

A study from the UK found that meat intake from 4-12 months was positively related to weight gain up to 12 months. The authors suggested that the association might be mediated via protein intake, because of the high protein concentrations in meat (Morgan et al., 2004).

A Danish study found that protein intake at 9 months of age was positively associated to height and weight, but not body fatness (BF% or BMI) at 10 years of age (Hoppe et al., 2004b). The authors’ conclusion was that while the data suggest that a high protein intake stimulates growth, it does not support the theory that it leads to a higher risk of overweight and obesity.

Further results from the DONALD study (Gunther et al., 2007a) are likely the first to show that a higher intake of protein during infancy or childhood might result in accretion of fat mass. Their main results were that a consistently high protein intake at the ages of 12 and 18-24 months was associated with both higher BMI and higher BF% at 7 years of age. Protein intake at 6 months did not show any association with the outcomes.

Two recent studies have investigated protein intake in the second year of life and BMI in childhood. While BMI at 6-18 months was found to be the strongest predictor of BMI at 4 years in a Swedish study, protein intake at 17-18 months and at 4 years were also among contributing factors (Ohlund et al., 2010). A study from Australia showed that protein intake at 18 months was positively associated with BMI and waist circumference at 8 years of age (Garden et al., 2011).

A study from the US studied diet and growth in children aged 2-8 years as predictors of BMI at 8 years. Mean protein, as well as mean fat intakes, between 2 and 8 years were found to be positive predictors of BMI at 8 years. Mean carbohydrate intake over the same time period was negatively related to BMI at 8 years (Skinner et al., 2004).

While prospective epidemiological studies give clear indications to support the early protein hypothesis, RCT have the potential, beyond epidemiological studies, to test the real effect of dietary protein on growth and BMI.

2.4.2 Randomized controlled trials

A multicentre randomized trial conducted in five European countries studied whether higher or lower protein intakes during the first year of life influenced growth until the age of 2 years (Koletzko et al., 2009b). Infants were randomly assigned to receive infant and follow-on formulas with lower (1.25 and 1.6 g protein/100 ml, respectively) or higher (2.05 and 3.2 g protein/100 ml, respectively) protein contents for the first year of life. The protein contents represented approximately the lowest and highest levels of the range accepted in the 1991 EU Directive on Infant and Follow-on Formulae (European Commission, 1991). For comparison, children exclusively breastfed for at least the first 4 months of life were followed. Differences in weight, weight-for-length and BMI between the formula groups emerged at 6 months and remained thereafter with a decreasing tendency towards the end of the study at 2 years of age, where the difference in BMI between the high (HP) and low (LP) protein groups was 0.3 kg/m^2. At 2 years of age the weight-for-length z score of infants in the LP group did not differ from that of the breastfed reference group. The authors suggested that lower protein intake in infancy might diminish the later risk of overweight and obesity via slower weight gain in infancy. Further follow-up is planned up to school age and it will be very interesting to see whether the randomization to LP and HP groups will have effects on BMI and overweight and obesity risk at school start.

Other intervention studies have not found an association between protein intake and growth. A Danish study (Larnkjaer et al., 2009a) found no effect of milk type (whole milk or infant formula) on growth between 9 and 12 months. However, drop-outs (17% in whole milk group and 6% in formula group) were shorter at 9 months than participants, which might have affected the results. Another study (Raiha et al., 2002) found no difference in BMI or weight and length gain between four feeding groups (breastfed vs. formula fed with different protein content and ratio whey/casein) during the first 4 months of life. The difference in protein contents between the study formulas was however smaller than in the multicentre randomized trial discussed above (Koletzko et al., 2009b) and the intervention period was also shorter.

To summarize, there is growing evidence that high protein intake in infancy is associated with higher BMI and increased risk of overweight and obesity later in life. While most studies in the context of the protein hypothesis have concentrated on total protein intake, a few studies have investigated whether intake of only animal or plant protein or protein from distinct food sources (e.g. dairy, meat and cereals) could be responsible for potential associations between early protein intake and later body fatness.

2.5 Protein sources

A prospective longitudinal pilot study from the Netherlands (Weijs et al., 2011) aiming at assessing the combined effects of high animal protein intake and high beverage sugar intake showed that infants in the highest tertile of animal protein intakes at 4-13 months of age had a more than 9 times higher risk of becoming overweight at 8 years than infants consuming less animal protein, independent of beverage sugar intake. Combined effects of high intakes of animal protein and beverage sugar were even more deleterious than independent effects of either one. The study did not take total protein or dairy protein into consideration.

Results from the DONALD cohort in Germany (Gunther et al., 2007b) indicated that higher total and animal, but not vegetable protein intakes (at the ages of 12 months and 5-6 years, stronger effects at 12 months) were positively related to body fatness (BMI and BF%) at 7 years of age. With respect to food groups, dairy, but not meat or cereal protein intake, was related to an unfavourable body composition at 7 years.

On the contrary, a cohort study from Australia (Garden et al., 2011) found a negative association between dairy protein intake at 18 months and BMI at 8 years, and a positive association between meat protein intake and BMI. The reason for the opposite findings of this study and the DONALD study is unknown. It might though be conceivable that it may be explained to some part by different patterns of consumption of protein sources due to cultural or age dependent issues. For example, it is possible that while dairy consumption of the 12 month olds in the DONALD study is characterized by a higher intake of fluid milk, e.g. formula, dairy consumption of the 18 month olds in the Australian study is characterized by a higher intake of solid milk products.

Overall, it seems probable that intake of animal protein has a stronger association with later risk of overweight and obesity than vegetable protein has. Studies on IGF-1 strengthen this assumption.

2.6 Biological plausibility of the protein hypothesis: IGF-1

IGF-1 is a polypeptide hormone that it synthesised in the liver upon stimulation by growth hormone (GH) and circulates in the plasma bound to specific IGF-binding proteins (IGFBPs). IGF-1 has anabolic effects: enhances glucose and amino acid uptake, inhibits protein breakdown and is involved in cell replication and proliferation, protein synthesis, carbohydrate homeostasis and bone metabolism. As such it has growth promoting effects on almost every cell in the body and plays a central role in stimulating systemic body growth, adipocyte differentiation and multiplication, and early development of overweight and obesity (Friedrich et al., 2008; Grohmann et al., 2005; Laron, 2001; Madsen et al., 2011).

GH and the IGFBPs determine the circulating levels and tissue availability of IGF-1 together with a factor especially important in infancy: nutrition (Grohmann et al., 2005). While energy intake and certain micronutrients might influence IGF-1, it is hypothesized that protein intake might be the most important dietary stimulator of IGF-1.

2.6.1 Effect of dietary protein on IGF-1 concentrations

Studies on 3, 4, 6 and 9 month old infants (Chellakooty et al., 2006; Madsen et al., 2011; Savino et al., 2005; Socha et al., 2011) have shown an inverse relationship between breastfeeding and IGF-1 concentration. Cross-sectional studies have shown positive associations between animal protein and milk on one hand and IGF-1 on the other hand at ages 2.5 and 7-8 years in both boys and girls (Hoppe et al., 2004c; I. Rogers et al., 2006; I. S. Rogers et al., 2005), and short intervention studies have shown stimulating effects of protein, animal protein and milk on IGF-1 in infants (Larnkjaer et al., 2009a; Socha et al., 2011), 8 year old boys (Hoppe et al., 2004a), and 10-11 year old children (Rich-Edwards et al., 2007). On the contrary, it has been proposed that protein from plant sources entail a rise in IGFBPs that block the activity of unbound IGF-1 (Krajcovicova-Kudlackova et al., 2005; McCarty, 1999).

It is likely that breastfeeding counterbalances milk intake, so it is difficult to determine whether the observed inverse relationship between breastfeeding and IGF-1 is caused by lower protein content in breast milk or by potential specific effect of breast milk through other factors such as hormonal modulation (Larnkjaer et al., 2012). A short intervention study on 8 year old boys, where half of the group drank 1.5 l skimmed milk per day for 7 days while the other half consumed the same amount of protein as 250 g low fat meat daily for 7 days found increased IGF-1 concentrations in the milk group only at the end of the study (Hoppe et al., 2004a). The authors interpret the findings as a suggestion that compounds in milk, and not a high protein intake *per se* stimulates IGF-1 concentration. Results from a prospective cohort study investigating the associations between milk consumption during pregnancy and infant size at birth support this, as they found milk protein and not non-dairy protein to be associated with infant birth measures (Olsen et al., 2007). A cross-sectional study on 7-8 year old children however found that the association of cow's milk/dairy products with IGF-1 was greatly attenuated on controlling for protein intake and that regression coefficients of dairy and non-dairy protein on IGF-1 were very similar (I. Rogers et al., 2006). They authors suggested that the apparent relationship between milk and IGF-1 could reflect an underlying association with protein rather than with milk *per se*, and further that protein *per se* rather than protein from any particular food source may be the important factor. It is evident that there are many unanswered questions with regard to effects of dietary protein on IGF-1 concentration.

2.6.2 Gender differences with regard to IGF-1

A pattern with an increase in IGF-1 concentrations from birth to about 2 months, followed by a decrease until 8-9 months and thereafter a gradual increase which is likely to continue during childhood until it peaks during puberty, around the age of 14 years in girls and 16 years in boys, has been suggested. In childhood, girls have significantly higher values of IGF-1 than boys, especially during the first years of life, whereas boys have higher IGF-1 values during late puberty, when the values start to decrease after the pubertal peak (Clayton & Hall, 2004; Larnkjaer et al., 2012).

Gender differences in IGF-1 concentrations in infancy as response to interventions have been observed but are conflicting as one study found stronger effects of a protein intervention on IGF-1 in girls than boys (Closa-Monasterolo et al., 2011) while another found effects in boys only (Larnkjaer et al., 2009a). A cross-sectional study on 7-8 year old children found higher intakes of cow's milk and dairy products to be significantly associated with IGF-1 concentrations in boys but not in girls (I. Rogers et al., 2006). It would therefore make sense to study the effects of diet on IGF-1 and its association with growth and BMI separately for boys and girls.

2.6.3 Programming of IGF-1 concentration

Studies on long-term effects of IGF-1 concentration in infancy have suggested a programming mechanism as breastfeeding is associated with higher IGF-1 levels at 7-8 years (Martin et al., 2005) and at 17 years (Larnkjaer et al., 2009b) while subjects receiving milk supplementation up to the age of 5 years had lower IGF-1 levels at 25 years of age in a randomized trial (Ben-Shlomo et al., 2005). It has been proposed that increased protein intake during infancy causes a long-term resetting of the growth hormone secreting pituitary resulting in lower IGF-1 levels later in life (Martin et al., 2005). It is not known when this resetting might occur, but the findings that breastfeeding is associated with higher IGF-1 levels at 7-8 years may indicate that the resetting has already taken place by early childhood (Martin et al., 2005).

2.6.4 Effect of IGF-1 on development of overweight and obesity

The above mentioned multicentre RCT with the primary objective of testing whether higher or lower protein intakes during the first year of life influence growth until the age of 2 years as well as overweight and obesity risk at school age (Koletzko et al., 2009b), found higher IGF-1 concentrations at 6 months in the HP than the LP group (Socha et al., 2011). Therefore, it can be figured that the IGF system played a significant role in mediating the effect of the higher protein intake on the increase in weight-for-height and BMI. The associations however seem very complex as two studies (Madsen et al., 2011; Ong et al., 2009) narrate a negative association between IGF-1 at 3 and 9 months and BMI at 12 and 18 months, respectively. In both studies, IGF-1 was positively associated with length gain and not weight gain. These findings seem to be conflicting with the hypothesis that high IGF-1 early in life is associated with later obesity, but it has been proposed that the reverse relationship may indicate an acceleration of decrease in BMI seen in children between 8-9 months and 6 years, which could result in an earlier AR (Larnkjaer et al., 2012; Madsen et al., 2011).

2.6.5 Age of adiposity rebound and puberty

A study from New Zealand strengthens this idea as it found children that were taller at 3 years to experience earlier AR, and thus proposed that age at AR might be partly regulated by linear growth (Williams & Dickson, 2002). IGF-1 is strongly associated with linear growth (Larnkjaer et al., 2012). A study previously discussed (Rolland-Cachera et al., 1995) found a negative association between protein intake at 2 years and age at AR, but two larger studies did not find protein intake at 18 or 12-24 months to be associated with timing of AR (Dorosty et al., 2000; Gunther et al., 2006). The evidence regarding the relationship between protein intake in infancy and timing of AR therefore seems rather weak, but might be mediated via IGF-1 and linear growth. An early AR has been found to be strongly associated with development of adult obesity in studies summarized in a recent review (Brisbois et al., 2012).

The ages at which girls reach AR and menarche have been found to be correlated, suggesting that AR could be a predictor of early maturity (Williams & Dickson, 2002). Observational studies suggest that children down to the age of 3 years with the highest intake of animal protein experience pubertal onset up to 7 months earlier than children with lower intake of animal protein (Cheng et al., 2012), and higher IGF-1 levels at 8 years have been found to be associated with earlier age at menarche (< 12 years) in girls (Thankamony et al., 2012). Early onset of puberty is considered a mediator to a number of diseases in adulthood, including hormone related cancers, a higher risk of all-cause mortality, metabolic syndrome, and cardiovascular disease (Cheng et al., 2012).

2.7 Summary

Childhood overweight and obesity are serious public health threats with both immediate and longer term health effects in addition to reductions in quality of life and social issues. Although they are ultimately assumed to be consequences of increased energy intake and decreased physical activity, dietary factors during the sensitive period of infancy and early childhood are potentially critical for later predisposition to obesity.

There is growing evidence that high protein intake in infancy and early childhood is associated with increased growth and higher BMI later in childhood. It has been proposed that protein from animal origin, especially dairy, rather than protein from plant origin might be responsible for potential associations. Studies on IGF-1 add strength to this notion.

Ten years ago an Icelandic study showed high protein intake at 9-12 months, attributable to high intake of regular cow’s milk, to be associated with higher BMI in 6 year old boys. Following these findings, the primary prevention strategy of revising infant dietary recommendations was implemented, emphasising breastfeeding and recommending limited consumption of a new product, follow-on formula (Stoðmjólk) with lower protein concentration than cow’s milk, from 6 months to 2 years. A new infant study conducted after the revised recommendations showed that dietary intake among infants had moved towards the revised recommendations. The manuscripts presented in this thesis investigate the effects of the revision of recommendation on protein intake in the latter half of the first year and answer the important questions on whether this primary prevention effort succeeded in lowering BMI and reducing the prevalence of overweight and obese Icelandic 6 year old children. They also report infant dietary predictors of BMI and IGF-1 in Icelandic 6 year olds, with a focus on sources of dietary protein, thus directing attention to potential risk factors for childhood and adulthood overweight and obesity.

3. METHODS

Methods related to my thesis are described in detail in the manuscripts (see chapter 4). More in-depth descriptions on the infant and follow-up studies are accessible in published papers (Atladottir & Thorsdottir, 2000; Gunnarsdottir & Thorsdottir, 2003; Thorisdottir et al., 2011).

3.1 Author's contribution

My contribution to the MSc project was divided into four work packages (WP):

WP1: Data collection in the follow-up study at 6 years in the 2005 cohort. Time: September 2011-February 2012.

The data collection was initiated in June 2011. When I started participating in the data collection, about half of the 219 eligible 6 year old children still needed to be contacted. Parents of 6 year old children were contacted by telephone and invited, on behalf of their children, to participate in the follow-up study. Participants living outside the capital area got the participation material sent by mail and were asked to accompany their children to the respective healthcare centre for anthropometric and blood measurements. Participants living in the capital area were visited at home with participation material, they were advised how to perform the weighed dietary assessment and a time for anthropometric and blood measurements was set. I went to a few schools that were not able to weigh participants' lunch and weighed for the children. The children and their parents were greeted at the Children's Hospital, participants were weighed and their height and blood pressure measured. The paediatrician was associated with blood testing and the participation material (informed written consent, food diaries, developmental inventory and parental questionnaire) was obtained from the parents. Participants were wished farewell with breakfast and a little gift. The blood samples were taken to the laboratory to be analysed immediately or to be refrigerated. Measures on weight and height between the ages 12 months and 6 years were obtained from the participating children's healthcare centres.

WP 2: Data processing. Time: February-March 2012.

The weighed food records were entered into the nutrient calculation program ICEFOOD. The results from the blood analysis were retrieved from the Flexlab program of Landspitali.

Participation material was entered into excel files. All data was imported in SAS along with data from the 1995 infant and follow-up study and the 2005 infant study. The databank was trimmed and organized.

Food sources of dietary protein in infancy were obtained from ICEFOOD, presented coded. The key can be seen in Appendix 1. When constructing food groups for the manuscript in chapter 4.2, I followed descriptions from the DONALD cohort (Gunther et al., 2007b) because I thought that the study was well designed, taking place in a setting quite similar to ours and the authors described clearly how and why they constructed their food groups. An exception was that infant formula was included in the calculation of dairy protein in our study on contrary to the DONALD study because I estimated the reasons for excluding it in the DONALD cohort not to be of relevance in our cohort. The food groups used in the manuscript in chapter 4.2 can be seen in Appendix 2.

WP 3: Statistical analysis and presentation of results. Time: April-November 2012.

- Poster presentation on the 10th Nordic Nutrition Conference in Reykjavik in June 2012: Thorisdottir B, Gunnarsdottir I, Thorisdottir AV, Palsson G, Halldorssson T, Thorsdottir I. *Growth and feeding in infancy and BMI at 6 years of age – Trends in a 10 years period.*
- Oral presentation on the Research Congress of the Icelandic Association for the Study of Obesity in Reykjavik in September 2012: Thorisdottir B. *Association between dietary habits of Icelandic infants and body mass index at six years of age* (In Icelandic: *Tengsl mataræðis á fyrsta aldursári við líkamsþyngdarstuðul sex ára barna).*
- Original scientific papers:
    - Thorisdottir B, Gunnarsdottir I, Thorisdottir AV, Palsson G, Halldorssson T, Thorsdottir I. *Infant dietary predictors of BMI at 6 years: Two population based studies conducted 10 years apart.* Manuscript to be submitted in January 2013.
    - Gunnarsdottir I, Helgadottir H, Thorisdottir B, Thorsdottir I. *Diet of six-year-old Icelandic children – National dietary survey 2011-2012.* Submitted in September 2012.
    - Thorisdottir B, Gunnarsdottir I, Halldorssson T, Thorsdottir I. *Protein sources in infancy as predictors for IGF-1 concentration and body mass index at the age of 6 years.* Draft manuscript to be submitted in January 2013.

I performed the statistical analysis and wrote the first draft of the poster presentation and the two papers on which I am the first author, with guidance and suggestions from supervisors and co-authors. Additionally, I critically reviewed the manuscript by Gunnarsdottir et al. and approved the final manuscript as submitted.

WP4: Writing MSc thesis. Time: November-December 2012.

4. MANUSCRIPTS

4.1 Infant dietary predictors of BMI at 6 years: Two population based studies conducted 10 years apart

4.2 Protein sources in infancy as predictors for body mass index and IGF-1 concentration at the age of 6 years

Manuscript, December 4th 2012

4.1 Infant dietary predictors of BMI at 6 years: Two population based studies conducted 10 years apart.

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Short title: Infant diet and childhood BMI

Keywords: Infant, growth, dietary proteins, body mass index, overweight, child

Abbreviations: BF – breastfeeding, BMI – body mass index, CI – confidence interval, E% – percent of energy, EBF – exclusive breastfeeding, IOTF – International Obesity Task Force, ISGEM – the Icelandic nutrient composition database, IQR – interquartile range, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SD – standard deviation, SFA – saturated fatty acids

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ABSTRACT

Background and objective: High protein intake in infancy has been associated with higher body mass index (BMI) in childhood. Icelandic infant dietary recommendations were revised in 2003, emphasizing prolonged breastfeeding and limited consumption of cow´s milk. Our objective was to assess the effects of the revision on protein intake in infancy and possible long term effects on BMI at 6 years.

Methods: Two prospective randomly selected cohorts, investigated with a 10 year interval prior to and after the revision of the infant dietary recommendations were recruited at birth and studied until 12 months and again at 6 years. Subjects were 90 and 170 children born in 1995-6 and 2005, respectively. Dietary intake at 9 and 12 months was assessed by weighed food records. Height and weight from birth to 12 months and at 6 years were measured.

Results: The main milk product consumed at ages 9 and 12 months shifted from cow´s milk in the former cohort to follow-on formula with lower protein content in the latter. Protein intake was significantly lower in the latter cohort, 11.9 percent of energy (E%) vs. 14.4 E% ($P<0.0001$) at 9 months and 14.6 E% vs. 15.6 E% ($P=0.016$) at 12 months. Relatively fewer children were classified as being overweight (including obese) in the latter cohort, 12% vs. 21% ($P=0.045$). Linear regression showed that cow´s milk and protein intake at 12 months were positive predictors of BMI at 6 years, presented as β 95%CI: 0.2 (0.0, 0.3) and 0.1 (0.0, 0.1), respectively, when adjusted for cohort, gender, energy intake, birth weight, duration of breastfeeding and mother´s education.

Conclusion: Emphasis made in the revised infant dietary recommendations from 2003 resulted in lower intake of cow´s milk and subsequent lower protein intake in the latter half of the first year. Changes in infant diet on the population level might have contributed to the lower overweight prevalence in the latter cohort than the former.

INTRODUCTION

Various factors in early life that influence or program the long-term propensity to obesity have been identified.(Brisbois et al., 2012; Druet & Ong, 2008; Monasta et al., 2010; Rooney et al., 2011) Infancy or early childhood might therefore be critical periods for primary obesity prevention.(Koletzko et al., 2009b; Singh et al., 2008; Summerbell et al., 2012) With regard to dietary factors, protein has received particular attention. Growing evidence from cohort studies and a randomized controlled trial supports the hypothesis that high protein intake in infancy stimulates growth and is associated with higher body mass index (BMI) in childhood.(Garden et al., 2011; Gunther et al., 2007a; Hoppe et al., 2004b; Koletzko et al., 2009b; Morgan et al., 2004; Ohlund et al., 2010; Rolland-Cachera et al., 1995; Scaglioni et al., 2000; Weijs et al., 2011)

In our previous analysis, high intake of protein at the age of 9-12 months was found to be associated with higher BMI in 6 year old boys.(Gunnarsdottir & Thorsdottir, 2003) At this time cow´s milk, for the most part full-fat cow´s milk, gradually replaced breast milk in the age range of 5-12 months and accounted for the high protein intake.(Atladottir & Thorsdottir, 2000) Based on these results, a follow-on formula with lower protein content than regular cow´s milk but same energy density was developed and made available at a fair price in every grocery shop in Iceland in ready to drink cartons. Furthermore, in 2003 the national infant dietary recommendations were revised, emphasizing breastfeeding and encouraging use of follow-on formula instead of cow's milk up to 2 years of age.(The Icelandic nutrition council and centre for child health services)

In the present study we investigated two prospective population based Icelandic cohorts of children born in 1995-6 and 2005, prior to and after publication of revised infant dietary recommendations. The objective was to assess the effects of the revision on protein intake in infancy and possible long term effects on BMI at 6 years.

MATERIALS AND METHODS

Sample recruitment

The study included randomly selected subjects from two Icelandic longitudinal birth cohort studies conducted with a 10 year interval (subjects in the former cohort born in 1995-6 and subjects in the latter cohort born in 2005). The inclusion criteria were the same in both studies, i.e. Icelandic parents, singleton birth, gestational length of 37-41 weeks, birth weight within the 10^{th}-90^{th} percentiles, no birth defects or congenital long-term diseases, and the mother had early and regular antenatal care. Methods in the two studies have been previously published in detail.(Atladottir & Thorsdottir, 2000; Gunnarsdottir & Thorsdottir, 2003; Thorisdottir et al., 2011) Subjects were thoroughly investigated from birth to 12 months of age and again at follow-up at 6 years. The mean weight and length at birth and at 12 months, intake of energy and macronutrients, as well as parental variables of the infants included in this current analysis did not differ from the infants in the original studies. This suggests that the random selection of participants resulted in a representative sample from the source population.

Informed written consent from the parents was obtained, and all individual information was processed with strict confidentiality. The studies were approved by the Icelandic Bioethics Committee, the Icelandic Data Protection Authority, and the Local Ethical Committee at Landspitali-The National University Hospital of Iceland (hereafter Landspitali).

Dietary assessment

At ages 9 and 12 months all food and fluids consumed were weighed for 2 or 3 consecutive days (48 or 72 hours) on accurate scales (PHILIPS HR 2385, Austria; PHILIPS HR 2385, Hungary) (precision 1 g). The breastfed infants were weighed in the same clothes before and after breastfeeding (Tanita 1581, Japan; Tanita model 1583, Japan or Sega model 336 7021099, Germany) (precision 10 g) to estimate the amount of breast milk consumed. An average daily consumption of food and nutrients was calculated using the Icelandic nutrient composition database (ISGEM). Special infant products, such as cereals and purées were added to the database for the purpose of this study. Nutrient losses due to food preparation were taken into account in the calculations. Total milk consumption was calculated by summing up consumption of breast milk, infant formula (intended for infants up to age 6 months), follow-on formula (intended for infants and children age 6 months to 2 years), and cow´s milk.

Growth and definition of overweight

Information on weight, length and head circumference at birth was gathered from the maternity wards where the infants received post-natal care. The participating families´ healthcare centers provided anthropometric measurements throughout infancy. In the present paper measurements conducted at birth and at 12 months were used. At 6 years of age, weight and height were measured at the Children´s Hospital, Landspitali using a Marel M series 1100 scale (Reykjavik, Iceland) (precision 0.1 kg) and Ulmer stadiometer according to Prof. Heinze (Ulm, Germany) (precision 0.5 cm). Children were classified as being normal weight, overweight or obese according to the International Obesity Task Force (IOTF) cut-off points for BMI for overweight and obesity defined to pass through BMI of 25 and 30 kg/m^2 at the age of 18.(Cole et al., 2000) Cut-off points of 17.55 kg/m^2 and 17.34 kg/m^2 for overweight and 19.78 kg/m^2 and 19.65 kg/m^2 for obesity were applied for 6 year old boys and girls, respectively.

Covariates

Information on breastfeeding was gathered monthly. An infant was classified as being breastfed if it was breastfed once or more a day. The definition of exclusive breastfeeding allowed in addition only water, vitamins e.g. A-D vitamin supplement, and medications for infant colic.(Atladottir & Thorsdottir, 2000) When the participants were 12 months of age the mother answered a questionnaire on socioeconomic and environmental factors. Weight and height of the mother was self-reported. The mother was defined overweight (including obese) if the BMI was greater than 25 kg/m^2. Higher education was set at schooling of 12 years or longer.

Statistical analysis

Statistical analyses were performed with SAS (Enterprise Guide 4.3; SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used for the participants´ characteristics and dietary consumption, presented as mean and standard deviation (SD) for normally distributed variables, median with interquartile range (IQR) for skewed variables, and ratios (%) for binominal variables. For comparison between two groups, independent t-test, Mann-Whitney *U* test, or chi-square was used.

To identify dietary predictors (milk products, energy or macronutrients) of BMI at 6 years in the former and latter cohorts, linear regression was used. Because the directions of the regression coefficients for the selected variables in both cohorts were the same, associations were tested and presented in the cohorts combined for simplicity and to increase exposure range and statistical power. The covariates included in the multivariate analysis were common predictors of childhood overweight;(Monasta et al., 2010; Rooney et al., 2011) birth weight, duration of breastfeeding, mother´s education, in addition to cohort (to incorporate different feeding practices) and gender. For these selected covariates the number of missing values ranged from 0 to 12%. Missing values were replaced by the median value for each covariate. Stability analysis revealed that the same conclusions were in all cases reached when using this substitution as compared with complete case analysis. We acknowledge that the distribution of BMI at 6 years was skewed, especially for the latter cohort. The regression analysis yielded same P-values when run on transformed data. Therefore we believe that the analysis presented with absolute values of BMI to facilitate interpretation is valid. The level of significance in the study was $P<0.05$.

RESULTS

In the former vs. latter cohort, 180 vs. 250 infants were invited to participate, 138 vs. 244 accepted and 100 vs. 219 were invited to the follow-up. Participants eligible for this study were subjects with dietary data at 9 or 12 months or complete anthropometric data in infancy and measurements on weight and height at 6 years (90 from the former cohort and 170 from the latter cohort, in all 260 subjects). Complete dietary registrations where obtained from 80 subjects at 9 and 12 months in the former cohort and 154 (at 9 months) and 137 (at 12 months) subjects in the latter cohort.

Table 1 shows the characteristics of the participating infants. Weight gain in the first year of life in the two cohorts was near identical (presented as mean (SD)): 6.2 (1.1) kg in the latter vs. 6.2 (1.0) kg in the former cohort, P=0.65. From 6 to 10 months of age, infant weight gain in the latter cohort was slower than in the former cohort: 1.3 (0.5) kg vs. 1.5 (0.6) kg, P=0.037. Other characteristics did not differ between the cohorts.

At 9 months, 37% of participants in the former cohort and 41% in the latter were still being breastfed. At 12 months, breastfeeding rate had dropped to 12% in the former cohort and 15% in the latter. Use of infant formula was very rare in both cohorts. At both 9 and 12 months of age, consumption of the recently available follow-on formula replaced cow´s milk to a large extent in the latter cohort compared to the former (Table 2). Total milk intake did however not differ between the cohorts, nor did intake of energy. Protein intake in the latter cohort was significantly lower than in the former cohort, replaced by a higher intake of carbohydrates. Differences in consumption of polyunsaturated, monounsaturated and saturated fatty acids (PUFA, MUFA and SFA), dietary fiber and added sugar were apparent between the cohorts.

Table 1. Characteristics of participants, comparison between former (born in 1995-6) and latter (born in 2005) cohorts.

	Former cohort (N=90)	Latter cohort (N=170)	P-value
Girls (%)	54	52	0.68
Birth weight[1] (kg)	3.8 (0.4)	3.7 (0.4)	0.15
Weight at 12 months[1] (kg)	10.0 (1.0)	10.0 (1.1)	0.89
Duration of EBF[2] (months)	4 (3)	4 (3)	0.50
Total duration of BF[2] (months)	8 (4)	8 (5)	0.95
Mother´s age[2] (years)	30 (8)	31 (9)	0.52
Mother´s BMI[2] (kg/m^2)	24.6 (5.9)	24.0 (5.6)	0.43
Overweight mother (%)	42	41	0.90
Mother ≥12 years of schooling (%)	73	81	0.21

Abbrevations: BF – breastfeeding, BMI – body mass index, EBF – exclusive breastfeeding, IQR – interquartile range, SD – standard deviation

[1]Presented as mean (SD)

[2]Presented as median (IQR)

Table 2. Intake per day of milk products, energy, macronutrients and fibre at 9 and 12 months, comparison between the former (born in 1995-6) and latter (born in 2005) cohorts.

	9 months			12 months		
	Former cohort (N=80) Median (IQR)	Latter cohort (N=154) Median (IQR)	*P*-value	Former cohort (N=80) Median (IQR)	Latter cohort (N=137) Median (IQR)	*P*-value
Breast milk (g)	0 (220)	0 (242)	0.70	0 (0)	0 (0)	0.18
Follow-on formula (g)	-	63 (285)	<0.0001	-	124 (284)	<0.0001
Cow´s milk (g)	232 (317)	0 (27)	<0.0001	262 (312)	17 (89)	<0.0001
Total milk[1] (g)	404 (322)	403 (317)	0.28	301 (333)	307 (212)	0.97
Energy (kcal)	760 (185)	745 (230)	0.36	864 (207)	821 (245)	0.13
Protein (g)	28.0 (13.7)	22.7 (9.7)	<0.0001	33.7 (11.7)	30.0 (13.5)	0.008
Protein (g/kg)	2.7 (1.1)	2.4 (1.1)	0.008	3.5 (1.3)	3.0 (1.0)	0.020
Protein (E%)	14.4 (5.8)	11.9 (4.5)	<0.0001	15.6 (4.2)	14.6 (4.1)	0.016
Fat (E%)	37.1 (8.6)	36.1 (8.3)	0.61	36.5 (7.9)	35.6 (7.7)	0.20
PUFA (E%)	2.5 (1.9)	2.9 (2.5)	0.26	2.6 (1.2)	2.9 (2.0)	0.023
MUFA (E%)	10.5 (4.2)	8.4 (7.8)	0.004	10.9 (3.0)	8.5 (5.0)	<0.0001
SFA (E%)	17.5 (7.0)	16.3 (4.9)	0.008	19.1 (4.8)	15.9 (4.3)	<0.0001
Carbohydrates (E%)	49.0 (8.9)	50.5 (7.5)	0.028	46.6 (8.2)	49.0 (7.6)	0.06
Fibre (g)	5.2 (3.5)	6.3 (3.8)	0.012	5.7 (2.9)	7.4 (3.9)	0.001
Added sugar (E%)	3.7 (5.7)	0.9 (2.4)	<0.0001	7.1 (4.9)	3.6 (4.0)	<0.0001

Abbrevations: E% – percent of energy, IQR – interquartile range, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids

[1]Total milk is the sum of breast milk, infant formula (intended for infants up to age 6 months), follow-on formula (intended for infants and children age 6 months to 2 years), and cow´s milk.

A small downwards trend was observed in BMI at 6 years of age (presented as median (IQR)): 15.5 (1.7) kg/m^2 vs. 15.8 (2.2) kg/m^2 in the latter vs. former cohort, P=0.15. Although the BMI at 6 years did not differ significantly between the cohorts, relatively fewer children were classified as being overweight or obese at 6 years of age in the latter cohort than the former (Figure 1), 12% vs. 21% (P=0.045). In both cohorts, 4% of children were classified as obese, but in the latter cohort there were a few children with extremely high BMI, higher than seen in the former cohort.

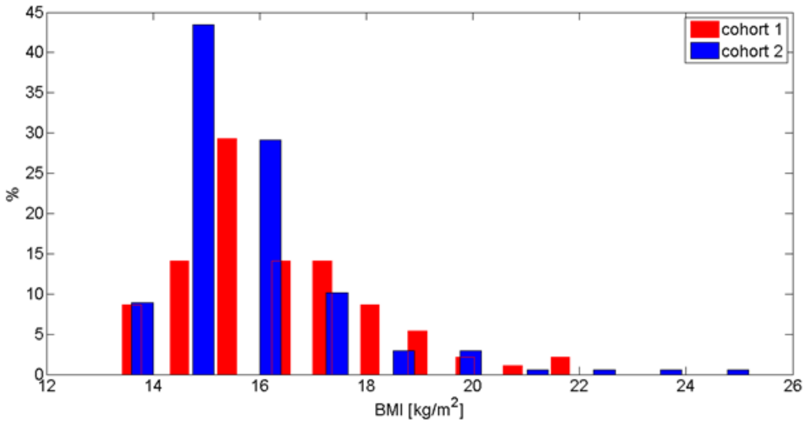

Figure 1. Distribution of body mass index (BMI) of 6-year-old children in the former (red) and latter cohort (blue). Cut-off points of 17.55 kg/m^2 and 17.34 kg/m^2 for overweight and 19.78 kg/m^2 and 19.65 kg/m^2 for obesity were applied for boys and girls, respectively. In the latter cohort, 150 children (88%) were classified as normal weight, 14 children (8%) as overweight and 6 children (4%) as obese. In the former cohort, 71 children (79%) were classified as normal weight, 15 children (17%) as overweight and 4 children (4%) as obese.

Table 3 shows fully adjusted models for the associations between intake of milk products, energy and macronutrients in infancy and BMI at 6 years in the former and latter cohorts combined. Intake of cow´s milk, total milk and dietary protein at 12 months were positive predictors of BMI at 6 years. When cow´s milk was excluded from the "total milk" group, total milk at 12 months was no longer associated with BMI at 6 years (β (95%CI)): -0.0 (-0.1, 0.1). Other variables presented in the table, as well as intake in infancy of PUFA, MUFA, SFA, dietary fiber and added sugar, were not associated with BMI at 6 years.

Children were divided into quartiles based on their intake of cow´s milk and protein at 12 months with the 25th vs. 75th percentiles for cow´s milk being 0 vs. 275 g/day, and the 25th vs. 75th percentiles for protein being 13.2 vs. 17.6 E%. Children in the lowest quartile of protein intake at 12 months had lower BMI at 6 years than children in the highest quartile (Table 4). BMI did not differ between the lowest and highest quartiles for cow´s milk.

Table 3. Associations between intake of milk products, energy and macronutrients and BMI at 6 years, combined cohorts.

	9 months (N=234)		12 months (N=217)	
	β (95%CI)	P-value	β (95%CI)	P-value
Breast milk (100 g)[1]	-0.1 (-0.2, 0.0)	0.13	0.0 (-0.2, 0.2)	0.76
Follow-on formula (100 g)[2]	-0.1 (-0.2, 0.1)	0.52	-0.0 (-0.2, 0.1)	0.74
Cow´s milk (100 g)[2]	0.1 (-0.1, 0.2)	0.28	0.2 (0.0, 0.3)	0.017
Total milk (100 g)[2]	-0.0 (-0.1, 0.1)	0.99	0.1 (0.0, 0.2)	0.049
Total energy (MJ)[3]	0.0 (-0.3, 0.3)	0.92	0.1 (-0.1, 0.4)	0.34
Protein (E%)[2]	0.1 (-0.0, 0.1)	0.16	0.1 (0.0, 0.1)	0.042
Fat (E%)[2]	-0.0 (-0.0, 0.0)	0.83	-0.0 (-0.1, 0.0)	0.18
Carbohydrates (E%)[2]	-0.0 (-0.0, 0.0)	0.63	0.0 (-0.0, 0.0)	0.86

Abbrevations: β – coefficient, BMI – body mass index, CI – confidence interval, E% – percent of energy

[1]Adjusted for cohort, gender, energy intake at 9 or 12 months, birth weight, mother´s eduction

[2]Adjusted for cohort, gender, energy intake at 9 or 12 months, birth weight, duration of breastfeeding, mother´s eduction

[3]Adjusted for cohort, gender, birth weight, duration of breastfeeding, mother´s eduction

Table 4. Body mass index in participants in the lowest and highest quartiles of consumption at 12 months, combined cohorts.

	Lowest quartile Median (IQR)	Highest quartile Median (IQR)	P-value
Cow´s milk (g)	15.5 (1.8)	15.8 (2.1)	0.10
Protein (E%)	15.1 (2.0)	15.8 (2.5)	0.01

Abbrevations: E% – percent of energy, IQR – interquartile range

DISCUSSION

This study on two prospective population based Icelandic cohorts of children born 10 years apart, prior to and after publication of revised infant dietary recommendations, shows major changes in infant dietary habits between the cohorts. The primary difference was the shift from cow´s milk as the main milk product consumed at ages 9 and 12 months to follow-on formula. The protein content of the follow-on formula is 1.8 g protein/100 g, or 10 percent of energy (E%), compared with 3.4 g protein/100 g, or 20 E%, in regular cow's milk. Energy density does not differ between the two products (67 kcal/100 g in follow-on formula and 68 kcal/100 g in cow´s milk), the protein indentation is mainly compensated for by carbohydrates (43 E% vs. 28 E%). The two cohorts reflect this, with lower intake of protein and higher intake of carbohydrates in the latter cohort than the former. We propose that the lower protein intake in the latter cohort may partly explain the slower weight gain between 6 and 10 months.(Larnkjaer et al., 2012) Other changes observed between the cohorts, although not related to BMI at the age of 6 years, were improved qualities of fat and carbohydrates, which may partly reflect favorable changes in the diet of the parents.(Thorgeirsdottir et al., 2011)

The only infant dietary variables associated with BMI at 6 years were cow´s milk and protein at 12 months. Our results suggest that children consuming more than 275 g cow´s milk per day, as half of the infants in the former cohort did, would have 0.3 kg/m^2 higher BMI at 6 years than children not consuming any cow´s milk, as common in the latter cohort. This difference was non-significant, but interestingly, difference in BMI between the cohorts, non-significant, was also 0.3 kg/m^2. Another interesting point is that the protein content of the follow-on formula (1.8 g protein/100 g) is quite similar as protein content in the lower protein (LP) follow-on formula in the multicentre randomized trial of the European Obesity Trial Study Group(Koletzko et al., 2009b) (1.6 g protein/100 ml), while protein content of regular full-fat cow´s milk (3.4 g protein/100 g) is similar as in the high protein (HP) follow-on formula (3.2 g protein/100 ml) in the randomized trial. In that study, mean difference in daily protein intake at 12 months in the HP and LP groups was approximately 5 g and difference in BMI between the groups at 2 years of age was 0.3 kg/m^2.(Koletzko et al., 2009b) In our study, mean difference in daily protein intake at 12 months is close to 4 g in the former and latter cohorts and the difference in BMI at 6 years is 0.3 kg/m^2 (non-significant). This might strengthen the assumption that lower intake of cow´s milk and subsequent lower protein intake in the latter cohort might have contributed to the downwards trend in BMI at 6 years and lower overweight prevalence.

The strength of this study is the longitudinal design and the detailed information about diet and growth at narrow age ranges during the first year of life and again at 6 years of age. Furthermore, the two cohorts are population based and representative of Icelandic children born during this period. In the present study considerable variation in complementary feeding practice was seen between the two cohorts and to some extent, the study may be considered a "natural intervention". To our knowledge, it is the first population based study including repeated measures of intake in an infant population born 10 years apart showing long-term effects on prevalence of overweight with decreased cow´s milk and protein intake during infancy. Our finding on lower prevalence of overweight in the latter cohort compared with the cohort studied 10 years prior is positive and is strengthened by results from an Icelandic nationwide study on 9 year old school children(Johannsson et al., 2006) and school surveillance in the capital area of Iceland(S. H. Jonsson et al.) which reported stabilization of overweight rates in the past decade. However, the findings of few extremely obese children in the latter cohort presented in the present study are alarming and indicate that despite downwards trend in the prevalence of overweight on the population level the number of severe obese children might still be growing. Further studies are needed in order to define risk factors related to severe obesity in young children. Furthermore, although parents and other caregivers adopted the guidelines on limited cow´s milk quite easily, breastfeeding duration between the cohorts did not increase, despite effort in emphasizing prolonged duration of breastfeeding. The relatively small sample size may be a limitation to the study, and although not related to BMI at the age of 6 years in our regression models we cannot exclude that improved quality of carbohydrates and fat may also have contributed to the trend towards lower prevalence of overweight at the age of 6 years.

Conclusions

The results indicate that lower intake of cow´s milk and subsequent lower protein intake in the latter half of the first year might have contributed to the downwards trend in BMI of 6 year old Icelandic children and lower overweight prevalence in the latter cohort than the former. Our study emphasizes the importance of monitoring infant nutrition in order to provide appropriate dietary guidelines and suggests that altered recommendations on infant nutrition and the introduction of a new option in complementary feeding practices may have contributed to the observed decrease in the overweight in the population studied.

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4.2 Protein sources in infancy as predictors for body mass index and IGF-1 concentration at the age of 6 years.

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Short title: Infant protein sources predicting IGF-1 and BMI at 6-y.

Keywords: dietary proteins, animal protein, infant, body mass index, insulin-like growth factor 1, child

Abbreviations: BF – breastfeeding, BMI – body mass index, CI – confidence interval, E% – percent of energy, EBF – exclusive breastfeeding, ICEFOOD – a nutrient calculation program, IGF-1 – insulin-like growth factor 1, IQR – interquartile range, ISGEM – the National Food Composition Database, SD – standard deviation

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Contributor´s Statement:

Birna Thorisdottir: Ms. Thorisdottir participated in data collection, carried out the initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

Ingibjorg Gunnarsdottir: Dr. Gunnarsdottir conceptualized and designed the study, supervised data collection, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Thorhallur Ingi Halldorsson: Dr. Halldorsson supervised the initial analyses, critically reviewed the manuscript, and approved the final manuscript as submitted.

Inga Thorsdottir: Dr. Thorsdottir conceptualized and designed the study, coordinated data collection, critically reviewed the manuscript, and approved the final manuscript as submitted.

What´s Known on This Subject

High protein intake in infancy has been associated with increased growth and higher body mass index (BMI) in childhood. It has been suggested that animal protein, in particular dairy, has a stronger association with growth than vegetable protein has. Less is known about the association between protein intake in infancy and insulin-like growth factor 1 (IGF-1) concentration in childhood.

What This Study Adds

Our study supports the hypothesis that the association between high intake of total protein and higher BMI in childhood is rather related to high intake of protein from animal sources than vegetable sources. Furthermore, the study suggests that high protein intake from animal sources, especially dairy, at 12 months predicts increased IGF-1 concentration at the age of 6 years, in girls only.

ABSTRACT

Objective: To study the association between total protein intake as well as protein from various dietary sources at the age of 12 months and BMI and IGF-1 at 6 years.

Methods: Subjects were 137 children studied from birth to 6 years of age. Dietary intake at 12 months was assessed by three day weighed food records. Information about height and weight during the first year of life and at 18 months and 6 years was gathered. IGF-1 was measured at 6 years of age.

Results: The positive association observed between total protein intake at the age of 12 months and BMI at the age of 6 years was attributable to protein from animal sources. Children in the highest quartile of animal protein intake at 12 months (consuming ≥11.9 percent of total energy (E%) as animal protein) had higher BMI at 12 months (0.7 (0.0, 1.3) kg/m^2), 18 months (0.7 (0.1, 1.3) kg/m^2) and 6 years (0.8 (0.2, 1.4) kg/m^2) than children in the lowest quartile (<7.7 E% from animal protein). The difference in BMI at 6 years was independent of breast milk intake at 12 months. Children still being breastfed at 12 months (19% of study sample) had lower BMI at 12 (-0.6 (-1.2, 0.0) kg/m^2) and 18 months (-0.7 (-1.2, -0.1) kg/m^2) than children breastfed for a shorter duration. Dairy protein intake at 12 months was a positive predictor of IGF-1 at 6 years for girls (5.4 (2.5, 8.2) µg/l), independent of current height or weight. The association between intake of dairy proteins and IGF-1 at 6 years for boys seemed to be in opposite direction to what was seen in girls.

Conclusions: Our results highlight the need for special emphasis in infant dietary guidelines aiming at avoiding excess animal protein intake in the complementary feeding period. Further, the results support prolonged duration of breastfeeding, into the second year of life. Our findings of a positive relationship between dairy protein intake at the age of 12 months and IGF-1 levels in 6-year-old girls may be of relevance. Increased IGF-1 might be an indication of early puberty that has been associated with negative long-term effects on health.

INTRODUCTION

Growing evidence from cohort studies and a randomized controlled trial supports the "early protein" hypothesis, that postulates that high protein intake in infancy is associated with increased growth and higher body mass index (BMI) in childhood.(Gunnarsdottir & Thorsdottir, 2003; Gunther et al., 2007a; Hoppe et al., 2004b; Koletzko et al., 2009b; Morgan et al., 2004; Ohlund et al., 2010; Rolland-Cachera et al., 1995; Scaglioni et al., 2000; Skinner et al., 2004) It has been proposed that dietary protein stimulates the secretion of insulin-like growth factor 1 (IGF-1), leading to rapid weight gain early in life, which has independent effects on overweight risk and adipocyte differentiation.(Koletzko et al., 2009a) Animal and vegetable protein is known to exert different metabolic effects in the body due to differences e.g. in composition of amino acids.(Krajcovicova-Kudlackova et al., 2005; McCarty, 1999) While most studies have concentrated on total protein intake, few studies suggest that protein from animal sources, in particular dairy, could be responsible for the "early protein" effects on BMI.(Gunther et al., 2007b; Hoppe et al., 2009; Hoppe et al., 2004c; Larnkjaer et al., 2009a; Weijs et al., 2011)

IGF-1 has several potentially opposing roles in relation to overall health. In adults, higher levels of IGF-1 have been associated with a reduced risk of osteoporosis, diabetes and possibly heart disease, but also with increased risk of several types of cancer.(I. S. Rogers et al., 2005) It has been shown that diet influences the circulating levels and tissue availability of IGF-1(Grohmann et al., 2005) and protein has been suggested as the most important dietary stimulator of IGF-1.(Larnkjaer et al., 2012) It is not known whether total protein or protein from certain food sources is the most important factor. The effects of protein sources on IGF-1 levels in childhood are potentially important as it may be that IGF-1 concentrations in childhood have a long-term influence on the risk of later disease. Studies are however few.(I. S. Rogers et al., 2005)

Using data from an Icelandic infant cohort, followed-up at six years of age, the objective was to study the effects of total protein and protein from distinct sources on childhood growth, BMI and IGF-1.

METHODS

Subjects

170 subjects were invited to participate in this population based longitudinal children study. They were participants in the Icelandic infant nutrition study from 2005-7 of randomly chosen sample who fulfilled the criteria set for the present study, i.e. complete information on food and nutrient intake at 12 months of age along with information on breastfeeding duration and complete data on infant size and growth parameters. 137 subjects (81% of eligible subjects) agreed to participate. As previously reported,(Thorisdottir et al., 2011) the inclusion criteria were Icelandic parents, singleton birth, gestational length of 37-41 weeks, birth weight within the 10th-90th percentiles, no birth defects or congenital long-term diseases, and the mother had early and regular antenatal care. Informed written consent from the parents was obtained, and all individual information was processed with strict confidentiality. The studies were approved by the Icelandic National Bioethics Committee, Data Protection Authority, and the Local Ethical Committee at Landspitali-The National University Hospital of Iceland (hereafter Landspitali).

Dietary Assessment

At 12 months of age all food and fluids consumed were weighed for 3 consecutive days (72 hours) on accurate PHILIPS HR 2385 scales (Hungary) (precision 1 g). The breastfed infants were weighed in the same clothes before and after breastfeeding (Tanita model 1583, Japan or Sega model 336 7021099, Germany) (both with precision 10 g) to estimate the amount of breast milk consumed. An average daily consumption of food and nutrients was calculated using a nutrient calculation program, ICEFOOD, based on the Icelandic nutrient composition database (ISGEM). Special infant products, such as cereals and purées were added to the database for the purpose of this study. Nutrient losses due to food preparation were taken into account in the calculations.

In addition to total protein intake, we also considered animal protein (excluding protein from breast milk because its effect on development of BMI is expected to differ from that of other animal sources and might confound potential associations) and vegetable protein intake. Animal protein intake was further divided into protein from food groups that have been previously studied in regard to associations with childhood BMI or IGF-1:(Gunther et al., 2007b) dairy protein (e.g. from cow´s milk, formulas, yoghurt, cheese, and ice-cream) and meat protein in addition to fish protein.

Biochemical Analysis and Anthropometric Measures

At 6 years of age participants came to the Children´s Hospital at Landspitali. Fasting blood samples were obtained and IGF-1 level analyzed with Immulite® 1000 Immunoassay System (Siemens, USA) (precision 1 µg/l). Weight and height were measured using a Marel M series 1100 scale (Reykjavik, Iceland) (precision 0.1 kg) and Ulmer stadiometer according to Prof. Heinze (Ulm, Germany) (precision 0.5 cm). Information on weight, length and head circumference at birth was gathered from the maternity wards where the infants received post-natal care. The participating families´ healthcare centers provided anthropometric measurements throughout infancy and childhood, i.e. at ages 6, 12, and 18 months.

Statistical analysis

Statistical analyses were performed with SAS (Enterprise Guide 4.3; SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used for the participants' characteristics and consumption of food and nutrients, presented as mean and standard deviation (SD) for normally distributed variables, median with interquartile range (IQR) for skewed variables, or ratios (%) for categorical variables.

Multiple linear regressions were used to study the effects of total protein and protein from different dietary sources on BMI and IGF-1 at 6 years. In models with IGF-1, we decided to split by gender because differences between boys and girls in relation to IGF-1 levels and response to interventions have been shown.(Closa-Monasterolo et al., 2011) In the regression models we chose the nutrient density approach, i.e. all protein variables were expressed as percentages of energy and total energy intake was additionally included. In models with BMI as endpoints we additionally included fat intake as percent of energy because it has been hypothesized that not only a high protein intake but also the typical simultaneous decrease in fat intake might predispose children to later obesity,(Rolland-Cachera et al., 2006) gender, maternal education and birth weight as these variables might influence BMI in childhood.(Monasta et al., 2010; Rooney et al., 2011) In model 2 we additionally adjusted for breastfeeding status at 12 months (yes/no) to be able to investigate the effects of other protein sources independent of breastfeeding. In models with IGF-1 as endpoints we included total duration of breastfeeding. For these selected covariates the number of missing values ranged from 0 to 6%. Missing values were replaced by the median value for each covariate. Stability analysis revealed that the same conclusions were in all cases reached when using this substitution as compared with complete case analysis. The level of significance in the study was $P<0.05$.

RESULTS

A general description of the study sample is given in Table 1. Weight and height at 12 months and IGF-1 concentration at 6 years differed between the genders, boys being heavier and longer at 12 months and girls having higher IGF-1 levels at 6 years. Table 2 summarizes the infants´ consumption of selected foods and the protein it provides. Of the 137 infants, 26 were still being breastfed at 12 months of age, 3 did not consume any animal foods, and they all consumed some vegetable foods. Foods from animal sources provided 65% of total protein, thereof dairy products 41% and meat and fish 24%. Foods from vegetable sources provided 32% of total protein and breast milk the remaining 2%.

Table 1. Characteristics of the 137 participants.

	Mean (SD) or %
Girls (%)	52
Infant variables	
Birth weight (kg)	3.8 (0.4)
Birth length (cm)	51.8 (1.6)
Weight at 12 months (kg)	10.0 (1.1)
Length at 12 months (cm)	76.8 (2.5)
Duration of EBF (months)[a]	4 (3)
Total duration of BF (months)[a]	8 (5)
Maternal variables[b]	
Age (years)	31.5 (5.2)
BMI (kg/m^2)	24.7 (4.5)
Higher education (%)	78
Variables at 6 years	
Weight (kg)[a]	22.0 (3.3)
Height (cm)	119.8 (4.5)
BMI (kg/m^2)[a]	15.5 (1.7)
IGF-1 (µg/l)	124 (42)

Abbrevations: BF, breastfeeding; BMI, body mass index; EBF, exclusive breastfeeding; IGF-1, insulin-like growth factor 1
[a]Variable not normally distributed, results presented as median (IQR)
[b]Mothers answered when participants were 12 months of age

Total and animal protein intakes at 12 months were positive predictors of BMI at 6 years (Table 2). Segmentation of animal protein into dairy and meat and fish protein inflated the confidence intervals, yielding non-significant associations with BMI, but left the central estimate of the prediction coefficient relatively unchanged. Vegetable protein and breast milk protein intake at 12 months were not found to be predictors of BMI at 6 years.

Table 2. Consumption of the 137 participants of selected foods at 12 months and the dietary protein they provide, as well as associations between intake of total protein and protein from various sources and BMI at 6 years.

	Food consumption (g/d)	Protein intake (g/d)	Protein intake (E%)	BMI at 6 years[d]	
	Median (IQR)	Median (IQR)	Median (25th. perc., 75th perc.)	β (95%CI)	P-value
Total protein		29.7 (13.5)	14.6 (13.0, 17.1)	0.1 (0.0, 0.2)	0.018
Animal foods[a]	398 (290)	19.2 (10.8)	9.8 (7.7, 11.9)	0.1 (0.0, 0.1)	0.021
Dairy products	364 (264)	12.1 (7.9)	5.9 (4.1, 8.0)	0.1 (-0.1, 0.1)	0.17
Meat and fish	31 (42)	6.5 (6.0)	3.2 (2.0, 4.8)	0.1 (-0.0, 0.2)	0.07
Vegetable foods[b]	262 (172)	8.8 (5.3)	4.4 (3.5, 5.7)	-0.1 (-0.2, 0.1)	0.22
Breast milk[c]	0 (0)	0 (0)	0 (0, 0)	0.1 (-0.3, 0.5)	0.67

Abbreviations: BMI, body mass index; CI, confidence interval; E%, percent of energy; IQR, interquartile range; perc., percentiles
[a] They main categories of animal foods are dairy products, meat, fish and eggs and these food sources provide "animal protein" (61% from dairy, 29% from meat, 9% from fish, 1% from eggs)
[b]The main categories of vegetable foods are cereal products (including bread, rice, oat, breakfast-cereals, biscuits), porridges, fruit and vegetables and these food sources provide "vegetable protein" (57% from cereals, 19% from porridges, 15% from fruits, 9% from vegetables
[c] The 26 children (19%) still being breastfed at 12 months of age consumed (median (IQR)): 153 (183) g breast milk/day, which gave 2.0 (2.4) g or 1.1 (1.4) E% protein from breast milk/day
[d]Linear regression, models adjusted for energy and fat intake at 12 months, gender, maternal education and birth weight

When split into quartiles of protein intake at 12 months (Table 3), children in the highest quartile of total protein intake had higher BMI at 12 months than children in the lowest quartile, and this tendency seemed to persist at 18 months and 6 years (model 1). The difference was somewhat attenuated when breastfeeding status at 12 months was incorporated in the models (model 2). Children in the highest quartile of animal protein intake at 12 months had higher BMI at 12 months, 18 months and 6 years than children in the lowest quartile (model 1). The difference in BMI at 6 years between the highest and lowest quartiles was independent of breastfeeding status (presented as β (95%CI)): 0.9 kg/m^2 (0.3, 1.6) (model 2). Differences in BMI between the highest and lowest quartiles of dairy protein and meat and fish protein at 12 months were not significant. Children in the highest quartile of vegetable protein intake at 12 months seemed to have lower BMI at 6 years than children in the lowest quartile, independent of breastfeeding status at 12 months. Children that were still being breastfed at 12 months had lower BMI at 12 months than children not breastfed (borderline significance, presented as β (95%CI)): -0.6 (-1.2, 0.0). The difference in BMI between children breastfed and not breastfed at 12 months was even more marked at 18 months (presented as β (95%CI)): -0.7 (-1.2, -0.1).

Table 3. Difference in mean BMI at ages 12 months (N=65), 18 months (N=58) and 6 years (N=66) between highest and lowest quartiles of protein intake at 12 months of age.

	12 months		18 months		6 years	
	Δ (95%CI)	P-value	Δ (95%CI)	P-value	Δ (95%CI)	P-value
Model 1						
Total protein (E%)	0.6 (0.0, 1.3)	0.042	0.6 (-0.0, 1.2)	0.07	0.7 (-0.0, 1.5)	0.06
Animal protein (E%)	0.7 (0.0, 1.3)	0.041	0.7 (0.1, 1.3)	0.015	0.8 (0.2, 1.4)	0.014
Vegetable protein (E%)	-0.4 (-1.2, 0.3)	0.23	-0.4 (-1.0, 0.3)	0.28	-0.7 (-1.3, 0.0)	0.05
Model 2						
Total protein (E%)	0.6 (-0.0, 1.2)	0.05	0.5 (-0.1, 1.1)	0.11	0.7 (-0.0, 1.5)	0.06
Animal protein (E%)	0.6 (-0.1, 1.2)	0.09	0.6 (-0.0, 1.2)	0.05	0.9 (0.3, 1.6)	0.007
Vegetable protein (E%)	-0.4 (-1.2, 0.3)	0.22	-0.4 (-1.0, 0.3)	0.27	-0.7 (-1.3, 0.0)	0.05

Abbreviations: CI, confidence interval; E%, percent of energy
Model 1 adjusted for energy and fat intake at 12 months, gender, maternal education and birth weight
Model 2 adjusted for energy and fat intake at 12 months, gender, maternal education, birth weight and breastfeeding status at 12 months

For girls, total protein, animal protein and dairy protein at 12 months were positive predictors of IGF-1 at 6 years (Table 4). When excluding dairy protein from either total protein or dairy protein groups, their associations with IGF-1 became non-significant. Additional testing revealed that dairy protein intake at 12 months was a positive predictor for IGF-1 in 6 year old girls independent of height, weight, or both at 6 years (presented as β (95%CI)): 4.2 (1.6, 6.8), P=0.002; 4.5 (1.9, 7.1), P=0.001; 4.1 (1.6, 6.7) P=0.002, respectively.

Table 4. Total protein and protein from different food sources at 12 months (as % of total energy) as predictors of IGF-1 at 6 years in boys (N=56) and girls (N=56).

	Boys		Girls	
	β (95%CI)	P-value	β (95%CI)	P-value
Total protein (E%)	-1.3 (-4.1, 1.5)	0.34	4.7 (1.5, 7.9)	0.005
Animal protein (E%)	-1.0 (-3.3, 1.2)	0.37	5.4 (2.5, 8.2)	0.0004
Dairy protein (E%)	-2.2 (-5.1, 0.7)	0.13	5.4 (2.5, 8.2)	0.0004
Meat and fish protein (E%)	0.6 (-2.8, 4.0)	0.71	0.0 (-5.5, 5.6)	0.99
Vegetable protein (E%)	1.0 (-5.0, 7.0)	0.74	-3.6 (-8.1, 1.0)	0.12

Abbreviations: CI, confidence interval; E%, percent of energy
Models adjusted for energy intake at 12 months and total duration of breastfeeding

DISCUSSION

Based on an Icelandic infant cohort with a relatively long breastfeeding duration and high protein intake in infancy, our results suggest that a higher intake of total protein and animal protein results in higher BMI in childhood. Our results also suggest that infants still breastfed at 12 months have lower BMI at 12 and 18 months than infants breastfed for a shorter duration and that a higher intake of vegetable protein intake at 12 months may result in lower BMI at 6 years. Our results are strengthened by results from studies reporting a protective effect of breastfeeding on BMI in childhood(Arenz et al., 2004; Harder et al., 2005) and studies reporting positive associations between animal protein intake in infancy and BMI in childhood.[13,14,26]

Dairy protein has received particular attention with regard to associations with later BMI. A study on the DONALD cohort in Germany found intake of dairy protein at 12 months, but not meat protein, to be associated with BMI and percent body fat at 7 years of age.(Gunther et al., 2007b) A short intervention study suggesting that cow´s milk but not meat intake or vegetable protein stimulates secretion of IGF-1 in children supported this finding.(Hoppe et al., 2004a) On the contrary, an Australian study found a negative association between dairy protein intake at 18 months and BMI at 8 years, and a positive association between meat protein intake and BMI.(Garden et al., 2011) In our previous analysis,[1] we found cow´s milk intake at 12 months to be positively associated with BMI at 6 years. Based on our present results, not finding associations between dairy protein and BMI at 6 years, we cannot confirm that the association between cow´s milk intake at 12 months and BMI at 6 years is mediated via protein. However, findings on a positive association between dairy protein and IGF-1 at 6 years in girls may suggest that the effects are mediated at least partly through protein.

Interestingly, our results suggest opposing effects of total protein and protein from various dietary sources on IGF-1 at 6 years between the genders. It seems as if the association between dairy protein and IGF-1 is negative for boys (non-significant) and positive for girls. We therefore speculate whether it is possible that two separate mechanisms underlie the associations between dairy protein intake at 12 months and IGF-1 at 6 years in boys and girls.

Observational studies suggest that children down to the age of 3 years with the highest intake of animal protein experience pubertal onset up to 7 months earlier than children with lower intake of animal protein.(Cheng et al., 2012) IGF-1 levels increase through childhood and peak during puberty.(Larnkjaer et al., 2012) In the Avon Longitudinal Study of Parents

[1]Thorisdottir B, Gunnarsdottir I, Thorisdottir AV, Palsson G, Halldorsson TI, Thorsdottir I. Infant dietary predictors of BMI at 6 years: Two population based studies conducted 10 years apart. Manuscript 2012-11-27.

and Children from UK, higher IGF-1 levels at 8 years were found to be associated with earlier age at menarche (< 12 years) in girls.(Thankamony et al., 2012) We propose that it might be possible that our findings of a positive relationship between dairy protein at 12 months and IGF-1 levels at 6 years in girls only may be an indicator of dairy protein in infancy sending girls into earlier puberty and that at 6 years, IGF-1 levels are already starting to elevate and prepare for the pubertal peak. The reason we do not see a positive association in boys might, if this theory holds, result from later puberty in boys than girls, with peak levels of IGF-1 occurring at a higher age,(Juul et al., 1994) and that IGF-1 levels at 6 years are therefore not starting to prepare for the pubertal spurt although dairy protein may be associated with an earlier puberty in boys as well.

Although not statistically significant, the association between intake of dairy proteins and IGF-1 at the age of 6 years was in the opposite direction for boys to what was seen in girls, where higher intake of dairy proteins at the age of 12 months was associated with lower IGF-1 concentrations. Lower IGF-1 concentrations in early adulthood has been seen in subjects receiving milk supplementation up to the age of 5 years.(Ben-Shlomo et al., 2005) A programming mechanism has been proposed: that increased protein intake during infancy causes a long-term resetting of the pituitary resulting in lower IGF-1 levels later in life.(Martin et al., 2005) Studies finding higher IGF-1 levels at 7-8 years(Martin et al., 2005) and at 17 years(Larnkjaer et al., 2009b) in children breastfed for a longer duration support this hypothesis. Further studies are needed to fully understand the association between protein intake in infancy and IGF-1, as well as the clinical relevance for long term health.

Conclusion:

Our results suggest that children with high intake of animal protein at 12 months have higher BMI in childhood than children with lower intake. They further indicate that breastfeeding at 12 months has protective effects on BMI at 12 and 18 months. Our results highlight the need for special emphasis in infant dietary guidelines aiming at avoiding excess animal protein intake in the complementary feeding period. Further, the results support prolonged duration of breastfeeding, into the second year of life. Our findings of a positive relationship between dairy protein intake at the age of 12 months and IGF-1 levels in 6 year old girls may be of relevance. Increased IGF-1 might be an indication of early puberty that has been associated with negative long-term effects on health.

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5. CONCLUSION

Parents and other caregivers of infants seem to have embraced the primary prevention effort in 2003 of revising infant dietary recommendations and introducing a new option in complementary feeding practices, the follow-on formula (Stoðmjólk), as lower intake of cow’s milk and a subsequent lower protein intake in the latter half of the first year was seen in the 2005 compared to the 1995-6 cohort. The results presented in this thesis suggest that the "natural intervention" may have contributed to lower overweight prevalence in the 2005 cohort than the 1995-6 cohort.

To my knowledge, studies presented in this thesis are the first to show desirable effects on overweight prevalence in a children’s population following a primary prevention strategy of upgrading infant dietary recommendations to match the latest scientific knowledge. They therefore emphasise the importance of monitoring infant nutrition in order to provide appropriate dietary guidelines. The findings of other desirable changes in the infants’ dietary intakes 10 years apart, improved qualities of fats, increased consumption of fibre and less added sugar intake, similar to findings described in the national dietary survey conducted among Icelandic adults in 2010/2011, suggest that Icelanders are getting increasingly aware of the importance of healthy eating throughout life and that they may therefore easily adopt upgraded dietary recommendations. The findings of few children with very high BMI at 6 years in the 2005 cohort however are alarming and indicate that despite desirable effects on overweight prevalence, the number of severe obese children might still be growing. Further studies are needed in order to define risk factors related to severe obesity in young children, as population based prevention approaches might not be applicable for them.

The results suggest that children with high intake of cow´s milk and dietary protein, especially animal protein, at 12 months have higher BMI in childhood than children with lower intake. Whether dairy products or protein *per se* are the important factors could not be determined. Our results highlight the need for special emphasis in infant dietary guidelines aiming at avoiding excess animal protein intake in the complementary feeding period. The results support prolonged duration of breastfeeding, and indicate than increased effort needs to be placed on promoting prolonged duration of breastfeeding into the second year of life.

The relevance of higher IGF-1 concentration among 6 year old girls with high intake of dairy protein at 12 months should be examined as high IGF-1 may be a suggestion of early puberty that may have negative effects on health later in life. The results might be of relevance for future research, aiming at understanding the mechanisms behind the relationship between dairy protein, adiposity rebound, IGF-1 and the clinical relevance for future health.

6. FUTURE PERSPECTIVES

Blood samples for participants in the 2005 cohort at 12 months of age have been kept frozen since 2006 and are as we speak in the process of being analysed for IGF-1. I hope that they may be able to answer questions e.g. on whether the evidently opposing associations between dairy protein intake at 12 months and IGF-1 were already apparent at 12 months, whether any programming is evident in our cohort etc. Ideally, we would follow the 2005 cohort up again at later times, to follow their ages at puberty and their development of BMI and IGF-1 throughout childhood and adolescence. Whether that will be possible, time will show. One question that is possible to answer with the existing data is: How well established are the dietary habits of infants at 12 months of age? This would be done by comparing food and nutrient intake at 12 months and 6 years and might give us a stronger base on how to identify and respond to adverse dietary habits observed already at 12 months of age.

Hopefully, a new infant study will be conducted in 2015. It is important to keep the National Food Composition Database and information about contents of infant foods as much up to date in order to get as precise results on infant dietary habits as possible. Regular monitoring on infant nutrition is vital to being able to update recommendations to give best practice and to follow whether recommendations are being followed. If guidelines aiming at avoiding excess animal protein intake in the complementary feeding period should be included in the Icelandic infant nutrition recommendations, it is essential to shape the presentation with great care. It is important that the general public does not interpret the recommendations in a way that dairy products should be avoided. The Icelandic recommendations for children from 2 years of age and adults (Public Health Institute, 2006) and the Nordic recommendations (Nordic Nutrition Recommendations, 2004) recommend regular consumption of milk and milk products as a part of a balanced diet. Moderate intake of milk and dairy is important for early growth (Brantsaeter et al., 2012), bone health (Huncharek et al., 2008) and provides infants with essential nutrients (Nordic Nutrition Recommendations, 2004). Should an upper limit for daily consumption of milk or dairy product be recommended, risk assessment should be performed to confirm that the recommendation does not have a negative effect on the several nutrients that milk provides, e.g. calcium, potassium, riboflavin and selenium (Nordic Nutrition Recommendations, 2004).

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APPENDIX 1

Fæðuflokkar
29.08.2012 Birna Þórisdóttir
Listi notaður við úrvinnslu gagna úr rannsókn á mataræði ungbarna 1995-6

1. Mjólk og mjólkurvörur
    1.1 Drykkjarmjólk: nýmjólk, léttmjólk, dreitill, undanrenna, rjómi, kakómjólk, kakó, ábrystir, mjólkurhristingur, kindamjólk o.s.frv.
    1.2 Sýrðar mjólkurvörur, sýrður rjómi, jógúrt, jógúrtdrykkir og skyr
    1.3 Mjólkurgrautar, mjólkurbúðingar, rjómabúðingar, tíramísú
    1.4 Nýmjólkurduft, undanrennuduft
2. Ostar
    2.1 Allir ostar úr mjólk eða mjólkurvörum
    2.2 Ostur úr soja eða öðru jurtapróteini
3. Ís
    3.1 Mjólkurís, rjómaís, jógúrtís
    3.2 Jurtaís
    3.3 Vatnsís (sorbet)
4. Kornmatur, brauð og kökur
    4.1 Ómalað og heilmalað korn. Hrísgrjón, maís og hafragrjón
    4.2 Mjöl
    4.3 Morgunverðarkorn, mjölgrautar
    4.4 Brauð, hrökkbrauð, tvíbökur, bruður, skonsur
    4.5 Kex (sætt og ósætt9, smákökur
    4.6 Kökur, tertur, ostakökur, kleinur
    4.7 Pasta, kús-kús
5. Grænmeti og kartöflur
    5.1 Nýir/frystir rótarávextir, nema kartöflur (t.d. rófur, sætar kartöflur, laukur, radísur o.s.frv.)
    5.2 Nýtt/ferskt grænmeti: stönglar, blöð, aldin (t.d. kál, tómatar, gúrkur, paprika, salat, eggaldin, brokkolí o.s.frv.)
    5.3 Nýjar kartöflur, kartöflumús
    5.4 Nýjar, frystar baunir, ertur
    5.5 Nýir sveppir
    5.6 Tómt
    5.7 Niðursoðið og niðurlagt grænmeti, tómatmauk
    5.8 Þurrkað grænmeti, kartöfluduft
    5.9 Franskar kartöflur
6. Ávextir, ber, hnetur og fræ
    6.1 Nýir/frystir ávextir
    6.2 Ný/fryst ber
    6.3 Hnetur, fræ, möndlur

    6.4 Niðursoðnir ávextir, ber, ávaxtagrautar, ávaxtamauk
    6.5 Þurrkaðir ávextir og ber
    6.6 Sultur
7. Kjöt og kjötvörur. Fuglakjöt
    7.1 Lambakjöt, kindakjöt (nýtt, fryst, saltað, reykt, hakkað)
    7.2 Nautakjöt
    7.3 Svínakjöt
    7.4 Hrossakjöt
    7.5 Hreindýra- og hvalkjöt
    7.6 Alifuglar
    7.7 Sjófuglar og aðrir villtir fuglar
    7.8 Fars, farsvörur, pylsur, bjúgu, áleggspylsur
    7.9 Innmatur, slátur, svið, kæfa
    7.10 Niðursoðin kjötvara
8. Fiskur, fiskafurðir og skeldýr
    8.1 Ferskur/frystur fiskur. Fiskhakk, hrogn, lifur
    8.2 Þurrkaður og hertur fiskur
    8.3 Fiskfars og farsvörur, fiskipate
    8.4 Saltfiskur, reyktur fiskur, siginn, kæstur og grafinn fiskur. Hákarl
    8.5 Niðurlagður og niðursoðinn fiskur og skeldýr
    8.6 Fersk og fryst skeldýr
9. Egg og eggjavörur
    9.1 Egg (ný, fryst, heil eða fljótandi)
    9.2 Þurrkaðar eggjavörur
10. Feitmeti: smjör, smjörlíki, olíur o.fl.
    10.1 Jurtaolíur, jurtafeiti (olía og smjör?)
    10.2 Fiskolíur, lýsi
11. Sykur, hunang, sælgæti
    11.1Sykur, púðursykur, flórsykur
    11.2 Hunang
    11.3 Sælgæti
12. Drykkir, nema mjólkurdrykkir
    12.1Kaffi, te, kakóduft
    12.2 Gosdrykkir, svaladrykkir
    12.3 Blandaðir ávaxta- og berjadrykkir, saft
    12.4 Hreinir safar, ávaxtasafar, berjasafar, grænmetissafar
    12.5 Íþrótta- og orkudrykkir
    12.6 Bjór, pilsner, maltöl
    12.7 Borðvín
    12.8 Millisterk vín, brennd vín, líkjör
    12.9 Vatn, sódavatn (með og án bragðefna)
13. Matarsalt, edik, ger, krydd og kraftur
    13.1Matarsalt, edik, krydd og kraftur
    13.2 Ger og hjálparefni

13.3 Gervisætuefni

14. Snakk: poppkorn, flögur o.fl.
    14.1 Poppkorn
    14.2 Flögur, skrúfur, kornstangir, annað snakk
15. Sósur, súpur og áleggssalöt
    15.1 Allar sósur og ídýfur: salatsósur, majónes og majónessósur, olíusósur, rjóma- og ostasósur, sinnep, tómatsósur, sósur úr grænmeti, uppbakaðar, jafnaðar sósur, súr-sætar sósur o.fl.
    15.2 Súpur, súpuduft
    15.3 Áleggssalöt, majónessalöt, salöt úr sýrðum rjóma
16. Tilbúnir réttir
    16.1 Pizzur, samlokur, pítur, brauðréttir, hamborgarar, pylsa í brauði
    16.2 Pastaréttir, lasagna
    16.3 Kjötréttir
    16.4 Fiskiréttir
    16.5 Grænmetisréttir
    16.6 Eggjaréttir
17. Fæðubótarefni, næringardrykkir, sérfæði
    17.1 Vítamín, steinefni, önnur fæðubótarefni
    17.2 Megrunar- og próteindrykkir, næringardrykkir, próteinstykki
18. Tómt
19. Ungbarnamatur
    19.1 Grautar
    19.2 Ávaxtamauk
    19.3 Grænmeti og kjöt
    19.4 Safar
    19.5 Stoðmjólk
    19.6 Sojajógúrt
    19.7 Brjóstamjólk
    19.8 AD-dropar
    19.9 Tómt
    19.10 Þurrmjólk

APPENDIX 2

Variable	Food groups (from Appendix 1)
Protein from breastmilk	19.7
Animal protein	1+2.1+3.1+7+8+9+12.1+16.2+16.3+16.4+16.6+19.3+19.5+19.10
Dairy protein	1+2.1+3.1+19.5+19.10
Meat and fish protein	7+8+16.2+16.3+19.3
Vegetable protein	2.2+3.2+4+5+6+12.3+12.4+14+16.5+19.1+19.2+19.4+19.6