Iodine status and growth in 0-2 year old children on cow's milk protein free diets

Rut Anne Thomassen

Master Thesis, Department of Nutrition,
Faculty of Medicine

UNIVERSITY OF OSLO

Iodine status and growth in 0-2 year old children on cow's milk protein free diets

Results from the MILKID study

Rut Anne Thomassen

Master Thesis, Department of Nutrition,
Faculty of Medicine

UNIVERSITY OF OSLO

2015

Iodine status and growth in 0-2 year old children on cow's milk protein free diets

http://www.duo.uio.no

Trykk: Reprosentralen, Universitetet i Oslo

Acknowledgement

The present work was conducted at the Department of Paediatric Medicine, Oslo University Hospital in conjunction with the Department of Nutrition, Faculty of Medicine, University of Oslo, from February 2014 to May 2015.

To be given the opportunity to study a field of nutrition and patients that you work with on a daily basis has been nothing but a pleasure, and first and foremost I want to thank the children and parents that participated in the MILKID study. The parent's eagerness to include their child in a study that involved blood sampling of the child as well as additional visits to the clinic was overwhelming.

It has truly been a privilege to plan and conduct this study together with my two supervisors Clinical Dietitian Janne Kvammen and Associated professor Christine Henriksen. I am sincerely grateful for all the effort you both have put in, for valuable guidance, encouragement and fruitful discussions.

I want to express my gratitude to Dr Med Jarle Rutveit for his input in planning the study and for constructive feedback. A special thanks goes to the Head of the Paediatric Nutrition department, Linn Helene for making it possible for me to do a master thesis. I would also like to thank my companion master student, Mari Eskerud for her effort and collaboration in recruiting patients, data collection and processing of the study material, and Pétur Júlíusson for his help with the growth data.

I am truly grateful for the motivation and valuable suggestions from Dr Runar Almaas during this process and for the useful input from Dr Hasse Bangstads. I want to raise a special thanks to my colleagues of the Nutrition Team at the pediatric clinic, OUS; Marianne Bartlie, Beint Bentsen, Sissel Moltu, Anne Charlotte Brun, Kari Torp Hansen, Gøri Perminow and Erik Hankø for valuable suggestions and support, and for assistance in the recruitment process, and Sunniva Nordang and Norunn Vetlesand for making sure I could take leave from work at short notice to study.

Last but not least, I would like to thank my family and friends for support and encouragement during this process, in particularly my parents who have cooked, cleaned and taken care of the children when needed. A special thanks to my dear husband Knut Erik; for continuous endorsement, valuable inputs in the research process and for never making me feel that my studying was a burden. And to my children, Marte and Johanne; thank you for keeping up with the situation, for never questioning why mum had to study during evenings, weekends and holidays, and for just laughing when we were out of essentials, such as bread and milk.

Oslo, October 2015

Rut Anne Thomassen

Abstract

Introduction: Allergy to cow's milk protein is the most common food allergy in children under the age of three years. A diet without milk or dairy products is potentially low in many nutrients and could put the child at risk of malnutrition and compromised growth. Dairy products are a major source of iodine in the Norwegian diet, and excluding dairy products may negatively affect iodine status. Insufficient iodine status during childhood could potentially be detrimental and put the child at risk for delays in mental development and poor growth.

Objectives: The main objectives of this study was to investigate iodine intake and status along with growth in infants and young children under two years of age who were on a cow's milk protein free diet and to identify factors affecting iodine status and growth in these children.

Methods: Fifty-seven infants and children under two years of age where included in a larger cross-sectional study on the nutritional status of children on cow's milk protein free diets (MILKID study). Two spot urine samples from were collected and analysed for iodine, together with a three day food record and a food frequency questionnaire on dietary sources of iodine. Urine iodine concentrations were compared to the WHO cut-off values for iodine deficiency. Weight, length and head circumference were recorded at birth and at inclusion in the study and compared to the Norwegian growth standard. Sub-group analyses were done on different feeding patterns, according to weaning status.

Results: One third of the children had iodine deficiency according to urine iodine concentration (UIC). Children who were mainly breastfed were at highest risk and 58 % was classified as deficient. Dietary factors associated with iodine were intake of enriched baby cereals ($r = 0.230$, $p = 0.02$) and reaching RDI for iodine through diet ($r=0.313$, $p = 0.03$). Children of mothers who took supplement with iodine had higher urinary iodine concentrations, though this was not significant. Growth in length was compromised in boys, 11 % of the infants had weight for age z-score (WAZ) and BMI for age z-score (BMIZ) of ≤ -2 and 5 % length for age z-scores (LAZ) of ≤ -2 at inclusion and this was associated with food refusal and low appetite, but not associated with iodine deficiency.

Conclusion: The present study demonstrates that the exclusion of cow's milk protein puts the child at risk of deficiency of iodine and poor growth, however the two conditions were not related. Infants reliant on breast milk as their main iodine source are at increased risk of iodine deficiency. Boys with feeding problems were at highest risk of growth failure.

Abbreviations

AA	Amino acid formula
ADHD	Attention deficit and hyperactivity disorders (ADHD)
As^{3+}	Arsenious acid
BMIC	Breast milk Iodine content
BMIZ	BMI for age z-score
Ce^{3+}	Cerous ion
Ce^{4+}	Ceric ion
CMP	Cow's milk protein
CMPA	Cow's milk protein allergy
CNS	Central nervous system
Cr	Creatinine
DBPCFC	Double-blind placebo-controlled food challenge
eHF	Extensively hydrolyzed formula
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
FAO	Food and Agriculture Organization of the United Nations
FCT	Food Composition Table
FPIES	Food protein induced enterocolitis syndrome
GI	Gastro intestinal
HA	Hypoallergenic formula
I	Iodine
I_2	Iodine (chemical formula)
ICCIDD	International council for control of iodine deficiency disorders
IgE	Immunoglobulin E
IGF	Insulin-like growth factor
IQ	Intelligence quotient
JK	Janne Kvammen
LAZ	Length for age z-score
MoBa	Norwegian mother and child cohort study
ME	Mari Eskerud
MUIC	Median urinary iodine concentration
NaCl	Sodium chloride
NIS	Sodium/iodine symporter
NNR	Nordic Nutritional recommendations
OUS	Oslo University Hospital
RAT	Rut Anne Thomassen
T_3	Triiodothyronine
T_4	Thyroxine, tetra-iodothyronine
Tg	Thyroglobulin
TSH	Thyroid stimulation hormone
UIC	Urinary iodine concentration
UiO	University of Oslo
UNICEF	United Nations Children's Fund
WAO	World Allergy Organization
WAZ	Weight for age z-score
WHO	World health organization

List of Tables

List of Figures

Table of contents

1 Introduction

1.1 Food allergy

Allergy to components of food in the diet affects between 2.5 - 6 % of children worldwide with the highest prevalence among the youngest age groups. Allergy to cow's milk, egg, wheat, soy, peanuts, tree nuts, fish and shellfish constitutes the majority of food allergic reactions (1-3).

The National Institute of Allergy and Infectious Diseases has defined food allergy as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" (4). An important differentiation in the management of hypersensitivity is that of allergy or intolerance. Food allergens are defined as the specific components of food or ingredients within food recognized by allergen-specific immune cells which then initiates specific immunological reactions, resulting in a great diversity of symptoms. Food allergens are typically proteins, but sometimes also chemical haptens. Intolerance is non-allergic food sensitivity as a result of enzyme deficiencies for example (5) .

There are no pharmaceutical interventions to prevent the allergic reactions. Treatment involves elimination the offending food from the child's diet, with the risk of negatively affecting the nutritional quality and hence the nutritional status of the child. Most food allergy commonly develops within the first three years of life which means that development and growth may be compromised. This is particularly true if the food excluded is a major contributor of essential nutrients (2, 6).

1.2 Cow's milk protein allergy

Children with cow's milk allergy must avoid all milk and dairy products and these products are major providers of many nutrients, including iodine. In many European countries, milk and dairy products are the main contributors of iodine (7). Iodine is essential for brain development and growth, and in particularly in the first few years of life deficiency of iodine could have detrimental effects (8, 9). This thesis has sought to investigate the effect of excluding cow's milk protein, as treatment for cow's milk protein allergy, on iodine status and growth in children under the age of two years.

1.2.1 Epidemiology

The most common of food allergies is Cow's milk protein allergy (CMPA). The prevalence varies widely in the literature and studies of prevalence are heterogeneous in many aspects making conclusions about true prevalence difficult. Most commonly CMPA is reported to affect 2-3 % of children under the age of three years; however, CMPA with gastrointestinal tract manifestations alone can be diagnosed in all age group (10). In a Norwegian study of the prevalence of adverse reactions to milk the point prevalence of cow's milk allergy was estimated to be 1.1 %; however the investigators speculated that this was an underestimate as unrecognized reactions were detected (11). In the latest rapport from the EuroPrevall birth cohort, the incidence of diagnosed CMPA in Europe was 0.54 % in children up to the age of two years. National incidences ranged from 1 % in Britain and the Netherlands to 0.3 % in Lithuania and Germany, and none in Greece (12). This study, however, did not include reactions that took place later than two hours after exposure to cow's milk protein (CMP), except for worsening of eczema within 48 hours of starting the challenge. It therefore failed to include children with late reactions, frequently seen in gastro intestinal allergy. The prevalence of CMPA is highest in the first two years of life and in several studies most children outgrow their allergy by age three. In a Danish study of CMPA in children from birth through the age of 36 months, CMPA was suspected in 6.7 % and confirmed in 2.2 %. In this cohort allergy to milk developed in the first year of life, and by the age of three years most of the allergic children were able to tolerate milk (13).

1.2.2 Signs and symptoms

CMPA reactions are often divided into two groups according to the immune response, that is Immunoglobulin E (IgE) and Non-IgE mediated. In a subject the reactions may be IgE mediated, non-IgE mediated, or mixed (14).

The reactions to CMP are numerous and can be of immediate or delayed onset. Immediate reactions occur from minutes up to two hours after allergen ingestion, whereas delayed reactions manifest up to 48 hours or even one week following ingestion. Several organ systems can be affected by a reaction to the proteins in milk most commonly the gastro intestinal tract, the respiratory tract and the skin (Table 1). The involvement of more than two organ systems increases the likelihood of CMPA (10).

CMPA symptoms commonly associated with IgE-mediated reactions include oral and perioral swelling, urticaria, angioedema, vomiting, diarrhea, eczema, rhinitis and anaphylaxis. The reactions are of typical quick onset and often immediately after ingestion of the offending food. Non-IgE mediated reactions include dysphagia, and food impaction, vomiting, regurgitation, dyspepsia, early

satiety, anorexia, and food refusal (delayed gastric emptying), diarrhea, rectal bleeding, failure to thrive, abdominal pain, severe colic and persistent constipation. The symptoms can be both immediate or delayed reactions, and mixed, and sometimes quite non-specific like irritability or failure to thrive, making the diagnosis more difficult (5, 10). Symptoms also vary with age (10).

Other examples of non-specific symptoms include chronic iron-deficiency anemia and severe shock-like reactions with metabolic acidosis characteristic for the "food protein-induced enterocolitis syndrome" (5, 10). Clinical symptoms and signs in the digestive tract may be due several effects of the allergy-induced inflammation, including dysmotility (10).

Table 1. Symptoms and signs related to CMPA

	Infant/Toddlers	Older Children	Immediate reactions
Gastro intestinal	Dysphagia Frequent regurgitation Colic, abdominal pain Vomiting Diarrhea ± intestinal protein or blood loss Constipation± perianal rash	Dysphagia Food impaction Regurgitation Dyspepsia Nausea, vomiting Diarrhea ± intestinal protein or blood loss Constipation Abdominal pain	Vomiting
General	Failure to thrive Iron-deficiency anemia Anorexia, refusal to feed Irritability Disturbed sleep Anaphylaxis Shock-like symptoms with severe metabolic acidosis, vomiting, and diarrhea (FPIES)	Iron-deficiency anemia Anorexia, early satiety Anaphylaxis Irritability Disturbed sleep	Anaphylaxis FPIES
Respiratory	Rhinitis Wheezing Chronic coughing (unrelated to infections) Asthma	Rhinitis Wheezing Chronic coughing (unrelated to infections) Asthma	Wheezing stridor Breathing difficulties
Skin	Urticaria Atopic eczema Angioedema (swelling of lips or eyelids) Rash	Urticaria Atopic eczema Angioedema (swelling of lips or eyelids)	Urticaria Angioedema

Adapted from Koletzko et al. 2012 (10) and Kneepkens and Meijer 2009 (15).
FPIES - food protein induced enterocolitis syndrome

1.2.3 Diagnostic procedures

There exist certain relevant laboratory methods for diagnosing CMPA, such as assessment of the level of IgE to CMP in blood, skin prick test and patch tests. The reliability of these test are limited apart from highly elevated IgE for CMPA of the immediate type reaction. Use of patch test for diagnosing non-IgE reactions has not been standardized, is resource consuming and predominantly used for research purposes (16).

Diagnostic elimination of CMP is commonly used when CMPA is suspected. The child avoids cow's milk protein in the diet for a limited time period, the duration depending on the manifestation of allergy. Three to five days are often adequate in children with immediate clinical reaction (e.g. vomiting or angioedema). For children with delayed reactions, and especially those with gastro intestinal reactions, the elimination may have to last at least two to four weeks in order to make judgement of the response. If there is no improvement in symptoms then CMPA is unlikely. Exceptions may occur, such as infants with major gastrointestinal symptoms on extensively hydrolyzed or soy formulas who may react to components in these formulas and are in need of an amino acid formula. In breastfed infants the mother must follow a CMP free diet for a minimum of 14 days in cases of delayed reactions. In cases of exclusively breastfed infants in poor condition the practice in many countries is to put the child on an amino acid formula while the mother expresses breastmilk in the period from starting a CMP-free diet to the breast milk is expected to be free of CMP (10, 17).

A double-blind placebo-controlled food challenge (DBPCFC) is the gold standard for the diagnosis of CMPA (15). The DBPCFC involves feeding the child foods with or without the offending food component, often in increasing amounts, and registering potential reactions. Such food challenges are time consuming and expensive. Open challenges are another option, where the child is simply given food with CMP by the parents after a period of exclusion. If no symptoms are elicited within two weeks of regular cow's milk feeding then CMPA can be excluded. In some cases the re-introduction of CMP to the child is postponed until the child is well and thriving again. A re-evaluation and CMP challenge every six months is often recommended to ensure that the child is not on an exclusion diet longer than necessary (10).

1.2.4 Treatment: The cow's milk free diet

Exclusion of CMP is the only treatment for CMPA. The exclusion of CMP from the diet is extensive and involves all types of milk, including milk from other animals like sheep and goats for example, all dairy products like yoghurts and cheese and all food products that contain milk proteins. Milk protein is used in manufactured foods commonly eaten by small children such as enriched baby cereals,

meat products and fish products. Dairy products are important sources of many nutrients in a child's diet and inappropriate substitutions can induce vitamin and mineral deficiencies, anemia, rickets, failure to thrive and kwashiorkor (6, 18, 19). In an estimate based on the Norwegian infant feeding survey of 12 month old children, 30 % of the protein intake, 50 % of the energy intake and 100 % of the calcium intake came from dairy products or foods containing milk proteins (20).

Delaying the introduction of weaning foods with high allergenic potential like fish, egg and wheat etc. is not recommended unless there is proven allergy to these foods (21) and will only lead to risks of compromising the diet even further. Although several guidelines from international organizations, like World Allergy Organization (WAO) and European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), mention that particular attention must be paid to prescribing nutritionally safe diets for children with CMPA, there exists no specific guidelines (10, 16, 22).

Because normal infant formulas contain cow's milk proteins, hypoallergenic formulas (HA) are recommended in the treatment of CMPA in infants and young children (10, 16). HAs are divided into two categories, extensively hydrolyzed formulas (eHF) and amino acid formulas (AA). The difference between eHFs and AAs are in the nitrogen source. In eHFs cow's milk proteins, either whey or casein, are extensively hydrolyzed by enzymes to remove the allergenic epitopes that cause the majority of symptoms in CMPA children. In contrast, AA formulas contain pure amino acids and are considered non-allergenic. However, AA formulas are highly expensive and often reserved for those with severe allergies (16).

Soy formulas are based on soy protein and were commonly used in Europe until about 10 years ago, when rising concern regarding potential harmful effects led to a debate around the safety of soy based formulas in infants with CMPA (23). In a position paper by the ESPGHAN Gastro intestinal (GI) committee soy based formulas are mentioned as an option for children older than 6 months who do not accept the taste of an eHF or in cases where the costs of eHF is a limiting factor (10).

The inclusion of hypoallergenic formulas (HA) or soy based formulas, as a substitute for normal infant formulas and cow's milk, improves the quality of the CMP free diet in particularly vitamin D, vitamin E, energy, protein, calcium, iron and zinc (1, 6, 24). Infants and children with CMPA are advised to maintain a milk substitute in the diet until two years of age; either breastmilk or a hypoallergenic formula (16). However, HAs are known for their bitter taste and low acceptance when introduced to older infants and children. Such non-compliance can be detrimental for the child and advice on how to introduce these formulas are often required.

Industrial drinks made of soy, rice, almond, coconut and oat for example are improperly called "milks" and used as substitutes in vegan diets among others. These products are unsuitable to meet the infant's nutritional needs and vary highly in their content of proteins, fat, energy and vitamins and minerals. In many centers they are not recommended for substituting cow's milk as a drink for children less than three years of age (10, 25, 26). Rice drink is not recommended as substitute for milk as a drink for children under the age of six due to the high content of the heavy metal arsenic (27).

When children with CMPA are breastfed the breastfeeding mother must exclude cow's milk protein from her diet. There is little documentation on the effect of such exclusion neither with regards to the nutrient content of the breastmilk nor of the nutritional status of the mother. The lactating mother is generally advised to take calcium supplement to ensure her own bone health.

1.2.5 Cow's milk protein free diets and nutritional status

Exclusion of CMP puts the child at risk of malnutrition and studies have shown CMPA children are smaller than their peers (24, 28-31). Growth is a marker of energy and protein nutrition. Weight is a sensitive marker of energy intake and is affected by dietary inadequacies earlier and to a greater extent than stature (18). However, inadequate protein supply and chronic energy deficit will subsequently delay stature. In a Norwegian study, growth was affected in children aged 6-10 years who had out-grown their CMPA but were still restricting cow's milk and dairy products. The previously CMPA children were shorter and slimmer than their peers. The difference in growth was evident at the age of one, two and four years and at time of observation. Lower intakes of calcium, riboflavin and protein were also found in the milk-restricting group (31). In a study of Norwegian two year old children with and without CMPA, children excluding cow's milk had lower intakes of energy, fat, protein, calcium, riboflavin and niacin (24). Low intakes of vitamin D and calcium are a concern in children with CMPA (18, 25). These nutrients are particularly important for bone mineral status and disturbance in bone metabolism have been found in children with CMPA (32).

To prevent malnutrition in children excluding CMP, professional dietary advice to ensure appropriate substitution of dairy products is essential. Several studies have found improved nutrient intake in CMPA children who receive dietary advice from a dietitian (6, 29). In a Finnish study of children with allergy to milk or a combination of milk and wheat, who had all received dietary advice from a dietitian, the average intake of different nutrients were well within the Nordic nutritional recommendation (NNR). However, compared to the average intakes of healthy Finnish children, the food allergic children received lower amounts of protein and carbohydrates and higher amounts of fat. The allergic group was also smaller than their peers (33). Another study looked at the nutrient

intake of children with food allergy before and after dietary counseling. 80 % of the group had CMPA. The investigators found significantly lower intakes of energy, protein, calcium and zinc compared to controls before dietary advice was given. Six months after dietary counseling there was a significant increase in energy, protein, carbohydrate, iron, fiber, calcium and zinc intake in the food allergic children. There was also a progressive decrease of the percentage of children with a weight for length ratio below two standard deviations (SD) after dietary counseling (19).

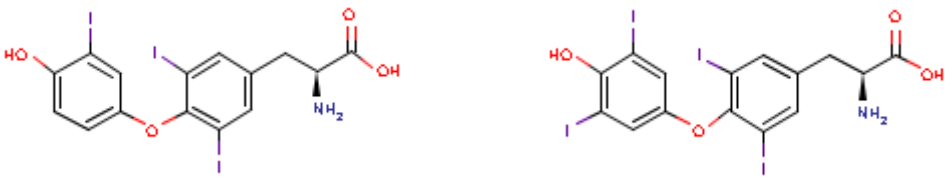
1.3 Iodine

Iodine is an essential trace element necessary for the production of thyroid hormones which play a vital role in growth and the development of tissues. Their actions are initiated through nuclear receptors which are found in most tissue. The thyroid hormones are involved in the regulation of basal metabolic rate and in macronutrient metabolism, as well as several processes in the central nervous system (CNS) (8).

Iodine is widely but unevenly distributes in the earth's environment. Most of the iodine is found in the oceans. Seawater has a concentration of 50 ug/L. Iodine ions in sea water are oxidized to elemental iodine, which volatilizes into the atmosphere and is returned to the soil by rain. The iodine cycling is, however, slow. Flooding and erosion depletes the surface soil of iodine and crops that grow on these soils will be low in iodine, leading to deficiencies in humans and animals that feed on these crops. Iodine deficient soils are common in mountainous regions and areas of frequent flooding (34).

1.3.1 Iodine function:

Iodine (I) is an essential trace element in humans. It is required for the production of thyroid hormones; triiodothyronine (T_3) and tetra-iodothyronine (T_4), also called thyroxine, in the thyroid gland (Figure 1).



Triiodothyronine (T_3) Thyroxine (T_4)

Figure 1 Chemical structure of the thyroid hormones (howMED 2011 (35)).

The thyroid gland is dependent on regular and adequate supply of iodine through the diet in order to produce these hormones. Iodine is transported into the thyroid follicles where it is oxidized to iodate. Iodate binds to the tyrosine residues in thyroglobulin to form a mixture of mono and di iodinate tyrosine which then coalesce to form T_3 and T_4 (36, 37) (Figure 2). The thyroid hormones are formed within the follicular cells of the thyroid gland and are released into the systemic circulation in response to thyroid stimulation hormone (TSH). T_4 is produced entirely by the thyroid gland, while the majority of T_3 is derived from the de-iodination of T_4 in other tissues such as liver and kidney. T_4 is receptor inactive and must be reduced to T_3 to evoke action signaled by thyroid hormone receptors (38). Thyroid hormones are important for cell differentiation, growth and maturation. They also affect the central nervous system (CNS) and maturation of glial cells, neuronal development and migration of cells in the CNS. The hormones are important for the maintenance of optimum function of particular neuronal systems throughout life. Thyroid hormones are also involved in regulation of metabolic rate, energy production and oxygen consumption in cells, protein and enzyme synthesis, thermoregulation, growth and sexual development (39).

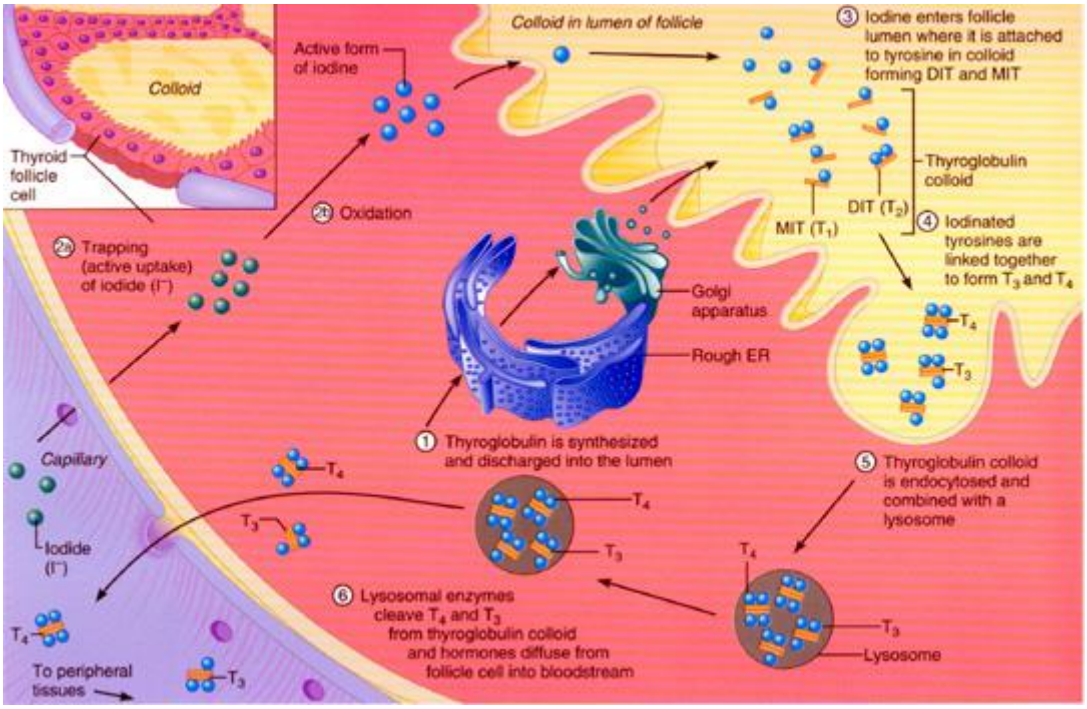


Figure 2 Thyroid hormone production in the thyroid gland (howMED 2011 (35)).

1.3.2 Absorption and Metabolism:

Iodine is ingested in several chemical forms, is reduced in the gut and absorbed as iodide. The absorption of iodine takes place in the stomach and duodenum, and is greater than 90 % in adults. The sodium-iodine symporter (NIS) is functionally expressed on the apical surface of enterocytes and mediates active iodine accumulation. Organically bound iodine is typically digested and the released

iodine absorbed, but some forms may be absorbed intact, for example thyroxin. Some iodine crosses the mucosa by diffusion. Iodine is cleared from the circulation by the thyroid and kidney. Renal clearance is fairly constant. The iodine clearance of the thyroid gland on the other hand varies with iodine intake and iodine status of the individual. The thyroid uptake is through its own NIS. If there is adequate supply of iodine, the thyroid gland will only take up 10 % of the absorbed iodine. In cases of chronic deficiency of iodine this fraction can exceed 80 % (34, 36). Figure 3 illustrates the function of NIS in the thyroid gland. During lactation, the NIS in the mammary gland concentrates iodine and secrets it into breast milk to provide iodine for the infant (34).

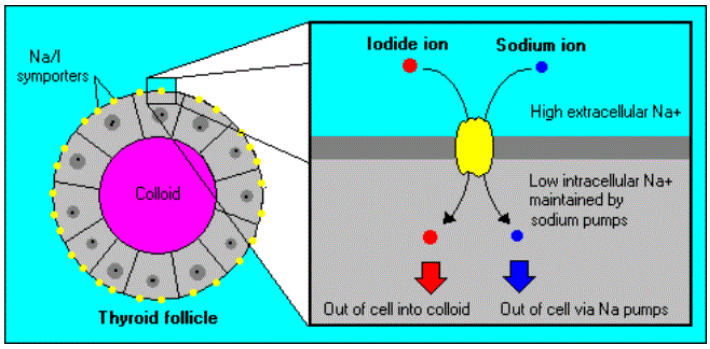


Figure 3 Uptake of iodine in the thyroid gland (howMED 2011 (35))

Plasma iodine has a half-life of about 10 hours under normal circumstances. Approximately 90 % of ingested iodine is excreted by the kidney in iodine sufficient subjects. If the intake is high then iodine excreted will be high, if the intake is low then the excretion will be low. Furthermore, low excretion of iodine can also be a sign of insufficient status, as the thyroid gland will absorb more iodine in conditions of iodine deficiency (40). The excretion of iodine in the urine therefore depends on both recent iodine intake and the iodine status of the individual and can vary considerably from day to day.

1.3.3 Iodine Deficiency:

Adequate thyroid hormone is needed for normal growth and neurological development of the brain. The World health organization (WHO) considers iodine deficiency to be the single most important preventable cause of brain damage worldwide. It is estimated that nearly one third of children globally are at risk of iodine deficiency (41, 42). Historically iodine deficiency has been associated with endemic goiter, thyroid failure and cretinism. However it is now recognized that iodine deficiency encompass a spectrum of disorders (Table 2)(40).

Historically iodine deficiency was a problem in Norway, causing endemic goiter in the population, especially those living in inland regions. Since the 1950's, iodine supplementation of cow fodder has been controlled by legislation and studies in the 1970s and 1980s showed satisfactory iodine status

in the Norwegian population (43, 44). Although iodine deficiency is often thought to be a problem of the developing world, industrialized countries are also affected with 50 % of Europe remaining mildly iodine deficient and iodine intakes falling in countries like the United States and Australia (34, 45, 46). In addition, even in countries that have achieved iodine sufficiency, the status of iodine nutrition in pregnant and lactating woman may still be inadequate due to higher requirements during this period of life. The iodine status in breastfed and weaning infants may be at risk for the same reasons (47). Inadequate iodine status was recently reported in pregnant women in the United Kingdom and in pregnant women in the Norwegian mother and child cohort study (MoBa) (46, 48).

Iodine deficiency disrupts the metabolism of thyroid hormones. When dietary iodine intake is inadequate, serum thyroid hormone levels initially fall. The pituitary gland senses low thyroid hormone levels and increase TSH secretion. TSH, in turn, stimulates the growth of thyroid cells, thyroidal iodine uptake and thyroid hormone synthesis. Enlargement of the thyroid, or goiter, in response to iodine deficiency, may occur at any age. If iodine deficiency is severe enough, thyroid hormone production will fall, resulting in hypothyroidism (40).

The level of T_4 decreases progressively with the severity of the deficiency in iodine. However, the level of T_3 follows different patterns of changes. In mild to moderate iodine deficiency, some adaption mechanisms such as increased iodine trapping, increased conversion of T_4 into T_3, and preferential synthesis of T_3 attempt to keep the concentration of T_3 in the normal range. Even in severe iodine deficiency, the level of T_3 is still normal in many tissues. However, the brain and pituitary gland T_3 level appears to be very sensitive to iodine deficiency and falls below normal even in mild and moderate iodine deficiency (8).

The consequence of iodine deficiency changes across the life span. In pregnant women, severe iodine deficiency have been associated with adverse outcomes like spontaneous abortion, stillbirth, congenital anomalies and infant mortality (34). Critically low levels of thyroid hormones associated with severe iodine deficiency causes neurological damage to the brain, particularly during the fetal and neonatal period, which can result in cretinism manifested by delayed motor and mental development (8).

Meta-analysis have shown that intelligence quotient (IQ) levels of children living in severely iodine-deficient areas are an average of 6-12 points lower than those living in iodine sufficient regions. The effects of mild to moderate deficiency are less well characterized, however, recent studies have shown that even mild maternal iodine deficiency in pregnancy may result in intellectual deficits (8, 49). Studies have found that children of mothers with mild iodine deficiency are more likely to have verbal IQ, reading accuracy and reading comprehension scores in the lowest quartile (46), and lower

scores for spelling, grammar and literacy (50). Significantly higher IQ was found in children with urinary iodine levels above 100 ug/L, with an increase in IQ with increasing urinary iodine content (51). Even if the iodine deficiency in these studies were classified as mild, the consequences were far from mild (51). Iodine deficiency has also been associated with hearing impairment, and behavioral and developmental disorders like attention deficit and hyperactivity disorders (ADHD) and autism (52, 53).

Studies of the effect of iodine deficiency on growth in children have had mixed results. A recent study demonstrated that iodine supplementation of iodine deficient children increased somatic growth rates (34). The effects of iodine deficiency on growth are likely mediated both by direct effects of thyroid hormone as well as by effects on secretion of growth hormone, insulin-like growth factor (IGF) -1 and IGF binding protein 3, all of which are increased by thyroid hormone (40).

Table 2 The spectrum of iodine deficiency disorders

Stage in life	**Effects**
Foetus	Abortions Stillbirths Congenital anomalies Increased perinatal mortality Increased infant mortality Neurological cretinism: mental deficiency, deaf mutism, spastic diplegia, and squint Myxedematous cretinism: mental deficiency and dwarfism Psychomotor defects
Neonate	Neonatal goitre Neonatal hypothyroidism
Child and Adolescent	Goiter Juvenile hypothyroidism Impaired mental function Retarded physical development
Adult	Goiter with its complications Hypothyroidism Impaired mental function

Zimmermann 2009 (34)

1.3.4 Methods of assessing iodine status:

There are several ways of assessing iodine status. Four methods are generally recommended: the goiter rate (thyroid size), urinary iodine concentration (UIC), serum TSH and serum thyroglobulin (Tg). Thyroid hormone concentration (T_3 and T_4) are poor indicators of iodine status. In iodine deficient population, serum T_3 increases or remains unchanged, and serum T_4 usually decreases. However these changes are often within the normal range (34).

1. Thyroid size:

The thyroid will increase in size in conditions of over and under nutrition of iodine. An increased thyroid gland is referred to as goiter. Inspection of thyroid size can be done either by palpation or thyroid ultrasonography. Palpation has poor sensitivity and specificity in areas of mild iodine deficiency and the use of ultrasonography of the thyroid is then recommended. However, interpretation of ultrasound is subjective and the technique can produce inter-observer errors in thyroid volume of up to 26 % (34).

2. Urinary iodine concentration (UIC):

Because more than 90 % of dietary iodine eventually appears in the urine, UIC is an indicator of recent iodine intake. UI does not provide direct information of thyroid function though a low value suggests a population at risk (34). UI is expressed as concentration (ug/L), in relationship with creatinine (Cr) excretion (ug I/g Cr) or as 24 hour excretion (ug/day). Most frequently the median spot urinary iodine concentration (MUIC) is used to determine population iodine status (40). This measurement cannot be used to determine an individual's iodine status as there is substantial day-to-day and diurnal variation in iodine intake. Hydration status will affect the result a spot measurement, however variations in hydration among individuals generally even out in a large number of samples and spot samples correlates well with 24 hour samples. Spot urinary samples analysed for iodine content is the recommended method for assessing iodine status in populations of infants and children if measured in a representative sample of the target group (54). The sample size for assessing iodine status by spot UIC depends on how large the day-to-day variation is. In a study on the number of spot samples needed in adult men to determine iodine status in a group, 125 samples was necessary for 95 % confidence with a precision range of ± 10 %. This was reduced to 100 if 24-hour urine was used. If the confidence interval was reduced to 90 %, then 86 urine spots were needed for the same precision range (55). Repeated spot samples in the same individual will reduce the effect of intra-individual variation in estimation of MUIC, and probably reduces the number of subjects needed to assess iodine status in a group (56). Alternatively age- and sex-adjusted iodine:creatinine ratio may be

used. However, creatinine estimations may be unreliable from spot samples, especially in malnourished subjects where creatinine concentration is low (57).

3. Thyroid stimulation hormone (TSH):

TSH is determined mainly by circulating levels of thyroid hormone, which in turn reflects iodine intake. TSH could therefore be used as an indicator of iodine nutrition. However, although serum TSH may be slightly elevated in iodine deficiency, the values often remain within the normal range. TSH is therefore an insensitive indicator. This is especially true in older children and adults. In newborns on the other hand, TSH is a sensitive indicator of iodine status (34).

4. Thyroglobulin (Tg):

Tg is a thyroid specific protein and precursor in the synthesis of thyroid hormones (58). When there is sufficient iodine, small amounts of Tg are secreted into the circulation, and serum Tg is normally < 10 ug/L. In iodine deficiency Tg in serum increases due to greater thyroid cell mass and THS stimulation. In intervention studies in children, Tg falls rapidly with iodine repletion. Tg is well correlated with the severity of iodine deficiency as measure by iodine in urine. In children it has been shown that Tg is a sensitive indicator for low iodine intakes and for excessive intakes of iodine (59). However, standardized assessment methods for assessment are not in place and are currently being developed.

The WHO recommends using median urinary iodine concentration (MUIC) for assessing iodine status in a population. In their latest recommendation, the WHO together with the International council for control of iodine deficiency disorders (ICCIDD) and the United Nations Children's Fund (UNICEF) proposed that a median level of more than 100 µ g/l urinary iodine should be considered as iodine sufficiency of children under two years of age (Table 3) (54) .

Table 3 Urinary iodine cut off values for children < 2 years of age

UIC ug/L	Iodine status
< 100	Insufficient
> 100	Adequate

Adapted from Zimmermann 2008 (57)

1.3.5 Iodine requirements:

Iodine requirements vary with age and stages of life. In order to maintain normal thyroid hormone production, most adolescents and adults need to ingest 150 ug of iodine per day (40). The daily recommendation for children is lower, however the iodine need is higher per kg body weight. The thyroid hormone turnover is highest during the first few months of life and during fetal life. The requirements therefore increase during pregnancy and lactation, as the diet needs to ensure good iodine status for both mother and offspring (40).

Recommendations on dietary iodine intake have been established by international expert groups and by several national organizations or governments. The recommendations differ somewhat due to differences in the end-points used to determine adequate intake (60).

The WHO/ICCIDD/UNICEF iodine recommendation for infants (Table 4) is based on the recognition that positive iodine balance in young infants is achieved only when the iodine intake is at least 15 ug/kg/day in full-term infants and 30 ug/kg/day in per-term infants. This corresponds to approximately 90 ug iodine per day, with the assumption of an average body weight of six kg at six months (47)

Table 4 WHO/ICCIDD/UNICEF recommendation for iodine intake in different age groups

Age	Total iodine intake in µg/day	Iodine ug/kg/day
0-11 months	90	15.0
1-6 years	90	6.0
7-12 years	120	4.0
Adults over 12 years)	150	2.0
Pregnant and lactating	200	3.5

Adapted from WHO,UNICEF,ICCIDD 2007 (61)
WHO = World Health Organization
UNICEF = United Nations Children's Fund
ICCIDD = the International council for control of iodine deficiency disorders

The Norwegian recommendation for dietary intake of iodine is based on the Nordic Nutritional recommendations of 2012 (NNR) and shown in table 5 (62). The recommendation for infants and children are based on goiter prevalence and urinary excretion in European children (63) and on extrapolations from adults based on energy and growth requirements. The NNR claims that in iodine sufficient populations, breastmilk will cover the needs of infants during the first months of life. No specific recommendation exists therefore for infants under the age of six months.

Table 5 NNR recommendation for iodine intake in different age groups	
Age	**Total iodine intake in µg/day**
6-11 months	50
12-23 months	70
2-5 years	90
6-9 years	120
10-17 years	150
Women	150
Pregnancy	175
Lactation	200
Men	150

Adapted from NNR 2014 (62)
NNR = Nordic Nutritional Recommendation

Infant balance studies could be used to calculate the iodine need of the breastfed infant. Full term infants fed 20 ug/kg/day of iodine were found to retain 7.3 ug/kg/day. In reference to a body weight at six months of seven kilos, the daily iodine retention in a six month old infant in positive balance is approximately 50 ug (34).

1.3.6 Dietary sources

The native iodine content of most foods and beverages is low. Foods of marine origin have higher iodine content. Major sources vary from country to country and depend on the supplementation policy of the region. Globally, iodization of salt has dramatically reduced goiter rates in many regions (34, 40). Salt fortification has not been required to reduce goiter in all countries. Iodine fortification of cow fodder has resulted in a relatively high concentration of iodine in milk and dairy products, which in turn has led to eradication of endemic goiter in some countries, like Norway, due to the relative high consumption of these products (43, 62). The iodine content of the soil in Norway is very low and for this reason cow fodder is supplemented with iodine that in turn makes dairy products an excellent source of this nutrient. Norwegian fruits, vegetables, bread and meat are generally low in iodine (64). National representative data on iodine content of food in Norway does not exist. The Norwegian food composition table includes values for iodine, however, many of the food items does not have known values of iodine and values are therefore missing from the table (65).

The main iodine sources in the Norwegian diet have been found to be milk and dairy products and fish and fish products, contributing to 80 % of total iodine intake (43). The iodine content of lean fish is more than double that of fatty fish. Other food groups provide only small amounts of iodine (Table 6). Eggs are high in iodine, however the consumption is low and hence the contribution is only

marginal. The iodine contribution of drinking water is negligible (43). Approximately 64-71 % of total iodine intake in Norwegian children comes from milk and dairy products, and 12-14 % from fish and fish products. Milk, as a drink, contributes to almost 50 % of total iodine intake in children. The contribution of iodized salt is expected to be low as regulations in Norway only permits the addition of 5 ug iodine per g of sodium chloride (NaCl)(64), however industrial salt used in food production is not supplemented with iodine (43, 66). It is recommended to reduce the intake of salt to 3 g per day and hence iodized salt would at most provide 15 ug/day (43). Adding salt to infant foods is not recommended (66).

There are a few studies published regarding the intake of iodine in different age groups in Norway. Dahl et al found that iodine intake among four year old girls and boys of 98 and 101 ug/day respectively, were above the recommended intake of 90 ug/day. Boys aged nine years had a mean dietary intake of iodine of 121 ug/ day, which was at the same level as the recommendation, however girls of the same age had lower intakes of 100 ug/day and hence did not reach the recommendation of 120 ug/day. The mean iodine intake in girls and boys aged 13 years of 90 and 109 ug/day were below the recommendation set at 150 ug/day. The lower iodine intake in 13-year-old girls was explained by a lower intake of milk (43). Brantseter et al found a median iodine intake of 141 ug/day without supplementation and 161 ug/dag with supplementation in pregnant women in the MoBa study and hence these women did not reach the recommended intake of iodine of 175 ug/day (48).

The iodine sources of infants and young children are somewhat different from the rest of the population. Most infants in Norway are fed enriched baby cereals, vegetable purees and fruit purees as their first foods (67). The rate of breastfeeding is high with over 70 % of the infants being breastfed at six months and 35 % at 12 months (68). The use of formula milk in the first few months is generally low with 25 % of the children being introduced to formula before the age of three months. Breastmilk iodine content and the content in baby cereals and formula milk affects the iodine status of infants and young children (69-71). Fish is generally not introduced until after six months of age, and the fish intake is low with 80 % of 12 month old Norwegian children consuming fish at a mean intake of 13 g per day in a national dietary survey from 2006 (20, 67). The iodine content of the diet of infants and children in Norway has not been assessed.

Table 6 Iodine content in Norwegian food

	Mean Iodine Content (ug/100g)
Lean fish	86
Fatty fish	40
Fish products	59
Fish as sandwich spread	33
Other fish products	12
Milk, all types	15
Yoghurt	8
Cream and cream products	11
Cheese, white	38
Cheese, whey	129
Eggs	45
Meat and meat products	2
Bread and cereals	3
Potatoes	2
Vegetables	2
Fruits and berries	2
Fats and oils	2
Water	0.2

Adapted from Dahl et al 2004 (43)

1.3.7 Groups at risk for iodine deficiency

The importance of milk and fish in the Norwegian diet for sufficient iodine intake puts certain groups of the population, such as people with allergy to milk or fish, vegetarians, especially vegans who do not consume fish, milk and dairy products and others with a low consumption of milk and fish, at risk of low intake of iodine (43). Infants could fall into this last category because cow's milk as a drink or in cooking is not recommended to children under the age of 10 months by the Norwegian government (66) and fish intake is generally low in this population or could be withheld from the diet because of fear of allergies. Plant foods, commonly introduced early in the weaning period, are poor in iodine and the use of iodized salt during the first year of life is undesirable. Breastfed weaning infants of mothers with low iodine intake could be at high risk of iodine deficiency if complementary food enriched with iodine (e.g. enriched baby cereals) or infant formula are not provided.

1.3.8 Breast milk Iodine content (BMIC):

The breastmilk content of several nutrients, such as vitamins A, D, B1, B2, B6, and B12, fatty acids, and iodine are dependent on maternal diet (72). Adequate maternal iodine intake is particularly important for the exclusively breastfed infant for whom breast milk is the sole source of iodine. Expression of the sodium iodide symporter (NIS) is up-regulated in the lactating mammary gland

which results in preferential uptake of iodide and iodine is concentrated into the breast milk at a gradient of 20-50 % higher than in plasma through the NIS (73). BMIC of human milk varies widely depending on maternal iodine intake and peaks six hours after maternal ingestion. Median breast milk iodine concentrations are reported to range from 5.4 to 2170 ug/L (median 62 ug/L) in worldwide studies (74). The exact cut-off for concentration of iodine in human milk has not been specified; however, values above 75 µg/l of milk has be considered as an index of sufficient iodine intake (75). Assuming an intake of 700-800 ml of breast milk per day in infants less than six months of age, a BMIC of 75 ug/L would supply the infant of 52.5 to 60 ug of iodine per day. BMIC was found to vary from 10-175 ug/L in studies from Europe (76). BMIC decreases during the first six months of lactation in iodine deficient subjects (77) and have been found to correlate with the urinary iodine content of the infant (71). In areas of insufficient iodine status, supplementation of the nursing mother has proved to be more effective than direct supplementation of the infant in ensuring adequate iodine status of the child (78).

1.3.9 Iodine toxicity

Most individuals tolerate high dietary intakes of iodine remarkably well, however as with iodine deficiency, iodine in excess can be harmful to the thyroid of infants and children and is associated with an increase in thyroid disorders (79). High exposure to iodine leads to an inhibition of the synthesis and release of thyroid hormone, through the so called the Wolff-Chaikoff effect. The Wolff-Chaikoff effect is transient. The Wolff-Chaikof effect does not develop until week 36-40 of gestation and premature infants are therefore vulnerable to the effects of iodine overload. Long-standing moderate iodine deficiency is accompanied by an accelerated trapping of iodine and by a decrease in the stores within the thyroid. Iodine supplementation to subjects with endemic iodine deficiency goiter can result in thyrotoxicosis in a small fraction of individuals at risk. Excessive iodine intake in children in high iodine areas is associated with impaired thyroid function (80, 81) .

1.3.10 Iodine and CMPA

Iodine in relation to CMP free diet has as far as we know never been studied. Little is therefore known about the iodine status of the CMPA child. Several case studies have found goiter and signs of goiter due to low iodine intake in children restricting cow's milk protein and in some cases also fish (82-85). Dairy products accounts for around 70 % of the iodine intake in young children (43) and the iodine concentration in breast milk is dependent on maternal iodine intake. Table 7 shows the iodine content in hypo allergenic formulas and cereals used by CMPA children in Norway. The amount of iodine in infant formulas and weaning food are regulated by a Commission Directive which allows 35 ug/100 kcal ready to use product (86). Calculations show that 350-700 ml of formula must be consumed to cover the recommended dietary intake of children six to 24 months of age. Enriched

baby cereals are supplemented with iodine, however at the time of writing there is only one such cereal without cow's milk protein on the Norwegian marked. Some parents buy CMP free enriched cereals from the Swedish marked where the selection is larger. Cereals that are not enriched with vitamins and minerals and other weaning foods are generally low in iodine (69). Breastfed infants with CMPA will be dependent on the iodine intake of their mothers who are also excluding cow's milk protein. The iodine nutrition of infants and young children with CMPA may therefore be at risk.

Table 7 Iodine content of hypoallergenic formula milks and cow's milk protein free baby cereals

		ug per 100 ml	ug per 100 kcal
Extensively hydrolyzed formula (eHF)			
Althera	(Nestle Nutrition)	10	15
Nutramigen 1	(Mead Johnson)	14	21
Nutramigen 2	(Mead Johnson)	12	18
Pepticate	(Nutricia)	12	18
Amino acid formula (AA)			
Alfamino	(Nestle)	11	16
Neocate LCP	(Nutricia)	13.8	21
Puramino	(Mead Johnson)	10	15
Formula > 1 year			
Neocate Advanced/Active	(Nutricia)	7	7
Non Enriched baby cereal			
Holle	(Dementer)	0.2	0.5
Enriched baby cereal			
Sinlac	(Nestle)	10	13
Enago	(Enago AB)*	20	24
Semper (milk free)	(Semper AB)*	20	23
Neocate Spoon	(Nutricia)**	15.3***	8.7

* available on the Swedish marked ** product for special medical purposes

***per portion of 60 ml water and 37 g powder

2 Aim

The main aim of this thesis is to investigate iodine status and growth in a group of children on cow's milk protein free diet by;

- Investigating iodine status at group level using urine spot samples.
- Investigating iodine intake by dietary record and food frequency questionnaire.
- Identifying dietary factors affecting iodine status.
- Assessing growth by weight for age z-scores (WAZ), length for age z-scores (LAZ) and BMI for age z-scores (BMIZ).
- Identifying dietary factors affecting growth.

The main hypothesis of this study is that children on a cow's milk protein free diet are at risk of iodine deficiency and compromised growth.

3 Subjects and methods

3.1 Overview

This thesis is part of the MILKID study, a cross sectional study on nutrient intake, growth and nutritional status in children under two years of age following a cow's milk protein (CMP) free diet. The study was conducted in the period from February 2014 to May 2015.

The study is a collaborative project between the Department of Nutrition at the University of Oslo (UiO) and the Department of Paediatric Medicine, Women and Children's Division at Oslo University Hospital (OUS).

All children following a CMP-free diet under two years of age were invited to the study. The invitation was made in conjunction with either a planned visit to hospital or by phone, after referral from hospital staff.

Diet and nutritional status were recorded after the child had followed a CMP-free diet for a minimum of three weeks. Parents had been given dietary advice on a CMP-free diet from a dietitian. The methods used in this thesis included:

- 3 day dietary record with household measure.
- A food frequency questionnaire.
- A questionnaire on background data.
- Anthropometric measurements.
- 2 urine spot samples

The parents completed the dietary record and questionnaires, and collected urine samples at home. Anthropometric measurements including weight, length and head circumference were done at a hospital outpatient clinic by hospital staff. An overview of the MILKID study is presented in figure 4.

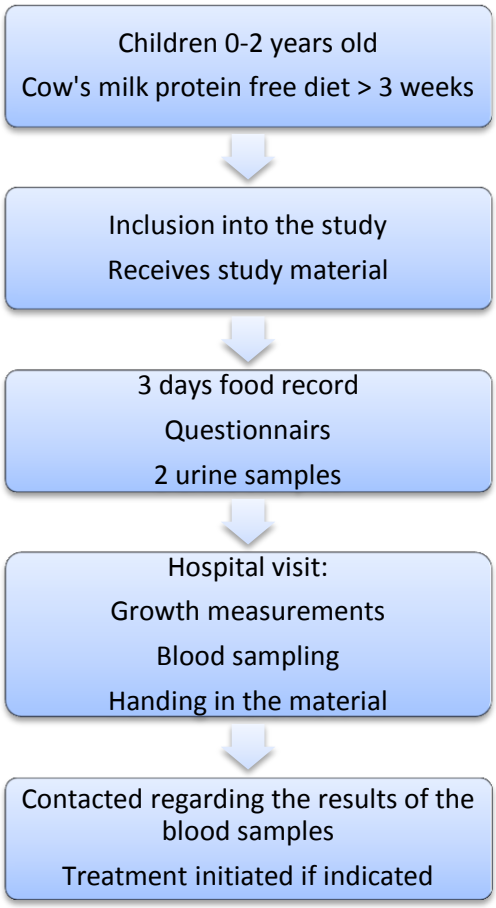


Figure 4 Overview of the MILKID study

3.2 Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway (REC nr. 2013/1579), and by the Head of the Department and the Research committee at OUS Women and Children's Division Department of Paediatric Medicine. Written consent was obtained from both parents before the child was included in the study program, and additional written consent was obtained from the breastfeeding mothers in order to collect data on the mother's diet. In accordance with good clinical practice each participant was randomly assigned an ID-number and all data collected on a participant was marked with this ID-number. The key connecting names and ID-numbers was kept in a locked compartment at the Department of Paediatric Medicine, OUS, and not removed from the hospital at any time. All digital material was stored on a designated research server in a folder only accessible by the study personnel. The investigators contacted the family maximum once by phone if information was missing or the patient

did not show up at the planned visit. The participation was voluntary and parents could withdraw consent at any time during or after the study.

3.3 Subjects

Patients under the age of two years were invited to participate in the study. Parents were informed either by a paediatric dietitian, doctor or research worker (master student).

The patients had to fit the following inclusion criteria to participate:

- Follow a cow's milk protein free diet.
- Age under two years.
- Born at term or no earlier than 37 weeks of gestation.

Patients fitting any of the following were excluded:

- Premature birth.
- Thyroid disease.
- Dependence of enteral- or parenteral nutrition.
- Use of medication that effect iodine status.
- Received iodine containing contrast the past 6 months.
- Suffering from other diagnosed illnesses affecting nutrient absorption or metabolism (e.g. Cystic Fibrosis).
- Parents in need of an interpreter (excluded due to financial limitations of the study).

3.4 Methods

3.4.1 Dietary advice

The Milk school (Melkeskolen) is an information class held by the dietitians every third week for patients who are diagnosed with cow's milk protein allergy (CMPA) or who are undergoing a diagnostic elimination of cow's milk protein. The two hourly session aims to ensure safe exclusion cow's milk protein while maintaining nutritional adequacy. The parents and carers are taught how to avoid cow's milk proteins in the diet by reading food labels and information about milk-free

substitutes and how to vary the diet to ensure nutritional adequacy is provided. Using hypoallergenic formula as a formula or cow's milk substitute and in the preparation of certain baby foods is recommended. Recommendations regarding dietary supplements are given. Mothers who breastfeed their CMPA child are required to follow a CMP free diet and are given advice on how to maintain energy and nutrient intake in their own diet.

3.4.2 Nutritional assessment

Nutritional assessment is necessary to identify nutritional status. In this study anthropometric measurement, dietary assessment and biochemistry were used to assess nutritional status.

Growth is the primary outcome measurement of nutritional status in children (87). The study included anthropometric measurements of growth at birth and at the time of the study. In children less than 36 months of age assessment of growth includes weight, length, and weight for length or Body mass index (BMI) and head circumference.

Values for birth weight, length and head circumference were taken from the child's health visitor card, a record provided to all children born in Norway or from their hospital record.

Measurements at the time of inclusion were performed most of the time by one of the research workers (Janne Kvammen (JK), Mari Eskerud (ME) and Rut Anne Thomassen (RAT)) and occasionally by a paediatrician or paediatric nurse.

The children were weighed on a digital infant scale, Data Baby scale 930 (Oriola, Espoo, Finland), to the nearest 0.005 kg. The child was without clothing or diaper. Weight was recorded lying down in most children, only large children were weighed sitting upright on the scale if they were too long to fit the scale lying down.

Recumbent length was measured without clothing using a length board with a fixed headpiece and a moveable foot piece perpendicular to the surface of the board, to the last completed millimeter (Figure 5). The length board stood on a flat stable surface. The parent laid the child flat with the head against the headpiece, eyes looking straight up, and helped to hold the child's head in place. The observer stretched the knees and the footboard was brought against the heels with ankles at 90^{o}. Length was recorded to the nearest 0.1 cm.

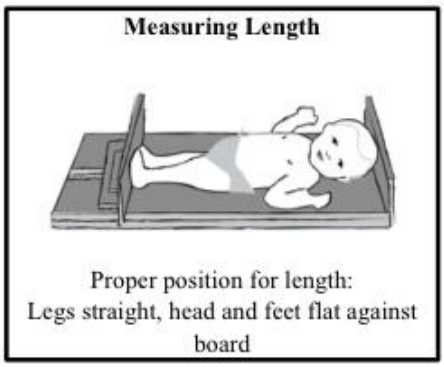


Figure 5 Illustration of measurement of length (WHO (88))

For measuring head circumference a poly-fiber measuring tape (Hoechstmass, Germany), was placed just above the glabella, the area between the eyebrows, and around the largest protuberance of the head (Figure 6). The observer was positioned laterally to the child. The tape was firmly pulled to compress hair and the head circumference was recorded to the nearest 0.1 cm. Three measurements were performed and the mean was used (89).

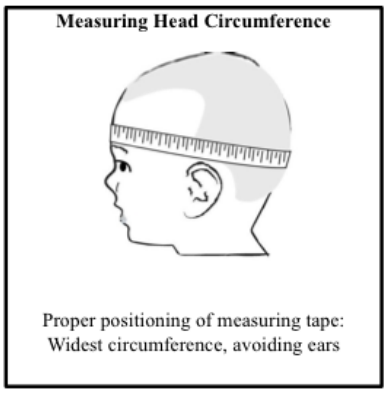


Figure 6 Illustration of measurement of head circumference (WHO (88))

The anthropometric measurements were plotted using the Norwegian growth chart by Júlíusson (89, 90) for weight for age, length for age, head circumference for age and weight for length. The Norwegian growth data was chosen over WHOs growth charts as it is shown to be more accurate for Norwegian children who are generally heavier, longer and have larger head circumference than the WHO standard (90). Z-scores for weight for age (WAZ) and length for age (LAZ) based on the Norwegian data were calculated with the hospital electronic growth charts (Vekstjournal), and z-scores for weight for length and BMI for age (BMIZ) were calculated by Pétur Júlíusson, Section for Paediatrics, Department of Clinical Medicine, University of Bergen, Norway, based the same cross-sectional sample (89).

Percentiles and Z-scores are used to assess anthropometric measurements and to evaluate children's growth and nutritional status. Z-scores have several advantages over percentiles. Z-scores are calculated based on the distribution of the reference population (mean and standard deviation) and thus reflect the reference distribution. They are also comparable across ages, gender and anthropometric measures. Z-scores can also be analyzed as a continuous variable in studies and they quantify extreme growth status at both ends of the distribution (91).

The WHO classification of z-scores (Table 8) were used to identify under- and over-nutrition.

Table 8 WHO standards for malnutrition

Outcomes	Anthropometric measures and	Indication of growth/nutrition
Infants and children (< 10 years)		
Stunting	LAZ ≤ -2	Chronic malnutrition
Underweight	WAZ ≤ -2	Acute malnutrition
Wasting/thinness	Weight for height ≤ -2 z-score or BMIZ ≤ -2	Acute malnutrition, current malnutrition
Overweight	Weight for height ≥ 2 z-score or BMIZ ≥ 2	Overweight

WHO 2012 (92)
WHO = World Health Organization

Data on nutritional intake was collected using a 3-day food record with estimated portion sizes. Food records have been found to provide the best estimate for young children of six months to four years old. The number of days needed to get a valid estimate of macro- and micronutrients in children younger than 24 months are between two to five days depending on the target nutrient (93, 94). The parents in the MILKID study were instructed to describe as detailed as possible, using household measures such as tablespoons, milliliters and weight, when known, all foods and beverages served to the child and the amount the child ate. Product name and manufacturer were recorded for ready-made meals. If the child used a hypoallergenic formula the parents were asked to record numbers of scoops and amount of water used in preparing the formula. The time of day was also recorded. Recipes of home cooked meals were provided.

The data on diet and nutritional supplements was entered into a Norwegian food and nutrient database, "Kostholdsplanleggeren" (95), launched by the Norwegian Food Safety Authority and the Norwegian Health authorities in 2014, and based on the Norwegian Food Composition Table (FCT)

(96). The Norwegian FCT provides information on the content of energy and 36 different nutrients of 1469 of the most commonly consumed foods in Norway. Details on foods not included in the database were provided by the manufactures and entered into the program by the research workers. When data on a product was missing, data for a similar product was used and corrections were made to fit the missing product.

Data on iodine content in food is not complete in the Norwegian FCT. The calculation of iodine content of foods that did not have a value for iodine in the FCT was done manually by the investigator using the mean values for different foods published by Dahl et al (table 6) (43).

If the child was breast fed each breastfeeding was recorded along with how long the feeding lasted. Breastmilk was not entered into the food and nutrient data base as we had not standardized how much breastmilk children consumed in a certain amount of time.

Two spot samples of urine, each minimum 5 ml, were collected during the study period, preferably on a day when the child's diet was recorded. The parents were instructed to take the samples on two separate days, avoid the first morning urine, and record the date and time of the sample. Two methods for urine collections were used:

- Urine collection pad (Euron Newcastle Uricol collector, United Kingdom). The method involves a Steriset with two urine collection pads, one 5 ml syringe and a urine specimen container. The urine sample is collected on the pads, which are laid in the child's nappy. Urine is then extracted from the pad using the 5 ml syringe and then transferred into the specimen bottle. The parents are instructed to check the child's nappy every ten minutes and to discard the pad if has been soiled by faeces. This method has been validated in infants (97).

- Urine sample collection bag (U-bag single specimen style, sterile, newborn, Briggs Healthcare, Waukegan, USA). The method uses a collection bag with adhesives on one end that is fixed over the baby's genital area. The area around the urethra is washed before fixing the bag. The parents then check the bag regularly for urine. The bag is emptied into the specimen bottle.

The parents were given equipment for both methods and were free to choose what method they used as some had problems when using the pad, others with the collection bag.

Urine samples were handed to the investigators at the follow up visit at the hospital, a few were sent by postal service. As iodine in urine is fairly stable, keeping the sample at room temperature will not affect the iodine content as long as the container is airtight. The specimen was then frozen to -18 degrees before sent to the lab for quantification of iodine.

The urine samples were analyzed by the Hormone Laboratory at Aker, OUS (Hormonlaboratoriet) using a colorimetric method based on Spectrometry with the Sandell-Kolthoff reaction after oxidation of interfering substances with ammonium-sulfate. Colorimetric measurements of the Sandell-Kolthoff reaction are performed with a Spectrophotometer, analyzing the catalytic effect of iodine on reduction and oxidation between arsenic and cerium.

Sandell-Kolthoff reaction:

$As^{3+} + I_2 \rightarrow As^{5+} + 2I^-$

$2Ce^{4+} + 2I^- \rightarrow 2Ce^{3+} + I_2$

(As^{3+} = arsenious acid, I_2 = Iodine, Ce^{4+} = Ceric ion, Ce^{3+} = Cerous ion)

The yellow colored ceric ion (Ce^{4+}) is reduced to colorless cerous ion (Ce^{3+}) by iodine ion (I^-). The time taken for the color disappearance is inversely proportional to the amount of iodine catalyzing it. The more iodine in the solution (here urine) the faster the colour disappearance i.e. the absorbance of the solution gives a measure of the iodine concentration. Iodine concentrations are determined from standard curve of absorbance versus iodine concentration (98, 99).

The lab uses microtiter plates in the process.

Iodine content is expressed as umol/L:

Lowest iodine value quantified by the laboratory:	0.2 umol/L = 25.4 ug/L
Values lower than 0.2 umol were expressed as:	<0.2 umol/L

Iodine has a molar weight of 127 g/mol.

The participants were asked to fill in two questionnaires. The first questionnaire, “Spørreskjema om bakgrunnsinformasjon” (appendix 1), had 14 question and sought to collect information on the diagnosis of CMPA, symptoms, other diseases and parental characteristics. If the child was breastfed there were questions regarding the breastfeeding mother’s intake of dairy products and dietary supplements.

The second questionnaire, “Spørreskjema om Kosthold” (appendix 2), was a semi-quantitative food frequency questionnaire with 26 questions. Information about breastfeeding (past and present), the use of hypoallergenic formula, time of introduction to solids, feeding development and difficulties, inclusion of plant milk, common baby foods and iodine rich food sources in the child’s diet was collected.

Both questionnaires were designed uniquely for this study by two of the investigators (JK, RAT) and have not been validated. Other investigators who had studied the diet of the same age group as in this study by FFQ’s were contacted in a search for already validated tools. However, due to the dietary restrictions and usage of CMP-free specialized products no food frequency questionnaire was identified to suit this group of cow’s milk allergic children.

3.4.3 Statistics

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22 (Armonk, New York). Normality was tested using Shapiro-Wiik test and normal Q-Q plots. Continuous variables are presented as means with standard deviation (SD) if normally distributed or medians with a minimum and maximum value range if not normally distributed, except for nutritional data which are presented as medians with interquartile range (25^{th}- 75^{th} percentile). Categorical values are presented as frequencies.

For non-normally distributed variables, groups were compared using the Mann-Whitney U test if there were two groups and the Kruskal Wallis test, if there were more than two groups, and the independent sample t-test was used for normally distributed variables. The Spearman rho correlations coefficient was used to assess the relationship between continuous variables. For categorical values the Chi square or Fisher’s test was used. The paired sample t-test was used to look at changes in continuous variables over time and the one sided t-test was used to compare the distribution of variables with the normal or expected distribution.

The level of significance for all statistical tests was 2-sided and significance level was set to 0.05.

We devided our study population into three groups according to feeding pattern at the time of the study. The three groups were; mainly breastfed (BF), partially BF and weaned. The breastfed children were devided into the two groups to destinguish between the children that were mainly receiving their energy and nutrient intake from breastmilk and those that were more reliant on complementary foods. The mainly breastfed children were defined as those receiving less than 50 % of their estimated energy requirement from food. We used the WHO/Food and Agriculutral organization of the United Nations (FAO) recommendation for energy reqirement for breastfed

children as reference value (100). WHO/FAO recommends approximately 80 kcal/kg/day for breastfed children under the age 12 months. Children receiving less than 40 kcal/kg/day from food and drinks other than breast milk, calculated from the food record, were classified as mainly breastfed.

The main endpoint for calculating sample size of this study was urinary iodine concentration. A standard deviation in UIC of 34 ug/L in was expected for this group of children. Using a confidence interval of 80 % and 5 % margin of error, 46 individuals with two spot urine samples, that is 92 spot samples, were needed to detect a difference of 20 ug/L between our group and the cut off level for insufficiency of 100 ug/L. 10 % was added to allow for dropouts from the study. In summary a sample size of 50 children was needed.

4 Results

4.1 Recruitment

In the period of January 2014 to May 2015, parents of 101 infants and children were approached and asked to participate in the study. 19 patients proved not to be eligible because they did not fit the inclusion criteria, five were premature, nine were no longer on a CMP free diet, one had received parenteral nutrition within the last four weeks, three had underlying diseases affecting absorption of nutrients and metabolism, and one was in need of interpreter. Of the 82 remaining patient, 19 (23%) did not want to participate, leaving 63 (77 %) infants and children for inclusion. Six of these patients dropped out before information had been collected, leaving 57 (70 %) patients in the study group. Six patients did not complete the urine samples. By May 2015, urine samples from 51 (62 %) patients had been collected (Figure 7).

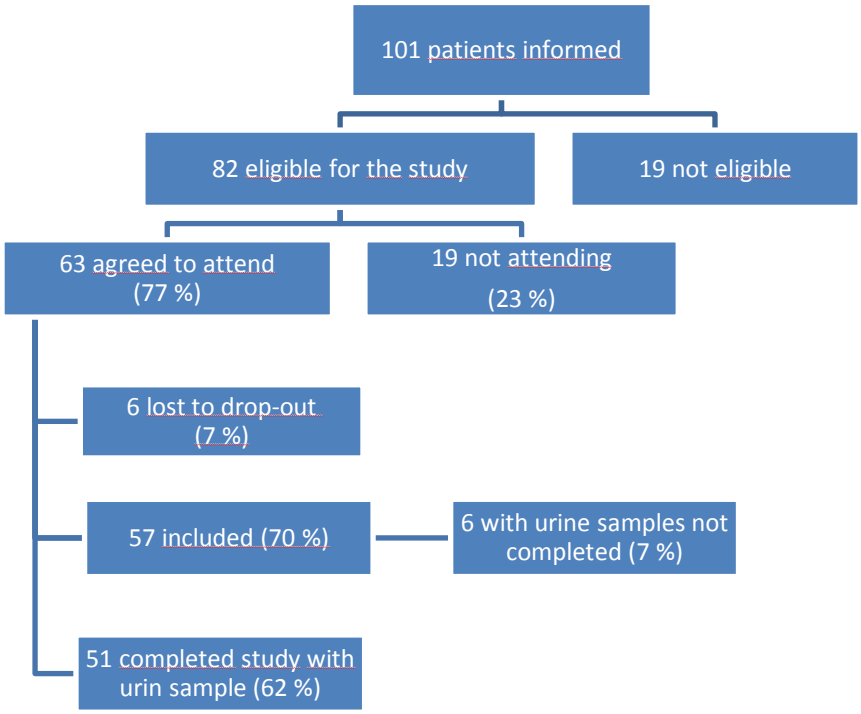


Figure 7 Recruitment of patients

4.2 Subject characteristics, symptoms and nutritional intake

4.2.1 Subject characteristics in the MILKID study

Subject demographic characteristics in the MILKID study are presented in Table 9. The study population consisted of 54 % boys and 46 % girls with a median age of nine months. 61 % were in the 6-11 months age range. Mean birth weight, length and head circumference were 3387 g, 50.6 cm and 34.9 cm, respectively. Mean LAZ at birth were close to zero indicating normal growth in utero. Mean WAZ and mean z-scores for head circumference at birth were both slightly lower than the normal population. Mean weight, length and head circumference at inclusion was 8699 g, 72.7 cm and 45.3 cm, respectively. Mean WAZ, LAZ, BMIZ and z-score for weight for length at inclusion were all below zero while mean z-score for head circumference was close to zero indicating normal growth. Median age of the mothers was 33 years, 84 % were of Scandinavian origin, 89 % had higher education and none were smokers.

Table 9 Subject characteristics

Characteristics	n	Mean/median	SD/Range	%
Child				
Age, months (median)	57	9	2-23	
0-5 months				12.3
6-11 months				61.4
12-23 months				26.3
Gender	57			
Boys				54
Girls				46
Anthropometry (mean)				
At birth:				
Birth weight, kg	55	3387	473	
Birth length, cm	47	50.6	2.09	
Birth head circumference, cm	24	34.9	1.26	
WAZ	55	-0.54	0.92	
LAZ	47	0.07	0.95	
z-score birth head circumference, cm	24	-0.30	0.99	
At inclusion:				
Weight, kg	57	8699	1718	
Length, cm	57	72.7	6.6	
Head circumference, cm	55	45.3	2.2	
WAZ	57	-0.41	0.99	
LAZ	57	-0.39	0.93	
BMIZ	57	-0.53	1.16	
z-score w/l	57	-0.48	1.14	
z-score head circumference	55	0.08	0.97	
Parents				
Mother's age (median)	55	33	23-42	
Mother's ethnicity	55			
Scandinavian				84
European				7
Non-European				9
Mother's education level	55			
12 years of school				11
<4 years of college/university				47
>4 years of college/university				42
Smoking	55			0

Range = minimum-maximum for median values.
SD = Standard deviations for mean values.
WAZ = z-score weight for age, LAZ = z-score length for age, BMIZ = z-score BMI for age
w/l = weight for length, BMI = body mass index

The patients had followed a CMF diet for a median of 17 weeks before inclusion. The median number of weeks from diagnosis to dietary counselling was 13, though there was a large rage from 0-52 weeks (Table 10).

Table 10 Number of weeks on cow's milk free diet and time from diagnosis to dietary counselling

	Median	Range
Number of weeks on diet (n=48)	17	4-84
Number of weeks from diagnosis to dietary counseling (n=47)	13	0-52

Range = minimum-maximum value

4.2.2 Symptoms, foods avoided and feeding difficulties in the MILKID study

Table 11 gives an overview of parent reported symptoms of CMPA in their child. Symptoms from the gastro intestinal (GI) tract were most common with colic and reflux reported in 74 % and 67 % of the subjects. Around half of the children had loose stools. Eczema was reported in 46 % of the children and sleep disturbance in 50 %. Growth faltering was a concern in 40 % of the cases. Median number of symptoms per patient was four, with 46 % of the parents reporting more than four symptoms of CMPA in their children (46 %). More than one organ system was involved in the allergic reaction to milk in 84 % of the children, most commonly two (46 %) and three (33 %) organ systems.

Table 11 Prevalence of symptoms of CMPA, organ system involved, distribution number of symptoms and distribution of number of organ systems involved in the MILKID study (n= 55)

Organ system	n	%
GI:		
Colic	39	71
Reflux	37	67
Loose stools	29	53
Bloody stools	24	44
Obstipation	14	26
Skin:		
Eczema	25	46
Respiratory:		
Anaphylaxis	1	2
Otitis Media	3	6
General and Subjective symptoms:		
Sleep disturbance	26	47
Growth faltering	21	38
Food refusal	18	35
Number of symptoms:		
Median number	4	
1-3 symptoms	18	33
4 symptoms	14	25
5-8 symptoms	23	42
Number of organ systems involved		
1	9	16
2	27	49
3	18	33
4	1	2

GI = Gastro intestinal

Most children had one or more symptoms from the GI tract, with or without symptoms from other organ systems. Only one child did not report GI symptoms, 10 % had one, 33 % had two, 35 % had three symptoms, 16 % had four and two percent had five symptoms from the GI tract. Median number of symptoms from the GI tract was three (Table 12).

Table 12 Distribution of number of symptoms from the GI tract in the MILKID study (n=55)

Number of symptoms from the GI tract	% of the group
0	2
1	11
2	33
3	35
4	16
5	2

GI = Gastro intestinal

The effect on symptom improvement of the CMP-free diet is illustrated in figure 8. In 89 % patients the diet gave improved symptoms, either partially (45.5 %) or fully (43.5 %). In two percent of the participants there was no improvement in symptoms and in nine percent the it was unclear if the diet improved symptoms.

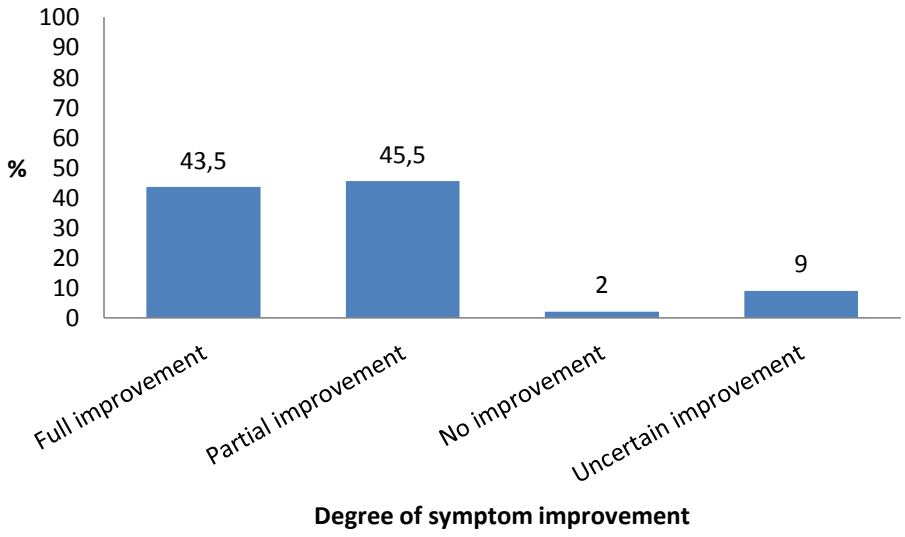


Figure 8 Effect of Cow's milk free diet on improvement of symptoms in the MILKID study (n=55)

The parents were asked to report which foods they excluded from their child's diet. Frequencies of number of foods avoided are shown in table 13. Median number of foods avoided including CMP was three. Excluding more than one potential allergen in addition to CMP was common. Excluding only CMP only was reported in 28 % of children. 19 % of children excluded two foods and 53 % excluded three or more foods in the diet.

Table 13 Distribution of number of foods avoided in the MILKID study (n=54)

Number of foods avoided	%
1	28
2	19
3	9
4	22
5	11
6	7
7	0
8	4

The most frequent food avoided along with CMP was nuts (65 %) followed by eggs (39 %) and soy (37 %), citrus fruit, fish and legumes (22 %) . Only four percent of the children avoided gluten (Figure 9).

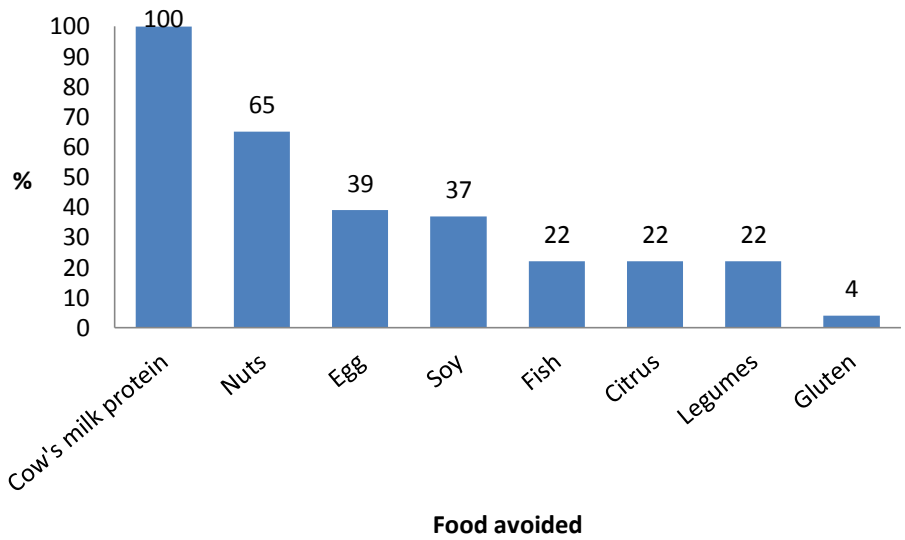


Figure 9 Distribution of foods avoided in the MILKID study group (n=54)

Feeding difficulties were assesed by a questionnaire of the most common feeding problems in this age group (Figure 10). The prevalence of feeding difficulties was high with 70 % of parents reporting one or more problems. Poor appetite was reported in 33 % of children, gaging og swallowing problems in 19 %, other food allergies by 17 % and problems with breast- og bottlefeeding by 15 %. 13 % of the parents reported weaning difficulties and 11 % perceived their child as a selective eater. 39 % of the parents reported problems not listed in the questionaire. 50 % of the parents reported more than one feedingproblem in their child (not shown).

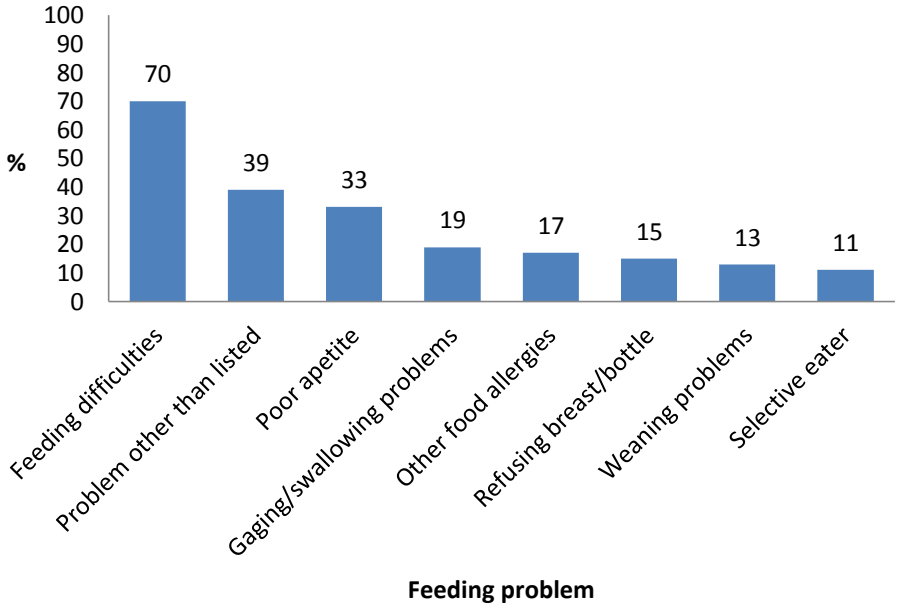


Figure 10 Prevalence of feeding problems in the MILKID study (n=54)

4.2.3 Breastfeeding status and feeding method

Table 14 gives an overview of breastfeeding status and time of introduction of food and drink. 58 % were breastfed while 42 % patients were not receiving breast milk at inclusion. Only two percent of the children had never been breastfed. Median age of introduction of other fluids and age of weaning onto complementary foods were both four months, with complementary food introduced no later than seven months.

Table 14 Breastfeeding status and age of introduction of food and drink in the MILKID study (n=55)

	%	Median	Range
Breastfed	58		
Weaned of breast milk	42		
Never breastfed	2		
Exclusively breastfed from birth	81		
Age of introduction of fluids other than breast milk, months		4	0-10
Age of introduction of solid food, months		4	3-7

Range = minimum-maximum value

Figure 11 illustrates different feeding patterns in the study group. Two percent of the children were exclusively breastfed and two percent received a combination of breast milk and hypoallergenic formula. 25 % were fed breastmilk, formula milk and complementary foods. 29 % were breastfed and complementary fed but received no formula milk. 38 % of the children were given complementary foods together with hypoallergenic formula and four percent were complementary fed only.

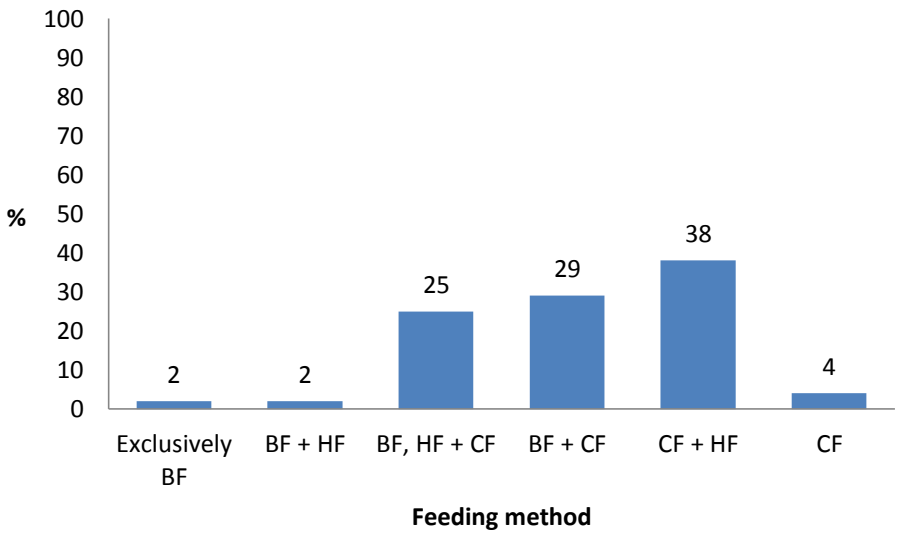


Figure 11 Distribution of feeding method in the MILKID study (n=55)
BF = breastfed, HF = hypoallergenic formula, CF = complementary feeding

Number of breastfeeding per day in the breastfed children is shown in figure 12. One child was breastfed more than 11 times per day. Median breastfeeding frequency was 3-5 times per day. Six percent of the breastfed children received breast milk 1-2 times per day, 53 % 3-5 times per day, 28 % 6-8 times per day and nine percent 9-11 times per day.

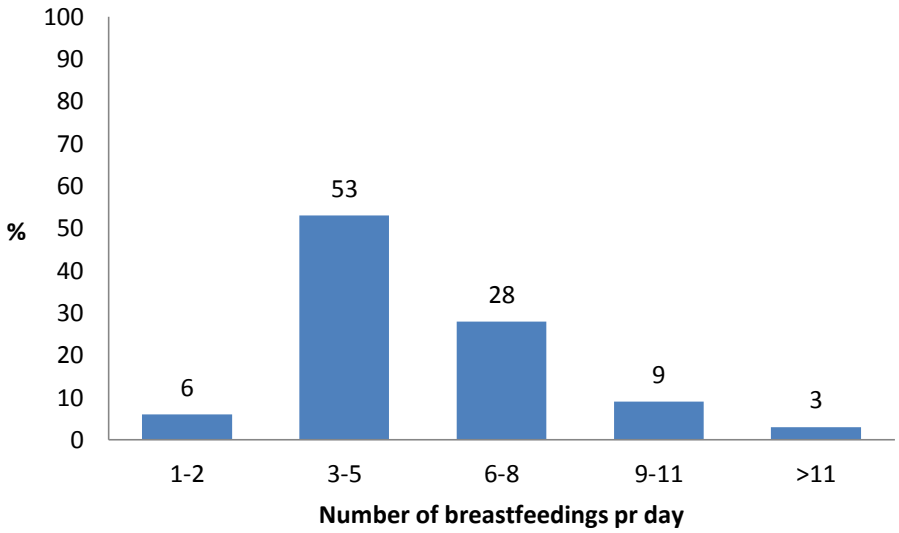


Figure 12 Distribution of number of breastfeeding per day in the MILKID study (n=32)

The children were divided into three different groups according feeding pattern. As table 15 shows, 24 % of the children were mainly breastfed, 33 % partially breastfed and 43 % were weaned and received no breast milk. There were differences in median age of the children between the three groups with the mainly breastfed children being significantly younger than the children in the two other feeding groups. There were small differences in gender in the different groups, however, these were not significant.

Table 15 Frequency, age and gender according to feeding pattern in the MILKID study (n=55)

	Mainly BF (n=13)	Partially BF (n=18)	Weaned (n=23)	P-value
% of the study group	24	33	43	
Median age, months	7	10	110	0.02*
Boys, %	46	61	52	ns

*Significant using Kruskal-Wallis Test
BF = breastfed

The distribution of number of breastfeedings in the two breastfed groups is shown in figure 13. Median breastfeeding frequency in the mainly breastfed group was 6-8 times pr day, while the partial breastfed children had a median of 3-5 times pr day. The difference was significant at the 0.001 level using Mann-Whitney U-test between the two groups.

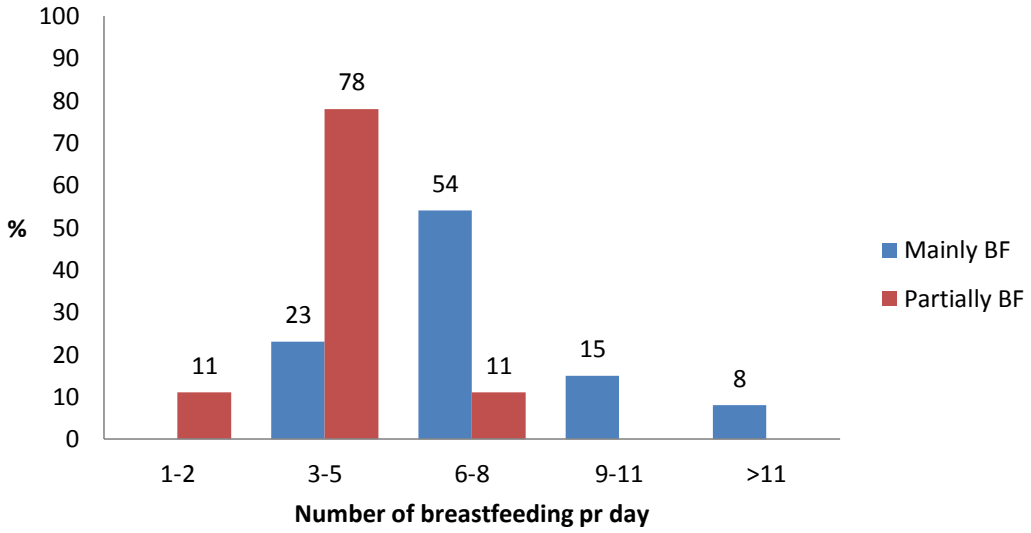


Figure 13 Distribution of number of breastfeeding per day according to feeding group in the MILKID study (n=32)
BF = Breastfed

4.2.4 Dietary intake

The prevalence of users of common weaning foods of the study population is shown in figure 14. Almost all children ate fruit (93 %) and vegetables were also commonly included in the diet (85 %). 70 % ate red meat and 69 % used a hypo allergenic formula. Bread and other cereals were eaten by 65 % and 61 % percent respectively. Enriched baby cereals were eaten by 56 %, fish by 44 %, poultry by 35 %, soy/oat products by 30 % and 17 % of the children included eggs in their diet.

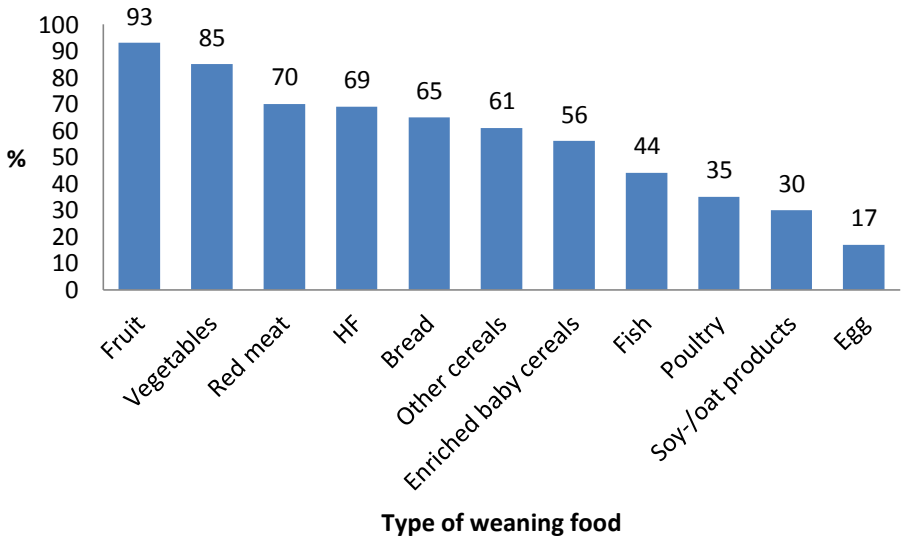


Figure 14 Prevalence of users of common weaning foods in the MILKID study (n=54)
HF = hypo allergenic formula

Median intake of the most common weaning foods and drinks in Norway in grams in relation to feeding pattern is shown in table 16. There were significant differences in the use of hypoallergenic formula (HF) between the feeding groups. The mainly BF children had a median intake of 0 g pr dag, the partly BF children 20 g pr day (140 mls) and the weaned group 63 g pr day (420 mls). There were differences in the median intake of enriched baby cereals between the groups, from 4 g (17 mls) in the weaned group to 23 g (100 mls) per day in the partly BF group, althoug this did not reach statistical significance. The same was seen for other cereals, bread, poultry and egg. There were significant differences in the intake of red meat, fish, fruit and vegetables between the groups. The weaned children had the highest intake of red meat. The partially BF group had the highest intake of fish, fruit and vegetables. There were significant differences in the intake of soy and oat products between the groups even if median values were similar, probably due to a large variation in intake within the groups.

Table 16 Median intake of different weaning foods in grams in relation to feeding pattern (n=54)

	Mainly BF (n=13)		Partially BF (n=18)		Weaned (n=23)		p-value
HF	0	(0-5.5)	20	(0-36)	63	(32-88)	>0.001*
Enriched baby cereal	13	(0-35)	23	(0-39)	4	(0-50)	ns
Other cereals	4	(0-12)	6	(0-22)	2	(0-20)	ns
Bread	0	(0-3)	6.5	(0-27)	19	(0-67)	ns
Red meat	0	(0-11)	7,5	(2-15)	12	(0-42)	0.03*
Poultry	0	(0-0)	0.8	(0-9)	0	(0-3)	ns
Fish	0	(0-0)	8	(0-34)	1	(0-13)	0.02*
Egg	0	(0-0)	0	(0-0)	0	(0-2)	ns
Fruit	50	(16-77)	121	(80-149)	67	(33-145)	0.01*
Vegetables	22	(11-45)	69	(54-89)	36	(4-61)	0.005*
Soy-/oat products	0	(0-0)	0	(0-34)	0	(0-43)	0.03*

*Significant using Kruskal-Wallis Test
Interquartile range (25th percentile to 75th percentile)
BF= Breastfed, HF= hypo allergenic formula

Type of HF in users is illustrated in table 17. Of the 40 children using a formula, 62 % used an extensively hydrolyzed formula (eHF) and 38 % an amino acid formula (AA). We tested the choice of treatment against other parameters, however we found no differences in type or number of symptoms, food allergens, or feeding difficulties and type of formula used (results not shown).

Table 17 Prevalence of type of formula used in the MILKID study (n=40)

Type of formula	n	%
Extensively hydrolyzed	25	62
Amino acid	15	38

4.2.5 Nutritional intake

Table 18 describes differences in energy and protein intake from complementary food and drink in relation to weight and percentage of total energy for protein, fat and carbohydrate between the feeding groups. Breastmilk is not included in these numbers. The three groups were similar for percentage energy from the different macronutrients and in line with current recommendations. Energy in kcal per kg and grams of protein per kg from complementary food and drinks were significantly different between the feeding groups. The weaned children received more energy and protein from complementary food and drink than the two breastfed groups. As expected the partially breastfed group had higher energy intake per kg and grams of protein per kg from complementary food and drink than the mainly breastfed group.

Table 18 Median intake of macronutrients in relation to weight and percentage energy from macronutrients from complementary food and drink in the different feeding groups

	Mainly BF** (n=13)	Partially BF** (n=18)	Weaned (n=23)	p-value
Kcal/kg	31 (24-35)	67 (52-90)	92 (80-107)	<0.001*
gram protein/kg	1.0 (0.6-1.2)	2.1 (1.8-2.8)	2.9 (2.1-3.3)	<0.001*
Energy % protein	11 (8-15)	13 (12-14)	12 (11-14)	ns
Energy % fat	33 (25-42)	31 (27-36)	34 (31-37)	ns
Energy% carbohydrates	52 (46-57)	55 (50-57)	53 (57-55)	ns

*Significant using Kruskal-Wallis Test **Breastmilk not included
Interquartile range (25th percentile to 75th percentile)
BF = breastfed

4.3 Iodine: status, nutrition and factors affecting status

4.3.1 Iodine status in the MILKID study

A total of 92 urine spots were collected from the participants that provided urine samples, two spot samples from 41 subjects and one spot sample from 10 subjects, resulting in 1.8 samples per individual. We used the mean value of the two spots for each individual subject in those cases where we had two spots.

Median urinary iodine content (MUIC) of the study group was 158.8 ug/L (<25 – 457 ug/L) and the prevalence of UIC < 100 ug/L, the cut off for insufficiency of iodine according to the WHO references for populations, was 31 % (Figure 15). Adequate status with values between 100-199 ug/L was found in 37 % and between 200-300 ug/L in 29 %. A UIC of over 300 ug/L was found in 12 %, though none had values of above 500 ug/L, which would indicate iodine excess.

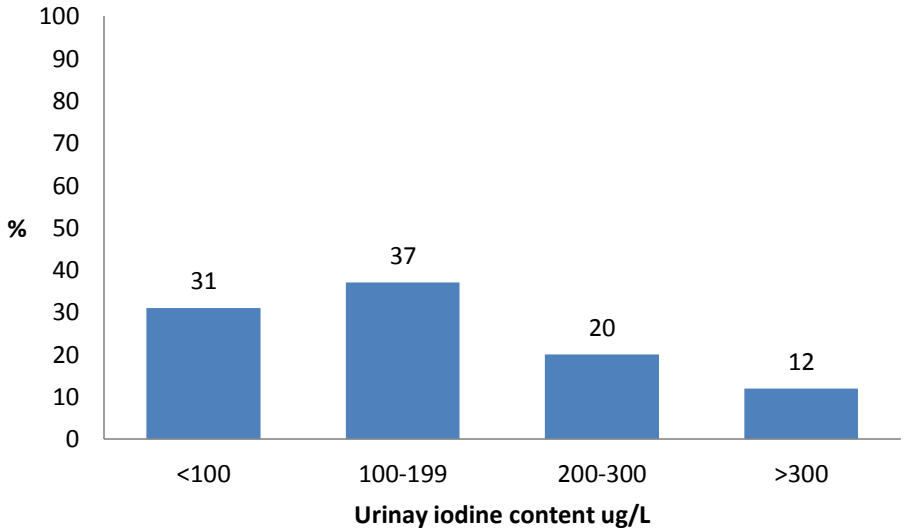


Figure 15 Prevalence of urinary iodine content in different categories in the MILKID study (n=51)

4.3.2 Iodine status and feeding pattern in the MILKID study

There were large differences in median urinary iodine content (MUIC) in the different feeding groups as shown in figure 16. The mainly breastfed group had a MUIC under the WHO cut off value for adequate status, while the two other groups had MUICs over the cut off value.

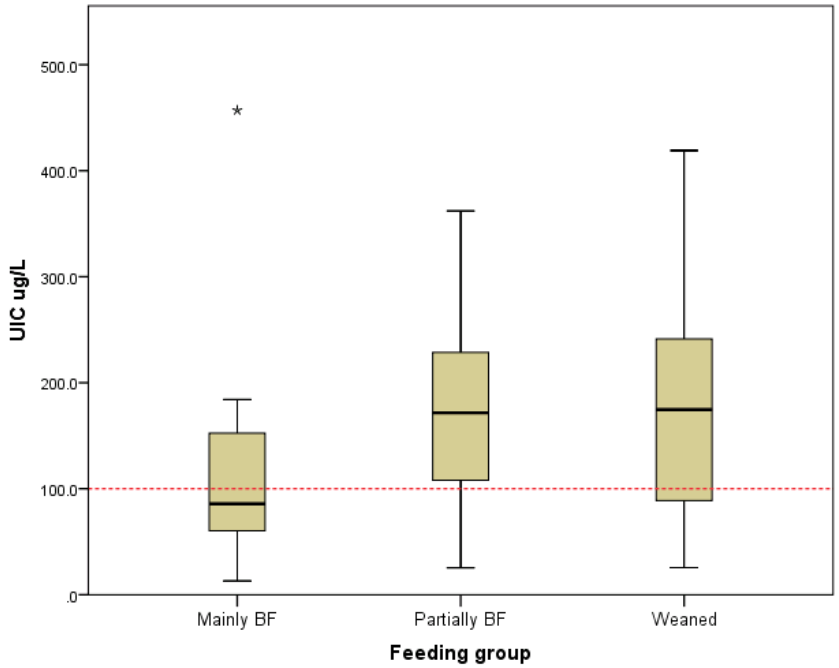


Figure 16 Urinary iodine content (ug/L) according to feeding pattern in the MILKID study
The horizontal line indicates the median, the box indicates the interquartile range (25th percentile to 75th percentile), the whiskers represent observations within1.5-times the inter quartile range and the stars indicate outliers. The red dotted line indicates the cut off value for insufficient status of 100 ug/L. BF = Breastfed

The mainly breastfed group had a MUIC of 85.8 ug/L, while the partially breastfed and weaned children had MUIC of 171.5 ug/L and 174.7 ug/L, respectively (Table 19). The difference in UIC between the groups was not significant. The mainly breastfed children had a high prevalence iodine insufficiency, with 58 % of the children having a value under 100 ug/L. The partially BF children had the lowest prevalence of insufficiency, with UIC of < 100 ug/L found in only 12 % of the children. The prevalence among the weaned children was 32 %. The difference in prevalence of UIC < 100 between the groups was significant.

Table 19 Median urinary content and prevalence of iodine insufficiency according to feeding pattern in the MILKID study.

	Mainly BF (n=12)	Partially BF (n=17)	Weaned (n=22)	P-value
MUIC, ug/L (range)	85,8 (<25-457)	171,5 (25,4-362)	174,7 (25,5-419)	ns*
% under 100 ug/L	58	12	32	0.03**

*Kruskal-Wallis test **Significant using Chi-square
Range = minimum-maximum value
BF = breastfed

4.3.3 UIC in breastfed infants in relation to maternal supplementation of iodine

Among the breastfeeding mothers, 33 % were taking supplements containing iodine. Half of the mothers of the mainly breastfed children were taking supplements with iodine compared to 23 % in the partially breastfed group (result not shown). The MUIC of mainly breastfed children whose mothers did not take supplements with iodine was 73 ug/L. In comparison the MUIC in children of mother's with iodine supplement was 121 ug/L. The difference, however, was not significant. There were differences between children of mothers who were and were not supplemented with iodine in the partially breastfed group, however both groups had MUIC indication iodine sufficiency. Table 20 summarizes the findings. None of the children were using iodine supplements.

Table 20 Median urinary content in breastfed children in relation to maternal supplementation of iodine in the MILKID study

		Mainly BF MUIC ug/L	**Range**	**Partially BF MUIC ug/L**	**range**	**p-value**
Maternal supplementation of iodine	yes	121	(<25-184)	197	(159-254)	ns*
	no	73	(32-457)	162	(25-363)	ns*

*Fisher's exact test
Range = minimum-maximum value
BF = breastfed

4.3.4 Dietary sources of iodine and iodine intake in the MILKID study

The main dietary sources of iodine in the diet of the MILKID study are described in the following figures and tables. Figure 17 gives an overview of the mean percentage contribution to iodine intake from different dietary sources in the study population. Hypoallergenic formula was the main contributor to the iodine intake in this population providing a mean of 42 % of total iodine intake, followed by enriched baby cereals which accounted for a median 27 % of iodine intake from diet. Fish provided nine percent, fruit and vegetables five percent, meat, poultry and egg six percent, bread and cereals four percent and other foods four percent of total iodine intake from diet.

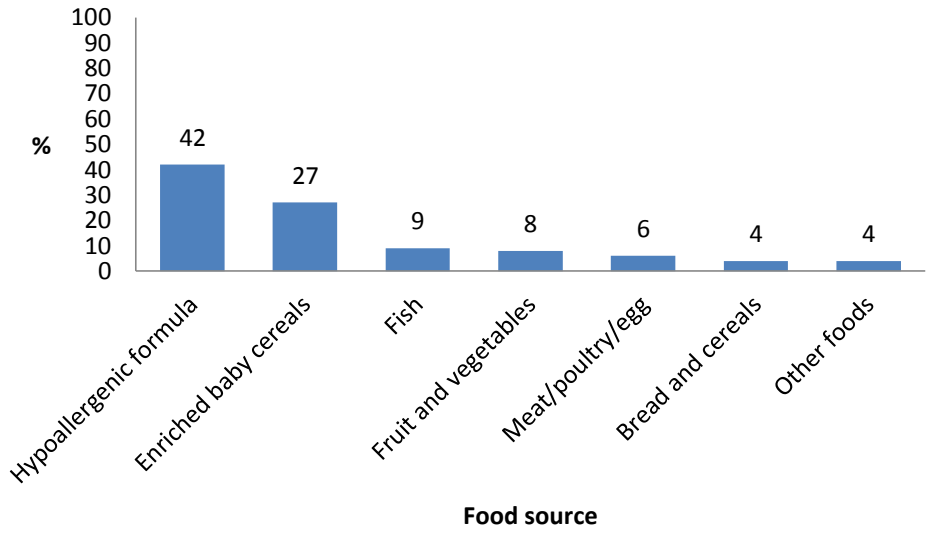


Figure 17 Distribution of the contribution to total iodine intake in percent from different food sources in the MILKID study

There were significant differences between the feeding groups both in terms of total iodine intake and contribution from different dietary sources (Table 21). Hypoallergenic formula provided more iodine to the weaned group than the two breastfed groups, and the difference between the groups was significant. There was a significant difference in the provision of iodine from fish with the partially breastfed group receiving more iodine from this food source than the weaned and the mainly breastfed group. The same was true for fruit and vegetables; the partially breastfed children had a higher intake of iodine from fruit and vegetables than the mainly breastfed and the weaned children and the difference between the groups was significant. Bread and other cereals contributed to significantly more iodine in the weaned and partially breastfed group than the mainly breastfed. The mainly breastfed group had a median iodine intake from food (breastmilk not included) that was lower than the two other feeding groups, and the weaned group had a higher iodine intake from diet than the partially breastfed children. The difference between the three groups was significant. There were no significant differences in the intake of iodine from enriched baby cereals, poultry, eggs and meat between the feeding groups.

Table 21 Iodine provision in ug from different food sources according to feeding pattern in the MILKID study

	Mainly BF (n=13)		Partially BF (n=18)		Weaned (n=23)		p-value
Hypoallergenic formula	0	(0-4.5)	16	(0-32)	43	(15-73)	<0.001*
Enriched baby cereals	6	(0-15)	7.9	(0-13)	1.3	(0-16)	ns
Fish	0	(0-0)	1.7	(0-9,6)	0.7	(0-2,6)	0.005*
Poultry	0	(0-0)	0.1	(0-0,9)	0	(0-0)	ns
Eggs	0	(0-0)	0	(0-0)	0	(0-0,2)	ns
Fruits and veg	1	(0.5-2)	3	(2.5-4.0)	1.9	(0.5-2.5)	<0.001*
Bread/other cereals	0,3	(0-0.5)	1,3	(0.4-2.4)	1.4	(0.3-2.9)	0.01*
Meat	0	(0-0.2)	0	(0-0.2)	0	(0-0.8)	ns
Iodine from diet	13	(8-26)	42	(27-69)	67	(48-91)	<0.001*

*Significant using Kruskal-Wallis test
Interquartile range (25th percentile to 75th percentile)
BF = breastfed

We looked in more detail at the provision of iodine from fish among the children that ate fish during the three days of food record (44 % of the total population). Half of the fish eating children ate salmon (50 %) followed by mackerel and cod (35 %) (Figure 18). Only two children ate herring and one ate tuna fish. Roe, a common spread in Norway was used by 12 % of the fish eating children. As seen from the figure 11 the most common fish spreads in Norway roe and mackerel, even if used in very small amounts, contributed to twice as much iodine as salmon. Cod was by far the largest contributor of iodine among fish eaters.

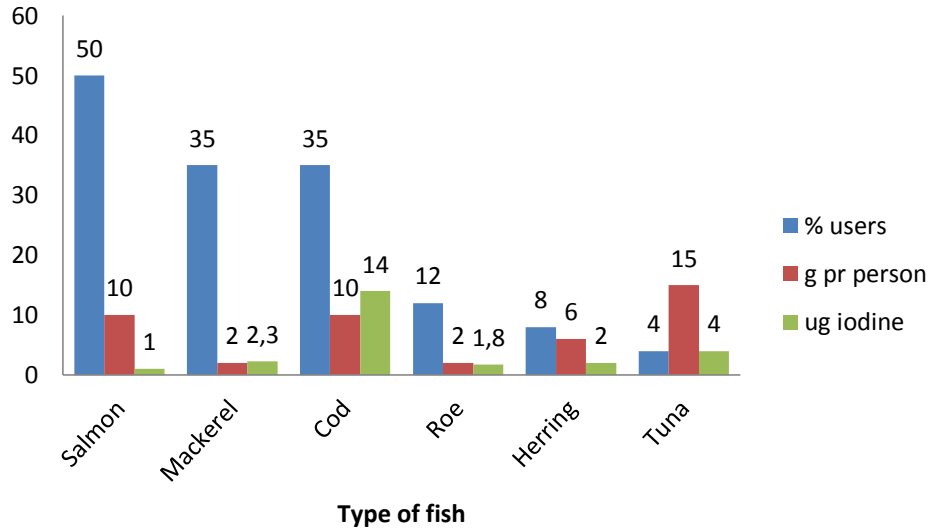


Figure 18 Prevalence of types of fish, median grams per day and ug iodine from the fish source in fish eating children (n=24)

.

The usage and avoidance of salt in the diet is shown in table 22. 56 % of the parents reported avoidance of salt in their child's diet and 75 % never added salt while cooking for the child. Median salt intake was 0.6 g per day. There is no recommendation for sodium and salt intake for children under two years of age, but the intake is well below the NNR of maximum 3-4 g in children two to nine years of age. The most commonly used salt type was sea salt (2 ug iodine per g) which is lower in iodine than the most common iodized salt in Norway (Jozo, 5 ug iodine per g).

Table 22 Prevalence of salt usage and median intake of salt and sodium (n=53)

	%
Avoiding salt	56
Adding salt in cooking	
3-6 per week	8
1-2 per week	8
< 1 per week	9
Never	75
Type of salt	
Sea salt	17
Seltin	4
Iodized salt (Jozo)	4

Median intake per day	amount
Salt g	0.6 (0.2-1,1)
Na mg	258 (135,7-499)

Inter quartile range (25th percentile to 75th percentile)
Na = Sodium

4.3.5 Associations between UIC and dietary factors.

Spearman's rho correlation coefficient was computed to assess the relationship between UIC in ug/L and iodine related dietary factors (Table 23). There was a significant positive correlation between the intake of enriched baby cereal and UIC. UIC was also positively and significantly correlated with the percentage of recommended daily intake (RDI), based on NNR, for iodine from diet. There were positive associations between UIC and energy intake per kg, micrograms of iodine from the diet and grams of hypo allergenic formula, however these associations were not significant.

Table 23 Correlation between dietary variables and urinary iodine content (UIC)

	UIC ug/L	p-value
Kcal/kg	0.230	ns
ug I from diet	0.267	ns
% of RDI for iodine	0.313	0.03*
g Hypo allergenic formula	0.212	ns
g Enriched baby cereals	0.340	0.02*
g Fish	-0.041	ns
g Fruit/vegetables	0.049	ns

*Significant using Spearman's rho correlation
UIC = urinary iodine content RDI = recommended daily intake (NNR)

4.3.6 Differences between genders in relation to iodine status

There were differences between the genders and iodine status (Table 24). Boys had significantly higher MUIC than girls. There was a significant difference in intake of enriched baby cereals between the genders, with boys eating significantly more of this food source than girls. There were no significant differences in median age, number of breast feedings or intake of hypo allergenic formula between groups.

Table 24 Differences in median urinary iodine content, age, number of breastfeeding, and iodine related dietary factors between the genders in the MILKID study

	Boys		Girls		p-value
MUIC, ug/L	171,5	(108-254)	111	(70-197)	0.02*
Age, months	8	(7-11)	9	(6-12)	ns
Number of breastfeeding per day	4	(4-7)	7	(4-7)	ns
g enriched baby cereals	30,5	(0-70)	0	(0-22)	0.03*
g Hypoallergenic formula	19	(0-49)	24	(3-71)	ns

*Significant using the Mann Whitney U test
Interquartile range (25th percentile to 75th percentile)
MUIC = median urinary iodine content

4.4 Growth in the MILKID study population.

4.4.1 Anthropometry in relation to gender.

Anthropometric measurements for boys and girls at birth and at inclusion are shown in table 25. Although the girls were slightly shorter and lighter than the boys, there were no significant difference in WAZ and LAZ at birth. Girls had significantly lower z-scores than boys for head circumference at birth. There were no differences between the genders in z-scores for anthropometric measurement at inclusion. For head circumference there was an increase of almost one standard deviation for z-score in girls from birth to inclusion, while the change was smaller for boys. Both genders had normal head sizes at inclusion (Table 25).

Table 25 Differences in anthropometric measures according to gender (mean)

	Boys n=31		**Girls n=26**		**p-value**
At birth					
Weight	3466	(547)	3283	(340)	
Length	51	(1.86)	50	(2.26)	
Head circumference	35	(0.88)	34	(1.47)	
WAZ	-0.39	(1.05)	-0.74	(0.69)	ns
LAZ	0.25	(0.82)	-0.15	(1.06)	ns
z-score head circumference	0.02	(0.68)	-0.91	(1.15)	0.05*
At inclusion					
Weight	9033	(1758)	8300	(1614)	
Length	73	(6.3)	72	(7.1)	
Head circumference	46	(2.2)	44	(2.1)	
WAZ	-0.46	(1.13)	-0.61	(0.79)	ns
LAZ	-0.35	(1.08)	-0.20	(0.73)	ns
z-score head circumference	0.22	(1.00)	-0.08	(0.94)	ns
BMIZ	-0.38	(1.18)	-0.72	(1.14)	ns
z-score w/l	-0.31	(1.14)	-0.69	(1.12)	ns

*significant using the independent sample t-test
WAZ = z-score weight for age, LAZ = z-score length for age, BMIZ = z-score BMI for age
w/l = weight for length, BMI = body mass index

The change in LAZ from birth to inclusion is shown in figure 19. There was very little change in LAZ for girls between birth and inclusion. For boys there was a significant drop in LAZ, indicating that growth in length in boys was negatively affected in this group of children.

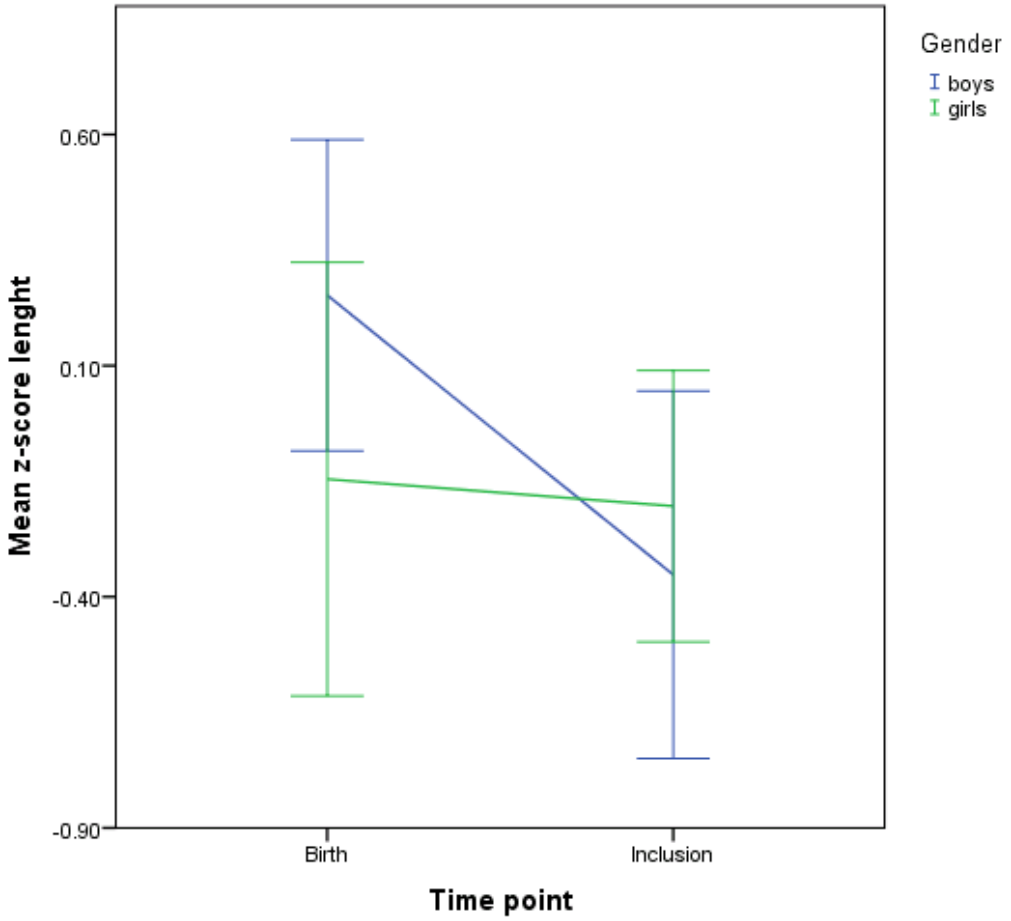


Figure 19 Changes in mean z-score for length for age from birth to inclusion in boys and girls

Wilcoxon signed rank test significant for boys, p=0.02
Error bars of 95 % confidence interval

4.4.2 Distribution of growth in the study population

Figure 20 a - d shows the distribution of WAZ and LAZ at birth and at inclusion for all the children in the study. The parameters were normally distributed. The curves for WAZ birth and WAZ and LAZ at inclusion were all shifted to the left indicating that the study population was slimmer than the normal population and had slower longitudinal growth.

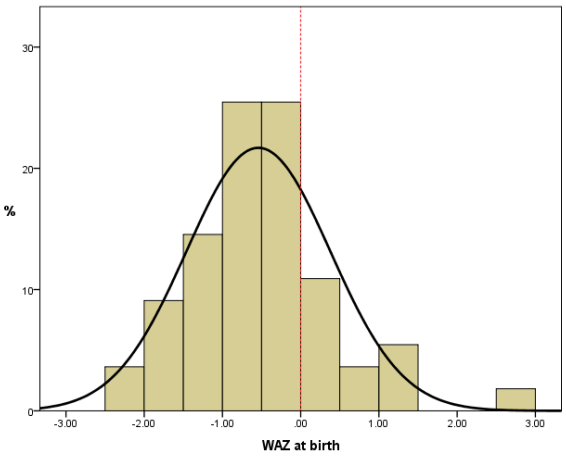


Figure 20a Distribution of WAZ at birth (n=55)

WAS = z-score for weight
Red dotted line indicates the peak of the distribution of the Norwegian growth standard

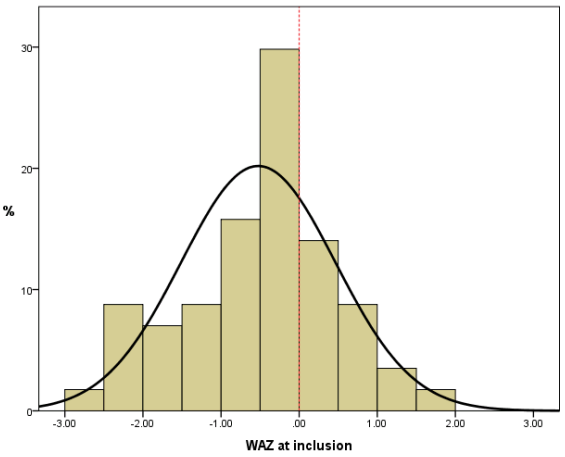


Figure 20b Distribution of WAZ at Inclusion (n=57)
WAS = z-score for weight
Red dotted line indicates the peak of the distribution of the Norwegian growth standard

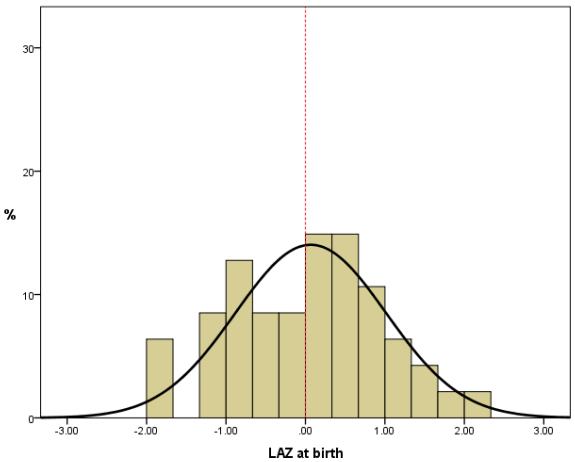


Figure 20c Distribution of LAZ at birth

LAZ = z-score for length
Red dotted line indicates the peak of the distribution of the Norwegian growth standard

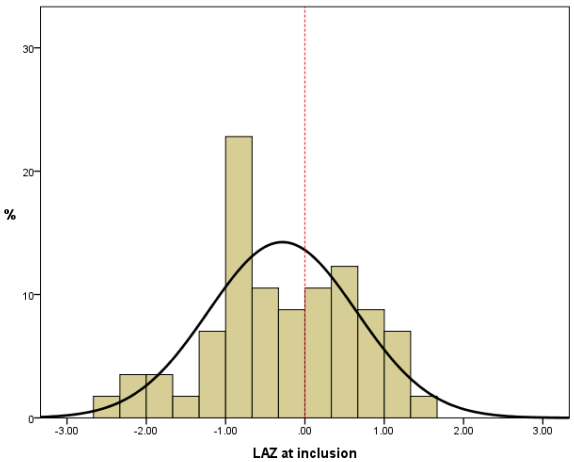


Figure 20d Distribution of LAZ at inclusion (n=57)
LAZ = z-score for length
Red dotted line indicates the peak of the distribution of the Norwegian growth standard

Distribution of BMIZ at inclusion is illustrated in figure 21. The curve was shifted to the left indication that the study population was slimmer with a lower BMI than the normal population.

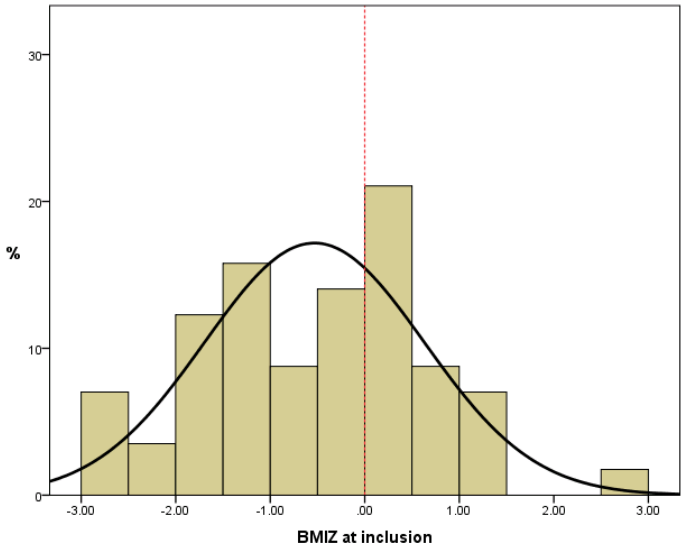


Figure 21 Distribution of BMIZ at inclusion (n=57)
BMIZ = z-score for BMI for age
BMI = body mass index
Red dotted line indicates the peak of the distribution of the Norwegian growth standard

4.4.3 Prevalence of malnutrition

The prevalence of WAZ, LAZ and BMIZ at inclusion is shown in table 26. 10.5 % of the children were underweight with a WAZ of ≤ - 2 and 10.5 % were wasted with a BMIZ of ≤ - 2. The proportion of children that were stunted, that is a LAZ of ≤ - 2, was 5.3 %. There were no children with WAZ or LAZ ≥ 2, however 1.8 % of the children had a BMIZ ≥ 2 indicating overweight.

When tested against the z-scores of the Norwegian growth standard the children in the MILKID study had significantly lower WAZ ($p<0.001$), LAZ ($p = 0.025$) and BMIZ ($p=0.001$) using the one sample t-test.

Table 26 Prevalence of z-scores ≤ -2 and ≥ 2 at inclusion in the MLKID study (n=57)

	≤-2	Normal	≥ 2
WAZ	10.5	89.5	0
LAZ	5.3	94.7	0
BMIZ	10.5	87.7	1.8

WAZ = z-score weight for age, LAZ = z-score length for age,
BMIZ = z-score BMI for age
BMI = body mass index for age

4.4.4 Factors affecting growth

Correlation coefficients between total number of symptoms, symptoms from different organ systems, feeding problems, number of foods avoided and weeks on diet and WAZ, LAZ and BMIZ at inclusion are shown in table 27. There were significant negative correlations between WAZ and BMIZ and number of general and subjective symptoms, and a significant negative correlation between BMIZ and total number of feeding problems. A significant positive association was found between WAZ and BMIZ and number of weeks on the cow's milk protein free diet.

Table 27 Correlations between disease factors and z-scores for growth at inclusion in the MILKID study

Correlation	WAZ	LAZ	BMIZ	p-value
Total symptoms	-0.191	-0.14	-0.205	ns
GI symptoms	0.023	0.015	0.077	ns
General/Subjective symptoms	-0.344*	-0.159	-0.357*	0.01, 0.008
Respiratory symptoms	-0.055	0.139	-0.110	ns
Total feeding problems	-0.101	0.127	-0.270*	0.05
Number of foods avoided	0.178	0.106	0.113	ns
Number of weeks on diet	0.284*	0.116	0.294*	0.04,0.03

*Significant using Spearman's rho correlation
WAZ = z-score weight for age, LAZ = z-score length for age, BMIZ = z-score BMI for age
BMI = body mass index, GI = gastrointestinal

In table 28 the difference in median z-scores between those with and without skin symptoms as well as food refusal as a symptom of CMPA is show. Skin symptoms did not negatively affect z-scores as the values were lower in the children who did not have eczema. In contrast children with food refusal as a symptom of CMPA had significantly lower z-scores for weight and BMI for age than those who did not refuse foods.

Table 28 Mean WAZ, LAZ and BMIZ at inclusion in subjects with and without skin symptoms and food refusal in the MILKID study

	WAZ	LAZ	BMIZ	p-value
Skin symptoms				
Yes	-0.39	-0.11	-0.47	ns
No	-0.58	-0.44	-0.51	
Food refusal (as a symptom of CMPA)				
Yes	-0.97*	-0.44	-1.04*	0.009, 0.01
No	-0.24	-0.21	-0.20	

*Significant using the independent sample t-test
WAZ = z-score weight for age, LAZ = z-score length for age, BMIZ = z-score BMI for age
BMI = body mass index
CMPA = Cow's milk protein allergy

Differences in WAZ, LAZ and BMIZ in subjects with and without different feeding difficulties are shown in table 29. Children with poor appetite had significantly lower WAZ and BMIZ. Children who were selective eater had lower BMIZ, however not significantly lower than the rest of the group. The same was true for refusing breast or bottle and BMIZ. We found no difference in WAZ, LAZ or BMIZ in subjects with and without weaning problems, chocking/swallowing problems and feeding difficulties in general.

Table 29 Differences in mean WAZ, LAZ and BMIZ at inclusion in subjects with and without feeding problems in the MILKID study				
	WAZ	LAZ	BMIZ	p
Feeding problems				
Yes	-0.58	-0.27	-0.65	ns
No	-0.33	-0.44	0.07	
Poor appetite				
Yes	-0.92*	-0.40	-1.00*	0.03, 0.02
No	-0.30	-0.28	-0.22	
Selective eater				
Yes	-0.75	0.12	-1.28	ns
No	-0.47	-0.38	-0.38	
Weaning problems				
Yes	-0.34	-0.35	0.26	ns
No	-0.53	-0.32	-0.53	
Chocking/Swallowing problems				
Yes	-0.58	-0.23	-0.75	ns
No	-0.49	-0.33	-0.42	
Refusing breast/bottle				
Yes	-0.57	0.05	-1.10	ns
No	-0.49	-0.39	-0.35	

*Significant using the independent sample t-test
WAZ = z-score weight for age, LAZ = z-score length for age, BMIZ = z-score BMI for age
BMI = body mass index

There were negative associations between urinary iodine content and WAZ, LAZ and BMIZ, however these were not significant correlations (Table 30).

Table 30 Correlation between z-scores for growth and urinary iodine content (UIC) in the MILKID study		
	UIC ug/L	**p-value**
WAZ	-0.188	ns
LAZ	-0.209	ns
BMIZ	-0.174	ns

Spearman's rho correlation
WAZ = z-score weight for age, LAZ = z-score length for age, BMIZ = z-score BMI for age
BMI = body mass index
UIC = urinary iodine content

5 Discussion

This study is the first to present iodine status in infants and children on cow's milk protein diets. One third of the study population was found to have urinary iodine content (UIC) under the recommended level and particularly infants that were mainly breastfed had a high prevalence of low UIC. Iodine status seemed to correlate positively with the intake of enriched baby cereals. The cow's milk allergic children tended to grow slower and be slimmer compared to the Norwegian growth standards. Boys had significantly reduced growth in length from birth to inclusion in the study. The prevalence of malnutrition was high and more than ten percent of the children had a z-score of less than two for weight and BMI for age, and this was associated with food refusal and poor appetite.

5.1 Methodological considerations

Dietary assessment methods are prone to over- and underestimation of true intake. The food record method has been shown to underestimate the energy intake in young children compared to energy expenditure measured with doubly-labelled water method (93). The length of observation is affected by within and between person variations. Ideally, observations over days, weeks or months are preferred. However, this would put a great burden on the respondent and shorter methods are therefore employed. The number of days needed to measure nutrient intake in groups of individuals is a function of the ratio of variation within individuals to the variation between individuals. The smaller the ratio is the fewer days are needed. The accuracy needed and the variability of the nutrient also plays a role when drawing conclusion about the optimal number of replicates needed. The ratio of within-subject variance to between-person variance was under one in a study of Finnish one year olds for most nutrients, and the ratio increased with increasing age of the child (101). This could in part be explained that for young children, like in the present study, the day to day variability in the diet is low. Estimates of energy intake was found in one study to require four to five days of recording in children six to 24 months, while another study found that two days were enough to get a good estimate (94, 101). For iodine intake, Erkkola found that two days were needed in children one year of age (101). In comparison 12 days was needed for 6 year old girls (101). For these reasons we chose 3 day food record for estimation of iodine intake in the MILKID study.

The parents and carers, e.g. nursery staff, are required to fill in the record for the child, and the more people caring for the child the higher the risk of bias. In addition changing the food habits during the registration is possible problems of the method. These factors could adversely affect the statistical precision and accuracy of intake data in our study (94, 102).In addition, parents and carers need to be educated in how to record the food by using household measurements. Using scales would have made the estimates more exact however, this is more time consuming and could lead to higher drop-out or underreporting (24).

The intake of iodine in the MILKID study had to a large extent to be calculated manually by using data of median iodine content in common foods in Norway. There is a risk that the iodine values for some of the products are not correct. In addition there is an increased risk of human error when calculations are done manually. However, the iodine content of most of the food manually calculated, such as fruit, vegetables, bread and meat is not high and the effect on the estimation of iodine intake is expected to be small.

The data on dietary intake of breastfed children, whether mainly or partially, is incomplete because we did not record the amount of breastmilk consumed and because of limited resources we could not analyse the nutrient content in breastmilk. This affects the results of the two breastfed groups and must be taken into account when interpreting our findings. The fact that we do not have data on the contribution of iodine from breastmilk does make it difficult to draw firm conclusions regarding the iodine intake of the breastfed infants.

Iodine in urine varies from day to day and is subject to variations in both diet and hydration (34, 40). This could bias the results of this study in that the number of subjects or spot urine samples may have been too low to even out such variations. Correction for hydration by adjusting for urine creatinine excretion could have been a solution. However, the cut off levels from the WHO is based on uncorrected levels, and investigators value of use of this method in infants (34). In addition we did not include a control group. The findings of urinary iodine status and iodine intake in this group could be similar to the normal population and not an effect of cow's milk protein free diet. However, our main interest was to find the status of this group of children and whether they were at risk of insufficiency, which would be important to address even if the same is found in a control group of children without CMPA. The iodine content of breastmilk and estimation of breastmilk intake would have strengthened the study, however due to financial constraints and increased burden on the participants we did not include such measurements.

Measurements of length, weight and head circumference were performed by several people, although most children were measured by one of the three research investigators (JK, ME, RAT) at inclusion. Between observer errors have been measured in other studies of growth at a magnitude of 0.56 cm for length and 0.4 cm for head circumference. Ideally one observer should be used, however clear instructions on how to do the measurements could reduce the risk of errors (89). The measurements for anthropometry at birth will always be subject to the risk of between observer errors.

The growth data are compared to the Norwegian growth standards and not a control group of same age and sex. When comparing of z-scores of a study population with the growth standards of a normal population, it is important to remember that these standards may not represent the true distribution in the normal population. This is because growth standards and curves are subjected to methods of curve-fitting and smoothing to derive related cut-off points for anthropometric measures (89, 91). This could lead to wrong conclusions being drawn in relation to growth. The authors of the Norwegian growth standard found that the normal population was in fact heavier than the adjusted curve (89) and so we may have underestimated the differences in WAZ and BMIZ of the MILKID study group and the normal population.

The study design has limitations. This study was a cross sectional study, which can be used to describe relationship between factors but not to establish causality and conclusions regarding the cause of a relationship cannot be drawn (103). Some of the data was collected retrospectively and could be subject to re-call bias.

5.2 Recruitment:

Children in this study were recruited at a tertiary health care center (Children's clinic, OUS). The majority of the participants were referred on from their general practitioner, and a few from private pediatricians. Patients with suspected or known cow`s milk allergy are routinely referred to our clinic from the general practitioners because the diagnosis and treatment of cow`s milk allergy in small children in Norway generally is judged as a specialist responsibility, not at least to secure adequate dietary advice in cooperation with the dietitians at the pediatric clinic. Thus even though the study was in a tertiary health care center, this may not imply any significant selection of more severely affected patients. Most parents had received dietary advice from a dietitian at a group session on CMP-free diet and had therefore been given information targeted at reducing the risk of nutritional

deficiencies of the exclusion of milk and dairy products. Even so, the participation rate was high with 70 % of eligible subjects included in the study and there is a high probability that the study group is representative of the cow's milk protein allergic (CMPA) population in our center (Figure 7).

5.3 Descriptive characteristics and nutritional intake

5.3.1 Descriptive characteristics

Median age of the children was nine months (Table 9) and is in accordance with studies of the natural history of food allergies which find that the prevalence peaks at one year of age. CMPA have been found to develop within the first year of life and with a prevalence in young children of 2-3 % (23). The children in this study were recruited at various stages of their allergy; as newly diagnosed and at follow up visits. Even so we found that 61 % of the children were between six and 12 months.

Boys were a little overrepresented compared to girls at 54 % and a little more than the national birth register of 51 % boys, however it was exactly the same as the number in the latest infant feeding report from Norway (104, 105). Mean birth weight and length were 3387 g and 50.6 cm respectively, both close to the birth weight and length of the general population (Table 9).

The median maternal age was 33 years (Table 9). This is higher the national average age (28.7 years) of women delivering (104) and also with the age (29.6 years) of the participants in the Norwegian Mother and Child Cohort Study (106). Almost 90% of the patients' mothers had higher education, such as college/university degree at lower or higher level. This is higher than found in the latest infant feeding report of 70 % with higher education (105), and could potentially mean this parent group may not be representative for the Norwegian parents of cow's milk allergic infants in general. The mothers were mostly of Scandinavian origin, 84 %, and only 16 % were from other European or non-European countries, which is close to the findings in the infant feeding report of 78 % with Norwegian origin (105).

One of the inclusion criteria was that the child had to have followed a cow's milk protein free diet for a minimum of three weeks. The reason for this was that we wanted to evaluate dietary intake and analyze the effects of CMP exclusion and not the effects of CMPA and the time from elimination of a food allergen to symptom improvement has been found to occur within four weeks in 98 % of patients (107). The median time on diet was 17 weeks, ranging from four weeks to 84 weeks (Table 10). Most of the children had in other words followed the diet long enough to expect almost full recovery of their CMPA symptoms if the exclusion was extensive enough.

There was a large lag time between diagnosis of CMPA and dietary counselling with the families having to wait a median of 13 weeks from diagnosis of CMPA to dietary counselling (Table 10). The milk-free diet group session is a low threshold offer that runs every three weeks and the delay of referral to this group was somewhat surprising. Prolonged waiting period before nutritional advice could potentially put the CMPA patient at risk of nutritional deficiencies and inadequate treatment of symptoms. Dietary advice from a nutritional expert such as a dietitian is crucial to ensure the exclusion diet is safe and nutritionally complete (6, 19, 108). The long delay before dietary advice for some of these children could possibly result in malnutrition and affect growth, as well as increase the risk of food aversions and feeding difficulties.

5.3.2 Symptoms, food avoidance and feeding difficulties:

The children experienced a large number of diverse symptoms of CMPA (table 11). 42 % had five or more symptoms of their food allergy, most commonly from two organ systems or more (table 11). Almost all children had symptoms from the GI tract with a median of three symptoms from this organ system (Table 12). Studies of food allergies and especially CMPA have found a multitude of symptoms in each subject. The involvement of several organ systems is not uncommon. According to the literature manifestations mainly occur at the level of the digestive tract (50%-60%), the skin (50%-60%), and the respiratory tract (20%-30%) (109). In a cohort of 119 infants with CMPA, Dupont et al found that 54% had skin reactions; 34% had gastrointestinal reactions; 44% had subjective symptoms, such as crying and irritability; and 3 % had airway reactions (110).

In the MILKID study, all, except for one child, had symptoms from the GI tract with more than two thirds of the subjects affected by colic (70 %) and reflux (67 %) and over half of the children reported to have had loose stools. Bloody stools, a typical symptom of proctocolitis, were a problem in 44 % of the children. Obstipation was seen in 26 % of children (Table 11). This is comparable with the prevalence of symptoms found in a large retrospective study of gastro intestinal symptoms of food allergy in a tertiary paediatric gastroenterology unit in Britain (111). As in the MILKID study group the prevalence of abdominal pain was high with 89 % of the children affected, 57 % had vomiting and 39 % rectal bleeding or bloody stools, which is close to what we found in our study. The prevalence of diarrhea at 80 % was much higher than what we found and they also report a higher prevalence of constipation (45 %) was reported. This could in part be due to differences in methodology and sampling. We included only children less than two years of age. The age group in the British study was not reported, however they did not exclude cases because of age. Gastro intestinal allergic symptoms are, in contrast to other symptoms of food allergy, found in all age groups and this could

potentially explain the difference in the numbers affected by e.g. constipation which is more often seen in older infants and children (112).

Eczema often coexists with gastrointestinal symptoms of CMPA. Eczema, or skin symptoms, was reported in 46 % of the children in our study (Table 11). This is similar to the findings in other studies of CMPA and gastrointestinal food allergy in children. Dupont et al found an incidence of 54 % of eczema in a study of CMPA while Meyer et al found an incidence of 41 % of coexisting eczema in children with gastro-intestinal manifestations of food allergy (110, 111). A review of CMPA reported a prevalence of 50-60 % of skin symptoms of CMPA (109). In a study focusing on children with atopic dermatitis 42 % of children had gastrointestinal symptoms (113).

Symptoms from the respiratory tract were few in the present study. Only one child had had an anaphylactic reaction to milk protein and six percent were affected by recurrent otitis media (Table 11). Wheezing or breathing problems were not reported in any of the subjects. Symptoms from the respiratory tract were reported to be 20-30 % in a review of studies of CMPA by Høst (109). In a smaller study and more selected group of infants with CMPA, 2.5% had airway reactions (110). A British study found asthma in 32 % of children with GI food allergy (111). The lack of respiratory symptoms could be due to the type of paediatric specialist through which the children were recruited from at our clinic, with a dominance of referrals of patients mediated by the paediatric gastroenterologists compared to those mediated by paediatric pulmonologists and paediatric allergists. Asthma is often found at a higher rate in older children, and this could also explain the lack of respiratory symptoms in our study (13). In addition, the wording of the questionnaire used to identify symptoms and the fact that symptoms of rhinitis were not included, which would be expected to be found in some cases in this age group of CMPA children, could explain the lack of symptoms.

Parents in the MILKID study reported a high rate of general and subjective symptoms like sleep disturbance, growth failure and feeding difficulties (Table 11). These symptoms are difficult to associate to a specific organ system, are often diffuse and may delay diagnosis (10). In this study 38 % of the children were reported to have faltering growth. Feeding difficulties are also well documented in the literature of food allergic children (114, 115) and 70 % of the parents reported problems related to feeding, with poor appetite as the most frequent problem (Figure 10). Food refusal was a symptom of CMPA in 35 % of the subjects in the MILKID study (Table 11). Feeding difficulties and growth failure often co-exist and will be discussed in detail later in this thesis.

The CMP-free diet's effect on improvement of symptoms was high with 89 % of the children having either full or partial improvement of symptoms (Figure 8). Only two percent reported no improvement in symptoms and in nine percent of the cases the parents were unsure in there was an improvement after initiating diet restriction. Failure of improvement of symptoms in this age group could be due to continued reactions to the extensively hydrolyzed formula and the need for an amino acid formula (116). The failure of response could also be because the diagnosis was not right or that the child had additional food allergy that was not diagnosed.

The concurrence of other food allergies with cow's milk allergy is common. Data on foods excluded from the child's diet in addition to CMP in the MILKID study was collected by a questionnaire. CMP alone was excluded in 28 % of the subjects, while 44 % excluded four of more foods (Table 13). The most common foods the children avoided were nuts (tree nuts and peanuts) with 65 % of the children avoiding this food (Figure 9). This is probably not because of allergy to nuts but rather avoidance due to the fear of chocking. The parents were not asked to explain why they excluded different foods and this has probably affected the result. In 39 % and 37 % of the children eggs and soy were avoided, respectively, and the prevalence for fish, citrus and legumes was 22 %. Only four percent excluded gluten. This is different from what was found in a British study where six percent avoided cow's milk protein alone, 15 % avoided soy together with CMP and 25 % avoided CMP, soy and egg (111). In a study by Bishop et al of Australian children with CMPA 58 % were found to have allergy to egg, 47 % to soy and 35 % to peanut (117). The difference between these finding and the findings of our study could be due to the fact that our children were younger and that a large proportion of the children may not yet have been introduced to these foods. Dietary elimination places a child at risk of malnutrition and cow's milk, soy, egg and wheat contribute significantly to nutrient intake (118). It is therefore important to introduce foods that are unnecessarily excluded. Excluding a large number of foods puts the child at greater risk of malnutrition and strict diets could affect growth and development in the child. Unnecessary avoidance of foods at an early age, may not only lead to nutritional deficiencies, but also an increase in feeding difficulties according to the literature (108, 118). As mentioned earlier, feeding difficulties were reported in 70 % of the children in the MILKID study and selective eating and weaning problems were reported in 11 and 13 %, respectively (Figure 10). In comparison, the prevalence of feeding problems in the infant feeding study of 12 month old children was 16 %, and selective eating and weaning problems were reported in 5 % and 4 %, respectively (20). In a study of children with GI food allergy 30-40 % were found to have feeding problems (114).

5.3.3 Diet and nutrition in the MILKID study

The median time for introduction of solids and fluids other than breastmilk was four months (Table 14). That is close to the mean age of 4,5 months for introduction of solids reported in the latest survey of infant feeding by the Norwegian health authorities (105). 58 % were still breastfed at this age and this is similar to what was found in the Norwegian infant feeding survey of children 12 months old where 56 % of the children were breastfed at the age of 10 months (20). In our study only two percent had never been breastfed and this is also in concordance with the infant feeding survey. Most of the children were fed solid foods with 25 % having a combination of breastmilk, hypo allergenic formula and complementary feeding, 29 % breastmilk and complementary feeding and 38 % complementary foods and hypo allergenic formula (Figure 11). Only two percent were exclusively breastfed, and two percent were breastfed together with hypoallergenic formula. Four percent were complementary fed without a breast- or formula milk. In other words, 65 % of the infants and children received formula milk. In the national infant feeding survey 62 % of the children were introduced to formula or other types of milk at nine months of age (105). Median frequency of breastfeeding was 3-5 times per day among the breastfed children with 53 % of children being breastfed at this frequency (Figure 12). This is comparable to the mean frequency of 3,5 breastfeeding per day found in the infant feeding survey of 12 month old infants in Norway (20).

We divided the children into groups according to feeding pattern; mainly breastfed, partially breastfed and weaned. 25 % of the children were mainly breastfed, 33 % were partially breastfed and 43 % were weaned (table 15). There was a significant difference in age between the three groups with the mainly breastfed being significantly younger with a median age of seven months compared to the two other groups with a median age of ten and 11 months, respectively. This is expected as exclusive breastfeeding is recommended until six months and gradual increase of complementary feeding is recommended in the second half of the first year of life (66). There was a significant difference in the frequency of breastfeeding between the mainly and partially breastfed groups which is only natural since the mainly breastfed children got most of their energy intake from breastmilk and hence needed more frequent breastfeeds (Figure 13). There were significant differences in the energy and protein provision from complementary food and drinks between the three groups as expected (Table 18). The energy intake of the weaned children was high at 92 kcal/kg per day and protein intake was almost 3 g/kg/day. This is higher than the recommendations for the general population of 80 kcal/kg, however, the infant feeding study also found high energy intakes in weaned children at the age of 12 months (20).

The dietary survey reviled a high prevalence of fruit and vegetable users with 93 % and 85 % of the children being fed fruit and vegetables respectively (Figure 14), which similar to what was found in

the Norwegian infant feeding study of 98 % of children eating fruit and 90 % vegetables at 12 months of age (20). The median intake of fruit was generally high in all feeding groups (Table 16). However, the partially breastfed ate almost double the amount of fruit of the two other groups, and there was a significant difference between the groups in fruit intake. The amount of fruit eaten by the partially breastfed group (121g) was comparable to what was found in the infant feeding study of 12 month old children of 135 g of fruit per day. The partially breastfed children ate more vegetables than the other feeding groups, and again the partially breastfed children's intake was close to findings of the infant feeding study of 60 g of vegetables per day (20). Red meat was eaten by 70 % of the children with the weaned and partially breastfed children eating more red meat than the mainly breastfed group and the difference was significant. The difference between the three groups for intake of vegetables and soy/oat products were also significant. The median intake of eggs, poultry and soy/oat products was generally low in all three groups (Table 16).

Enriched baby cereals were used by 56 % of the children (Figure 14). This differed from the infant feeding study where 82 % of children ate enriched baby cereals at 12 months of age (20). There was no significant difference in median intake between the three feeding groups, with the mainly breastfed eating a median of 13 g (57 ml of prepared product), the partially breastfed 23 g (100 ml of prepared product) and the weaned group 4 g (17 ml of prepared product) per day (Table 16). The intake in all groups were much lower than the 225 ml per day found in the infant feeding study (20). The differences in age between our study and the infant feeding study could explain some of the difference as the MILKID study included children from two months to two years while the infant feeding study looked at the intake when the children were 12 months. However the difference is so large that it is safe to say that the intake of enriched baby cereals was low in the MILKID study population. Part of the explanation for such a low intake could be that there exists only one brand of dairy free enriched baby cereal on the Norwegian marked at present. A few children were given CMP-free enriched baby cereal bought in Sweden. The alternatives to enriched cereal are non-enriched cereals bought in so called health stores. The intake of this type of cereal was generally low in our study population (table 16).

We found that 44 % of the children in our group included fish and fish products in their diet (Figure 14). This is much lower than the findings in a national survey of Norwegian 12 month old children where 80 % where consuming fish and fish products (20). Our survey did include some very young children of under six months of age, however the majority where older and at an age where fish could well be consumed. We have no data on potential allergy to fish, and though none of the families listed fish as an allergen when asked about concurring food allergies, fish was avoided by 22 % of the children (Figure 9). It may be that the families are avoiding fish as a precaution rather than

because of an actual allergy. The intake of fish was a median of 0-8 g per day and the difference between feeding groups was significant with the partially breastfed children eating more than the two other groups (Table 16). Comparatively, the intake of fish was found to be 13 g per day at 12 months in the infant feeding study (20). The intake of fish is discussed further in the iodine section of this discussion.

A hypoallergenic formula (HF) was used by 69 % of the children (Figure 14) and the weaned children were drinking more than the two breastfed groups, with a median intake of 63 g per day (i.e. 420 ml) compared to a median intake 20 g in the partially breastfed (i.e. 130 ml) and 0 g in the mainly breastfed (Table 16). The difference between the feeding groups in intake of HF was significant. Most of the children, 62 %, were treated with an extensively hydrolyzed formula (eHF) while 38 % used an amino acid formula (AA) (Table 17). It is known that in a subset of children with CMPA, eHF is not tolerated (116). The frequency varies between 2- 18 %, and definition of intolerance to eHF in these studies are often lacking and the explanation for treatment failure is not clear. Petrus et al observed in the course of the Dutch EuroPrevall Birth Cohort Study that more infants with incomplete symptom resolution were receiving eHF than expected from the literature. In their study of 49 children with confirmed CMPA, treatment failure with eHF was found in 51 %. Median time on eHF before switching to an amino acid formula was four weeks. The authors found “gastro intestinal discomfort” to be the only predictor of incomplete resolution of symptoms on eHF. The explanation could be that there is still cow’s milk protein epitopes present in eHF while an AA formula consist of a pure amino acid mixture not derived from CMP and is therefore considered non-allergenic (116). The choice of formula could be a sign of severity of the problem. In a British study of gastro intestinal food allergic children, Meyer et al found that 43 % of the children were using hypoallergenic formula and of those 53 % were using an amino acid formula. They argued that this reflected the severity of patients seen in their center (111). Amino acid formulas are often used as a diagnostic tool in children with suspected CMPA, and there is a chance that the children in our study were not switched to a eHF when such a trial was completed, thereby explaining the high number of AA formula users in our study group. As our children had mainly gastro intestinal symptoms they are comparable to the children found to fail treatment in the Petrus study and the high number of AA users would then be expected and highlights the severity of patients treated in our center.

5.4 Iodine: status, nutrition and factors affecting iodine status

5.4.1 Iodine status of the MILKID study group

One third of the children (31 %) in the MILKID study had a UIC value of under 100 ug/L (Figure 15), defined as insufficient iodine status, and are consequently at risk of the negative effects of mild to moderate iodine deficiencies (34, 51, 119). Iodine is required for the production of thyroid hormones which again affects myelination of the central nervous system. This myelination continues throughout childhood, particularly in areas affecting higher-order cognition and intelligence (40). With this in mind, identifying the risk factors for insufficient iodine status in this population is crucial. In turn it could provide health professionals caring for patients with CMPA with the tools to target dietary advice in order to prevent the detrimental effects of mild- to moderate iodine deficiencies in at risk patients.

MUIC of over 100 ug/L is suggestive of sufficient iodine status in populations of infants and children under the age of two years according to the WHO (Table 3) (54). The MUIC in the MILKID study was 158.8 ug/L and indicative of sufficient iodine status in this group of children less than two years of age avoiding cow's milk protein. Both sufficient and insufficient status has been found in studies of infants and toddlers from Europe and other countries in the developed world. Similar to our study sufficient iodine status was found in French children age ten months (181 ug/L) and two years (134 ug/L) (120), Belgian children six months to three years (101 ug/L) (121) and Swizz one year olds (103 ug/L) (71). Insufficient status was found in Swizz infants of six months (91 ug/L) (71) and infants and toddlers under two years of age in New Zealand (67 ug/L) (122). Switzerland, Belgium and Norway are countries considered to be iodine sufficient whereas New Zealand is known for insufficient status at least in some areas. The authors of the Belgian study suggested that MUIC cut for iodine sufficiency in young infants and children under three years of age should be raised to 180-225 ug/L. They argued that if the recommended intake of iodine is 90 ug per day, then UIC of an iodine replete child of less than three years, when urinary output is as low as 400-500 ml per day, would be 180 ug/L (i.e. 90 ug per 500 ml). In view of this they argued that their population of infants and children six months to three years old with a MUIC of 101 ug/L was in fact deficient in iodine (121). Our population had a MUIC of under 180 ug/L and would from Delange's point of view be insufficient. A group from Spain suggested from their findings in a study of iodine status in school children and IQ that the value for sufficiency should be raised to 150 ug/L as this level increased the IQ several points in their study population (51). The WHO and ICCIDD, however, did not change their reference values

in 2007 which was after the publications above and 100 ug/L still stands as the international cut-off between sufficiency and insufficiency of iodine in infants and children under two years of age (95).

5.4.2 Iodine status and feeding pattern:

We found significant differences in iodine status in the different groups of feeding pattern (Figure 16). MUIC in the mainly breastfed group was 85,8 ug/L and hence indicative of insufficient in iodine (Table 19). In contrast the partially breastfed and weaned children had sufficient status with a MUIC of 171.5 and 174.7 ug/L, respectively. The difference in MUIC between the mainly breastfed group and the two other feeding groups was not significant. 58 % of the children in the mainly breastfed group had a UIC of under 100 ug/L, while this was true for only 12 % of the partially breastfed and 32 % of the weaned children. The difference in prevalence between the groups was significant. This is in concordance with other studies of breastfed and weaned children and iodine status. Andersson et al. found in their study of Swizz weaning infants that breastfeeding was associated with lower UIC of 82 ug/L compared to 105 ug/L in the formula fed children (71), and Skeaff et al. found significantly lower iodine concentration in breastfeeding infants (44 ug/L) compared to formula fed (99 ug/L) in New Zealand (122).

The breastfeeding infant is dependent on the content of iodine in breast milk, which in turn is dependent on the breastfeeding mothers' iodine status. A summary of studies from Europe found that the countries with the lowest breast milk iodine concentrations also had low iodine values in breastfed infants' urine (123). In breastfeeding mothers a UIC of 100 ug/L is regarded as sufficient to ensure iodine status for both mother and child. A recent study by Seldal and co-workers of 76 Norwegian breastfeeding women, three months postpartum, found a MUIC of 60 ug/L which is considerably lower than the recommended value of 100 ug/L, and 76 % of these women had a UIC below 100 ug/L (124). This indicates that only one quarter reached the recommended dietary intake (RDI) of iodine for lactating women. Research on iodine status in postpartum Norwegian women is limited and this is the only study on iodine status in lactating Norwegian women. The breastmilk content of iodine was not measured. Reports on correlations between maternal UIC and breastmilk iodine content are inconsistent (125). In areas of iodine sufficiency, however, breast milk iodine concentration correlates with maternal urinary iodine level (75). There have been few studies of the iodine status of young women of childbearing age in Norway. A study 48 Norwegian women with school-aged children found a MUIC of 79.8 ug/L in the mothers, indicating mild deficiency in this population. In addition a significant correlation between UIC and intake of dairy products was seen (126). Brandsether et al found pregnant women in Norway to be iodine deficient with a MUIC of 69 ug/L (values less than 150 ug/L is defined as iodine deficiency in this group), and that not using iodine

containing supplements a long with low intake of milk and milk products, fish and egg were associated with suboptimal intake of iodine in the MoBA study (48).

UIC of infants and young children in Norway has, as far as we know, not been studied. The breastfed children in our group would in view of the above be at increased risk of iodine deficiency as their mothers are excluding all milk and dairy products, important sources of iodine, from their diet, as this is the treatment for the child's allergy. Mulrine and co-workers found that breastmilk iodine concentration decreased substantially in the first six months postpartum in a mildly deficient population (77). This could potentially put the young breastfed CMPA child further at risk of iodine deficiency since the Norwegian government, in line with the WHO, recommends exclusive breastfeeding until six months of age (66), and hence the iodine status of the child would solely depend on the mother's iodine intake and status during this period. Our study did find significant negative associations between breastfeeding and iodine status of the child. In light of the above findings of potentially mildly iodine deficiency in young women in Norway both during pregnancy and lactation (48, 124) and perhaps preconceptionally (126), the CMPA children is at exacerbated risk of the detrimental effects of mild- to moderate iodine deficiency during the vital first years of life from conception until the weaning diet is well established.

Maternal iodine supplementation during breastfeeding has been found to affect the iodine status of the infant and nursing mother as well as breastmilk content (77), however a few studies found no impact of iodine supplementation on breastmilk iodine content (75). Supplementing the mother rather than the child was found to be more effective in a study of Moroccan children (78). In our study, breastfed children of mothers who took supplements with iodine had higher MUIC (Table 20) however, this was not significant. The low number of children in each group, in addition to lack of data on amount of iodine in the supplement or frequency of supplementation could have affected the result. It seems warranted to advise breastfeeding mothers to take a supplement with iodine when restricting CMP.

5.4.3 Dietary iodine sources and iodine status:

The main iodine source in the MILKID study was hypoallergenic formula, which provided a mean of 42 % of the total iodine intake (Figure 17). Enriched baby cereals contributed to a mean of 27 %. This is to our knowledge the first study on the iodine sources of young infants and children in Norway. In a study of four year olds, Dahl et al found fish to account for 13 % and fruit and vegetables 5 % of total iodine intake respectively, while 70 % of the iodine in this age group came from dairy products (43). Studies of weaning infants have found formula milk, enriched baby foods (mainly cereals), and

breastmilk to be the main sources of iodine (69, 71). A study from Germany on iodine in complementary weaning foods found enriched baby cereals to yield appropriately high amounts of iodine, compared to other weaning foods and baby formulas (69). The authors commented that many of these products were added sugars. This is true for the only enriched milk protein free baby cereal on the Norwegian marked (Sinlac) and may lead to parents to abstain from feeding it to their child. In line with our results, the German group found that very few of the other commercial baby foods (fruit/vegetable purees and dinners) were fortified with iodine (69). In an estimate from New Zealand intake of infant formula dominated the iodine exposure of 6-12 month old infants followed by dairy products (70).

There were significant differences in the contribution of iodine from different food sources between the feeding groups. The weaned children received more iodine from formula than both groups of breastfed children and the difference was significant (table 21). As expected, total iodine intake from diet was significantly lower in the mainly breastfed group compared to the two other groups. Reports on actual iodine intake in infants and young children are generally missing from the scientific literature. A study of German toddlers (10-36 months) found low iodine intakes in all children and the intake of iodine fell with increasing age. The reduction in iodine intake was concurrent with a decrease in formula usage and the usage of fortified foods (127).

Andersson and co-authors reported that weaning infants were at risk of inadequate iodine status if not formula fed, and infants that were formula fed and had iodine fortified complementary foods were at lower risk (71). In a study from New Zealand, only percentage of energy from formula milk was significantly associated with UIC (122) and as shown above infant formula was the dominating source of iodine for infants in the New Zealand Total Diet Study (70). In our study group the intake of hypoallergenic formula was positively correlated with UIC (table 23), however the correlation did not reach statistical significance. There was neither significant association with percentage of energy from formula, nor the percentage of total iodine from formula and iodine status (results not shown).

Contrary to other studies of infants and iodine status, where infant formula is correlated with UIC, we found that the intake of enriched baby cereal correlated significantly and positively with UIC (table 23). Our findings could be a result of differences in weaning traditions. In Norway fortified baby cereals are a major part of the infant's diet and an important source of micronutrients. This is probably due to the traditionally low use of infant formula and high rate of breastfeeding during the first year of life.

Intake of fruit and vegetables only weakly correlated with UIC, and the intake of fish was surprisingly negatively, though not significantly, associated with iodine status. However, fish was only eaten by

44 % of the population, and the most common fish was salmon, eaten by 50 % of the fish eaters (Figure 18). Cod and mackerel were consumed by 35 % of fish eaters and roe by 12 %. Both mackerel and roe are common spreads in the Norwegian diet. The median intakes varied from 10 g for salmon and cod to 2 g for mackerel and roe. Salmon is not as good a source of iodine as cod, mackerel and roe and provides only 10 ug iodine per 100 g compared to 140 ug in 100 g of cod, and 75 ug per 100 g of mackerel and roe (43). The frequent use of salmon as a fish source could explain the negative correlation as the contribution from salmon was only a median of 1 ug per day in fish users (Figure 18). Low intake of sea fish was also found in a study of iodine in German pre-school children three to six years old, with less than 50 % of the population eating sea fish and hence fish contributed marginally to iodine supply (two percent of mean iodine intake). The investigators had previously found that fish significantly predicted iodine excretion in children 6-12 years old. The authors concluded that encouragement in fish consumption could effectively improve the children's iodine supply (128). The contribution of iodine from fish in Norwegian four year olds was found to be 13 % in a study by Dahl et al (43). As mentioned earlier, the intake of fish was only 13 g per day in the infant feeding study of 12 month old Norwegian children (20).

We tested for egg, chicken, bread, meat and oat products as well as the usage of salt in the diet but found no significant associations with UIC (results not shown). Andersson et al found that the MUIC of infants receiving some iodized salt in complementary foods was higher but not significantly different from the MUIC of infants not receiving such foods (71). 60 % of the children in their study were given foods with iodized salt, while in our study 56% of the parents avoided salt in their child's diet and 75 % never added salt while preparing food for their child (Table 22). A German study of toddlers found milk and iodized salt to account for 80 % of the estimated iodine supply (127). Salt could potentially be an important iodine source for CMPA children, however the general recommendation is to reduce salt in the diets of infants and children (66). In addition, amount of iodine in iodized salt in Norway is very low compared to that of other countries and would not be expected to contribute to significant amounts (129).

The bioavailability of iodine in a food source is important for uptake in the body and hence could affect the iodine status of an individual, especially if the food constitutes a major part of the diet. Iodine and iodate have high bioavailability and are found in human milk and cow's milk (130). In infant formula milk however, studies have found that more than half of the iodine is bound to high molecular weight organic molecules(131). The bioavailability of organic iodine, especially iodine associated with macromolecules, is low (130). The absorption of iodine from formulas could therefor potentially be low and hence explain the lack of significant correlation between the intake of formula and UIC in our population. We do not have data on the bioavailability of iodine in hypoallergenic

formulas and it may be that they differ from normal infant formulas and that the bioavailability in these types of formula is in fact high.

The RDI for iodine is dependent on age and differs between countries. The Norwegian RDI is based on NNR 2012 which states that children 6-12 months should receive 50 ug per day and children above 12 months 70 ug per day from the diet (Table 5). Due to the recommendation of exclusive breastfeeding until the age of six months, the Norwegian government has no recommendation for children under the age of six months (62). We found a significant correlation between the percentage of RDI for iodine from diet and UIC in the study population (Table 23).

Some studies have found differences between gender and UIC (127) which has been associated with differences in energy intake in boys than girls. We did find a difference between genders in our population (Table 24). The boys had a significantly higher MUIC (171.5 ug/L) compared to girls (111 ug/L). No difference was found in age, number of breastfeeding or intake of infant formula between the groups, however, the boys ate significantly more enriched baby cereal than the girls.

5.5 Growth

5.5.1 Growth in the MILKID study group

Poor growth is a concern in food allergic children. Growth failure could be due to the exclusion of foods important for the intake of macro- and micro nutrients which in turn leads to malnutrition and/or it could be caused by the allergic reaction itself because of an ongoing inflammation in the body, which could affect growth (108).

We found mean WAZ and LAZ at inclusion to be slightly lower than the normal population (z =-0.41 and z= -0.39 respectively) (Table 9) (89). Other studies have found reduced z-scores for food allergic children compared to controls or normal growth curves. In a study of food allergic children from Brazil with a median age of 10 months, of which 92 % had CMPA, the authors found significant lower WAZ (z= -0.95) and LAZ (z =-0.41) than in a control group of non-allergic children (132). However, in a British study of food allergic children with a median age 27 months from 13 different centers, the median WAZ and LAZ were found to be 0.22 and -0.06 respectively (118) and in a study from France of food allergic children, mean age 4.7 years, mean WAZ and LAZ were 0.1 and 0.2 respectively, though significantly lower than the control groups (29). The mean WAZ and LAZ in our group of CMPA children were higher for weight and similar for length compared with the Brazilian children. The children in the British and French study both had higher WAZ and LAZ compared our patients,

however in these studies the children were older. Young children are particularly vulnerable of growth failure as the first few years of life is a period of rapid growth. A group form the US, however, did not find growth impairment in food allergic children under two years of age but significant effects on growth in 2-5 year olds (18). In a retrospective study of Norwegian children with and without food allergy there were no differences in height at one year of age but the allergic children had lower weight, and by the age of two, the children excluding milk had lower height and weight than their peers. The effect of milk exclusion on growth was still evident at the age of 6-10 years of age (31).

Weight is a sensitive measure of energy intake and is affected by dietary inadequacies earlier than length. However, protein deficiencies or chronic energy deficiency will eventually affect length. Mean LAZ was 0.07 at birth and -0,39 at inclusion (Table 9). The distribution of LAZ shifted from right to left in this time period (Figure 20 c-d) indicating suboptimal growth in length and that the MILIKD study population was shorter than the normal population of Norwegian children. There was a significant fall in LAZ for boys in from birth to inclusion, indicating poor longitudinal growth in this group of children (Figure 19). The girls LAZ change only slightly (Table 25). It is normal to see both falls and climbs in z-scores for length during the first two years of life. As we do not have measurements after inclusion and hence we do not know if this poor growth in length continued in the boys or if the finding is due to normal changes in percentile for length. However, the fall was large and significant and one could speculate that this signals chronic malnutrition or perhaps the effect of an ongoing inflammation process or both.

Mean WAZ was -0.54 at birth and -0.41 at inclusion in our group (Table 9) and hence did not change much during this course of time. The curves were shifted to the left suggesting a greater prevalence than normal of low weight in these children (Table 20 a-b). This is in contrast to the findings of weight in the Norwegian growth study where the curve for weight was skewed to the right at all age groups (89).

The mean BMIZ at inclusion was -0.53 (-0.38 for boys and -0.69 for girls) and -0.48 for z-score weight for length (w/l) (-0.31 for boys and -0.72 for girls) (Table 9 and Table 25). This is higher than found in Brazilian food allergic children (z-score w/l = -0.85), but lower than found in the British (z-score w/l = 0.5) and the French (z-score w/l = -0.2) studies. The distribution curve for BMIZ was shifted to the left, indicating that a large proportion of the children in the study group had low weight for height (Figure 20). In the Norwegian growth study by Júlíusson et al a positive skewness was observed for BMI for all age groups (89), hence the children in our study were thinner than the normal population
.

Using z-score of -2 as a cut of for malnutrition and z-score of 2 as a cut of for overweight, we found that the prevalence of z-scores of ≤ -2 was approximately 11 % for WAS, 5 % for LAZ, and 11 % for BMIZ (Table 26). This indicates that a large proportion of these children were undernourished. We found no WAZ or LAZ of ≥ 2, however approximately 2 % of the children had BMZI for age of ≥ 2, indicating overweight or obesity in these children. Meyer et al found z-scores of ≤ - 2 in 9 % for WAZ, 11 % for LAZ and 4 % for z-score weight for length in the British study of food allergic children (118). Flammarion et al found z-scores of ≤ - 2 in 9 % for WAZ, 7 % for LAZ and 5 % for weight for length in French children with food allergy. Both studies found a larger proportion of children with WAZ of ≥ 2 (9 % and 10 %) and z-score of weight for length ≥ 2 (both 8 %) than we found in the MILKID study. Compared to the British and French studies the children in this study had a higher prevalence of low z-scores for weight for length (BMIZ), a sign of acute malnutrition, and we found a lower prevalence of overweight and obesity. Both the British (118) and French (29) children had a higher prevalence of low LAZ for age than we found, which could be an indication of chronic malnutrition or poor protein intake.

There were no significant differences between genders for most anthropometric measures at inclusion (Table 25), except for head circumference which was significantly different at birth; boys having significantly higher z-scores for head circumference (0.02) than girls (-0.91). At inclusion the difference was no longer significant for z-scores for head circumference between the genders.

5.5.2 Factors affecting growth in the MILKID study

We looked at factors in the CMPA child that could potentially affect growth (Tables 27 and 28). Neither total number of symptoms, involvement of skin, gastro intestinal or the respiratory tracts, nor number of foods avoided correlated significantly with growth. This is different from what has been found in several studies of food allergies where growth failure and poor nutritional status significantly increases with the number of foods avoided in the diet (29, 118). In the MILKID study it was not differentiated between food avoidance due to suspected or feared allergies and avoidance due to the chronological age of the child, as many of these children were included in the early period of introduction to complementary foods. This could of course affect the result. Having subjective symptoms did correlate significantly and negatively with all growth parameters. However, the subjective symptoms included growth failure and could have caused of the positive result. Number of weeks on the diet was significantly positively correlated with WAZ, LAZ and BMIZ, and this does give reason to question if the poor weight gain, growth and low BMI seen in our group could be a sign not of nutritional deficiencies of the exclusion diet but of under-treated CMPA causing persistent

intestinal inflammation due to factors like continuous antigen challenge, non-compliance with diet, under diagnosed food allergy or antigen remnants in the hypo allergenic formula (29, 108). The presence of other allergic disorders, like atopic dermatitis, have been shown to affect growth (2) and it has been suggested to be due to raised caloric and protein requirements in children with such co-morbidity (28, 108). We found no difference in growth between children with or without skin and respiratory affection in our study. Other investigators have come to the same conclusion; Flammarion et al found no difference in growth between children with or without atopic dermatitis and asthma in a study of French CMPA patients (29). Intestinal inflammation as part of the disease process could negatively affect growth, both through possible reduction of nutrient absorption and/or increased requirements of the inflammation process (28, 118). All the children in the MILKID study, except for one, had gastro intestinal symptoms. Although this could explain the effect on growth in CMPA, we found no association between the number nor type of GI symptom and growth parameters (result not shown).

Feeding difficulties could impact weight gain, which may lead to growth faltering and in the long run affect longitudinal growth and cognitive development. Feeding problems and food allergies have been associated in studies of food allergic children. Poor feeding skills and/or maladaptive feeding behaviors such as food aversion, food refusal, neophobia and anxiety during feeding could lead to inadequate nutrient intake. 30-40 % of children with food protein induced gastro allergy were found to have feeding difficulties in a large retrospective study of 437 food allergic children, and faltering growth was higher in the group with feeding difficulties than in those without (114). In another study of children with CMPA and healthy controls, the allergic children had significantly higher scores for fuzzy eating and feeding difficulties, however the majority was within the normal range and did not affect growth (115). We found a significant negative association between food refusal and WAZ and BMIZ (table 28). Children reported to have poor appetite had significantly lower WAZ and BMIZ than those with normal appetite (table 29). There was a large difference in BMIZ between selective eaters and non-selective eaters, however the difference did not reach statistical significance. For other feeding parameters there were no differences in anthropometric measures between those with or those without. Although there seems to be an association between feeding problems and reduced growth, we know little about the causation.

5.5.3 Iodine status and growth

Severe iodine deficiency in pregnancy is known to cause growth failure in the offspring. The relationship between postnatal iodine status and growth is less clear. Studies of growth and iodine deficiency have mixed results, though recent studies have found increased somatic growth rates in iodine deficient children that are supplemented with iodine. The effects are thought to be mediated

by thyroid hormone directly and the secretion of growth hormone, insulin-like growth factor (IGF)-1 and IGF binding protein-3 which are all increased by thyroid hormone (34, 40). In our study we found an inverse correlation between growth and iodine deficiency, however, the correlations were not significant (table 30). It may be that the children with growth restriction in the MILKID study were given more detailed advice on how to ensure good nutritional quality of the diet and so had achieved good iodine status. In addition there might be other causes for poor growth in these children with CMPA, such as inflammation in the gut resulting in increased requirements for nutrients (28, 118).

6 Conclusion

This present work is the first to present iodine status in infants and children with allergy to cow's milk protein. One third of the study population was found to have urinary iodine content (UIC) in ug/L under the recommended level. Infants who were mainly breastfed had a high prevalence of low UIC of 58 %, significantly higher than children that relied on complementary foods as their iodine source. Breastfed children of mothers who used supplements with iodine had higher, though not significantly higher median urinary iodine content (MUIC).

Urine iodine concentration correlated positively with the intake of enriched baby cereals and with reaching the RDI of iodine through complementary food and drinks. Contrary to other studies of iodine status in infants and young children, the use of formula milk did not significantly correlate with iodine status in this study.

The cow's milk allergic children tended to grow slower and be slimmer compared to the Norwegian growth standards. Boys had significantly reduced z-score for length from birth to inclusion in the study indicating poor longitudinal growth. The prevalence of malnutrition was high and more than ten percent of the children had a z-score of under two for weight and BMI for age. This is higher than found in other comparable studies of cow's milk allergic children from Europe.

There was a high prevalence of 70 % of parent reported symptoms of feeding problems in the children with cow's milk allergy. An association with food refusal and poor appetite and reduced z-scores for weight and BMI for age was found.

Iodine excretion in the urine is subject to variations in both diet and hydration. We did not correct for hydration status and cannot exclude that the differences found between groups is in part due to differences in the hydration status of children who have a high intake of breast milk and those who eat more solid foods. Intake and iodine content of breast milk were not measured and this makes it difficult to draw firm conclusions about the effect of diet on iodine status in this study.

Although the prevalence of malnutrition was high and an association between poor growth and feeding problems was identified, we cannot draw conclusion about the causation. The impact of the disease process itself must not be disregarded and could potentially explain these findings.

7 Future perspectives

The present work suggests that cow's milk allergic children are at risk of iodine deficiency, especially if their iodine intake is dependent on breastmilk. Ensuring good iodine sources, such as enriched weaning foods and advising nursing mothers to include good, non-dairy sources of iodine like sea fish, eggs and iodized salt in their diet, as well as take supplement with adequate iodine seems warranted.

There exist as far as we know no data on the iodine status of the general population of infants and young children under the age of two years in Norway. The iodine content of breast milk in Norwegian women has not established and there is little data on the iodine status of lactating women. One study found high incidence of insufficient status of women in Norway post-partum (124). In view of the Norwegian government's recommendation of exclusive breastfeeding until the age of six months and the knowledge that iodine content in breast milk decreases during the first six months of breastfeeding in mildly deficient subjects, there is an urgent need to establish whether iodine nutrition of young infants is sufficient in Norway today.

There is general trend towards increased focus on diet and health in the Norwegian population. In their quest for infant foods that are unsweetened, organically produced and of an ecological nature parents may prefer baby cereals from producers, such as Holle, that do not enrich their products with vital vitamins and minerals. These cereals need to be supplemented with formula milk to ensure sufficient nutrition of the child. However formula milk is not used at a high rate in Norway, especially not in breastfed infants. In light of this there could potentially be certain groups of infants in the general population that are at high risk of iodine deficiency. There is also a rising trend towards vegetarianism and vegan diets among young women of childbearing age which may put their offspring at risk of iodine deficiency and the subsequent effects.

In relation to food allergic children we know little about the diet of CMPA children that are managed by general practitioners or private paediatricians, who may not receive dietary advice from a dietitian. There is a lack of data on the iodine status and diet of older children with CMPA. All of these groups would be of interest for further research.

8 References

1. Meyer R, De Koker C, Dziubak R, Godwin H, Dominguez-Ortega G, Shah N. Dietary elimination of children with food protein induced gastrointestinal allergy - micronutrient adequacy with and without a hypoallergenic formula? Clinical and translational allergy. 2014;4(1):31.
2. Cho HN, Hong S, Lee SH, Yum HY. Nutritional status according to sensitized food allergens in children with atopic dermatitis. Allergy, asthma & immunology research. 2011;3(1):53-7.
3. Venter C, Arshad SH. Epidemiology of food allergy. Pediatr Clin North Am. 2011;58(2):327-49, ix.
4. Valenta R, Hochwallner H, Linhart B, Pahr S. Food Allergies: The Basics. Gastroenterology. 2015;148(6):1120-31.e4.
5. Vandenplas Y, De Greef E, Devreker T. Treatment of Cow's Milk Protein Allergy. Pediatric gastroenterology, hepatology & nutrition. 2014;17(1):1-5.
6. Christie L, Hine RJ, Parker JG, Burks W. Food allergies in children affect nutrient intake and growth. Journal of the American Dietetic Association. 2002;102(11):1648-51.
7. Rasmussen Lea. Iodine Intake and Food Choice. In: Victor R. Preedy GNBaRW, editor. Comprehensive Handbook of Iodine: Elsevier Inc.; 2009.
8. Bougma K, Aboud FE, Harding KB, Marquis GS. Iodine and mental development of children 5 years old and under: a systematic review and meta-analysis. Nutrients. 2013;5(4):1384-416.
9. Delange F. The role of iodine in brain development. The Proceedings of the Nutrition Society. 2000;59(1):75-9.
10. Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. Journal of pediatric gastroenterology and nutrition. 2012;55(2):221-9.
11. Eggesbo M, Botten G, Stigum H. Restricted diets in children with reactions to milk and egg perceived by their parents. The Journal of pediatrics. 2001;139(4):583-7.
12. Schoemaker AA, Sprikkelman AB, Grimshaw KE, Roberts G, Grabenhenrich L, Rosenfeld L, et al. Incidence and natural history of challenge-proven cow's milk allergy in European children - EuroPrevall birth cohort. Allergy. 2015.
13. Host A, Halken S, Jacobsen HP, Christensen AE, Herskind AM, Plesner K. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2002;13 Suppl 15:23-8.
14. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. The Journal of allergy and clinical immunology. 2010;126(6 Suppl):S1-58.
15. Kneepkens CM, Meijer Y. Clinical practice. Diagnosis and treatment of cow's milk allergy. European journal of pediatrics. 2009;168(8):891-6.
16. Fiocchi A, Brozek J, Schunemann H, Bahna SL, von Berg A, Beyer K, et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines. The World Allergy Organization journal. 2010;3(4):57-161.
17. Cianferoni A, Spergel JM. Food allergy: review, classification and diagnosis. Allergology international : official journal of the Japanese Society of Allergology. 2009;58(4):457-66.
18. Mehta H, Ramesh M, Feuille E, Groetch M, Wang J. Growth comparison in children with and without food allergies in 2 different demographic populations. The Journal of pediatrics. 2014;165(4):842-8.
19. Berni Canani R, Leone L, D'Auria E, Riva E, Nocerino R, Ruotolo S, et al. The effects of dietary counseling on children with food allergy: a prospective, multicenter intervention study. Journal of the Academy of Nutrition and Dietetics. 2014;114(9):1432-9.
20. Lande B, Andersen, L. F. Spedkost 12 måneder. Landsomfattende kostholdsundersøkelse blant spedbarn i Norge. Oslo: Sosial- og helsedirektoratet; 2005.

21. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B, et al. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. Journal of pediatric gastroenterology and nutrition. 2008;46(1):99-110.
22. Meyer R, Venter C, Fox AT, Shah N. Practical dietary management of protein energy malnutrition in young children with cow's milk protein allergy. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2012;23(4):307-14.
23. Host A, Halken S. Cow's milk allergy: where have we come from and where are we going? Endocr Metab Immune Disord Drug Targets. 2014;14(1):2-8.
24. Henriksen C, Eggesbo M, Halvorsen R, Botten G. Nutrient intake among two-year-old children on cows' milk-restricted diets. Acta paediatrica (Oslo, Norway : 1992). 2000;89(3):272-8.
25. Giovannini M, D'Auria E, Caffarelli C, Verduci E, Barberi S, Indinnimeo L, et al. Nutritional management and follow up of infants and children with food allergy: Italian Society of Pediatric Nutrition/Italian Society of Pediatric Allergy and Immunology Task Force Position Statement. Italian journal of pediatrics. 2014;40:1.
26. Carvalho NF, Kenney RD, Carrington PH, Hall DE. Severe Nutritional Deficiencies in Toddlers Resulting From Health Food Milk Alternatives. Pediatrics. 2001;107(4):e46.
27. Matportalen. Åtvarar mot risdrikk til barn under seks år: Matprotalen; 2013. Available from: http://www.matportalen.no/matvaregrupper/tema/drikke/aatvarar_mot_risdrikk_til_barn_under_seks_aar.
28. Isolauri E, Sutas Y, Salo MK, Isosomppi R, Kaila M. Elimination diet in cow's milk allergy: risk for impaired growth in young children. The Journal of pediatrics. 1998;132(6):1004-9.
29. Flammarion S, Santos C, Guimber D, Jouannic L, Thumerelle C, Gottrand F, et al. Diet and nutritional status of children with food allergies. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2011;22(2):161-5.
30. Vieira MC, Morais MB, Spolidoro JV, Toporovski MS, Cardoso AL, Araujo GT, et al. A survey on clinical presentation and nutritional status of infants with suspected cow' milk allergy. BMC Pediatr. 2010;10:25.
31. Karlsen MB, Loken EB, Mevold K, Bueso AK, Halvorsen R. [Growth and dietary intake among children with previous cow's milk allergy]. Tidsskrift for den Norske laegeforening : tidsskrift for praktisk medicin, ny raekke. 2005;125(22):3104-7.
32. Ambroszkiewicz J, Rowicka G, Chelchowska M, Gajewska J, Strucinska M, Laskowska-Klita T. Biochemical markers of bone metabolism in children with cow's milk allergy. Arch Med Sci. 2014;10(6):1135-41.
33. Berry MJ, Adams J, Voutilainen H, Feustel PJ, Celestin J, Jarvinen KM. Impact of elimination diets on growth and nutritional status in children with multiple food allergies. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2015;26(2):133-8.
34. Zimmermann MB. Iodine deficiency. Endocrine reviews. 2009;30(4):376-408.
35. howMED. Hormones of Thyroid Gland 2011 [20.08.2015]. Available from: http://howmed.net/physiology/hormones-thyroid-gland/.
36. SACN. SACN Statement on iodine and health 2014 [21.10.2014]. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/339439/SACN_Iodine_and_Health_2014.pdf.
37. Ahad F, Ganie SA. Iodine, Iodine metabolism and Iodine deficiency disorders revisited. Indian J Endocrinol Metab. 2010;14(1):13-7.
38. Horn S, Heuer H. Thyroid hormone action during brain development: more questions than answers. Mol Cell Endocrinol. 2010;315(1-2):19-26.
39. Smith JW, Evans AT, Costall B, Smythe JW. Thyroid hormones, brain function and cognition: a brief review. Neurosci Biobehav Rev. 2002;26(1):45-60.
40. Pearce EN. Iodine deficiency in children. Endocrine development. 2014;26:130-8.

41. Andersson M, Takkouche B, Egli I, Allen HE, de Benoist B. Current global iodine status and progress over the last decade towards the elimination of iodine deficiency. Bulletin of the World Health Organization. 2005;83(7):518-25.
42. Pearce EN, Andersson M, Zimmermann MB. Global iodine nutrition: Where do we stand in 2013? Thyroid. 2013;23(5):523-8.
43. Dahl L, Johansson L, Julshamn K, Meltzer HM. The iodine content of Norwegian foods and diets. Public health nutrition. 2004;7(4):569-76.
44. Frey H, Rosenlund B, Storli U. [Urinary excretion of iodine in some Norwegian population groups 1971-1972]. Tidsskrift for den Norske laegeforening : tidsskrift for praktisk medicin, ny raekke. 1974;94(15):982-7.
45. Andersson M. Iodine defi ciency in Europe : a continuing public health problem. WHO, editor: WHO Library Cataloguing-in-Publication Data; 2007.
46. Bath SC, Steer CD, Golding J, Emmett P, Rayman MP. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Lancet. 2013;382(9889):331-7.
47. Delange F. Iodine requirements during pregnancy, lactation and the neonatal period and indicators of optimal iodine nutrition. Public health nutrition. 2007;10(12A):1571-80; discussion 81-3.
48. Brantsaeter AL, Abel MH, Haugen M, Meltzer HM. Risk of suboptimal iodine intake in pregnant Norwegian women. Nutrients. 2013;5(2):424-40.
49. Qian M, Wang D, Watkins WE, Gebski V, Yan YQ, Li M, et al. The effects of iodine on intelligence in children: a meta-analysis of studies conducted in China. Asia Pacific journal of clinical nutrition. 2005;14(1):32-42.
50. Hynes KL, Otahal P, Hay I, Burgess JR. Mild iodine deficiency during pregnancy is associated with reduced educational outcomes in the offspring: 9-year follow-up of the gestational iodine cohort. The Journal of clinical endocrinology and metabolism. 2013;98(5):1954-62.
51. Santiago-Fernandez P, Torres-Barahona R, Muela-Martinez JA, Rojo-Martinez G, Garcia-Fuentes E, Garriga MJ, et al. Intelligence quotient and iodine intake: a cross-sectional study in children. The Journal of clinical endocrinology and metabolism. 2004;89(8):3851-7.
52. Vermiglio F, Lo Presti VP, Moleti M, Sidoti M, Tortorella G, Scaffidi G, et al. Attention deficit and hyperactivity disorders in the offspring of mothers exposed to mild-moderate iodine deficiency: a possible novel iodine deficiency disorder in developed countries. The Journal of clinical endocrinology and metabolism. 2004;89(12):6054-60.
53. Hamza RT, Hewedi DH, Sallam MT. Iodine deficiency in Egyptian autistic children and their mothers: relation to disease severity. Archives of medical research. 2013;44(7):555-61.
54. Andersson M, de Benoist B, Delange F, Zupan J. Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation. Public health nutrition. 2007;10(12A):1606-11.
55. Andersen S, Karmisholt J, Pedersen KM, Laurberg P. Reliability of studies of iodine intake and recommendations for number of samples in groups and in individuals. The British journal of nutrition. 2008;99(4):813-8.
56. Charlton KE, Batterham MJ, Buchanan LM, Mackerras D. Intraindividual variation in urinary iodine concentrations: effect of adjustment on population distribution using two and three repeated spot urine collections. BMJ open. 2014;4(1):e003799.
57. Zimmermann MB. Methods to assess iron and iodine status. The British journal of nutrition. 2008;99 Suppl 3:S2-9.
58. Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Carle A, Pedersen IB, et al. Thyroglobulin as a marker of iodine nutrition status in the general population. European journal of endocrinology / European Federation of Endocrine Societies. 2009;161(3):475-81.
59. Zimmermann MB, Aeberli I, Andersson M, Assey V, Yorg JA, Jooste P, et al. Thyroglobulin is a sensitive measure of both deficient and excess iodine intakes in children and indicates no adverse effects on thyroid function in the UIC range of 100-299 mug/L: a UNICEF/ICCIDD study group report. The Journal of clinical endocrinology and metabolism. 2013;98(3):1271-80.

60. Ristic-Medic D, Novakovic R, Glibetic M, Gurinovic M. EURRECA-Estimating iodine requirements for deriving dietary reference values. Critical reviews in food science and nutrition. 2013;53(10):1051-63.
61. WHO/UNICEF/ICCIDD. Assessment of iodine deficiency disorders and monitoring their elimination : a guide for programme managers. 3 ed. Geneva, Switzerland: WHO; 2007.
62. Councile of Nordic Ministers. Iodine. Nordic Nutrition Recommendations 2012. 2014.
63. Delange F, Benker G, Caron P, Eber O, Ott W, Peter F, et al. Thyroid volume and urinary iodine in European schoolchildren: standardization of values for assessment of iodine deficiency. European journal of endocrinology / European Federation of Endocrine Societies. 1997;136(2):180-7.
64. Dahl L, Meltzer, H. The Iodine Content of Foods and Diets: Norwegian Perspectives. In: Victor R. Preedy GNBaRW, editor. Comprehensive Handbook of Iodine: Nutritional, Biochemical, Pathological and Therapeutic Aspects: Elsevier Inc; 2009. p. 345-52.
65. Mattilsynet. Matvaretabellen Mattilsynet; 2014. Available from: www.matvaretabellen.no.
66. Statens råd for ernæring og fysisk aktivitet. Anbefalinger for spedbarnsernæring. Statens råd for ernæring og fysisk aktivitet; 2001.
67. Øverby N. Spedkost 6 måneder. Landsomfattende kostholdsundersøkelse blant 6 måneder gamle barn. Helsedirektoratet, Mattilsynet og Universitetet i Oslo; 2008.
68. Lande B., Helleve A. Amming og spedbarns kosthold. Landsomfattende undersøkelse 2013. Helsedirektoratet; 2014.
69. Alexy U, Drossard C, Kersting M, Remer T. Iodine intake in the youngest: impact of commercial complementary food. European journal of clinical nutrition. 2009;63(11):1368-70.
70. Thomson BM, Vannoort RW, Haslemore RM. Dietary exposure and trends of exposure to nutrient elements iodine, iron, selenium and sodium from the 2003-4 New Zealand Total Diet Survey. The British journal of nutrition. 2008;99(3):614-25.
71. Andersson M, Aeberli I, Wust N, Piacenza AM, Bucher T, Henschen I, et al. The Swiss iodized salt program provides adequate iodine for school children and pregnant women, but weaning infants not receiving iodine-containing complementary foods as well as their mothers are iodine deficient. The Journal of clinical endocrinology and metabolism. 2010;95(12):5217-24.
72. Valentine CJ, Wagner CL. Nutritional management of the breastfeeding dyad. Pediatr Clin North Am. 2013;60(1):261-74.
73. Leung AM, Pearce EN, Braverman LE. Iodine nutrition in pregnancy and lactation. Endocrinol Metab Clin North Am. 2011;40(4):765-77.
74. Dorea JG. Iodine nutrition and breast feeding. Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS). 2002;16(4):207-20.
75. Azizi F, Smyth P. Breastfeeding and maternal and infant iodine nutrition. Clinical endocrinology. 2009;70(5):803-9.
76. Allergies) ENPEPoPoDPNa. Scientific Opinion on Dietary Reference Values for iodine. EFSA journal. 2014;12(5):57.
77. Mulrine HM, Skeaff SA, Ferguson EL, Gray AR, Valeix P. Breast-milk iodine concentration declines over the first 6 mo postpartum in iodine-deficient women. The American journal of clinical nutrition. 2010;92(4):849-56.
78. Bouhouch RR, Bouhouch S, Cherkaoui M, Aboussad A, Stinca S, Haldimann M, et al. Direct iodine supplementation of infants versus supplementation of their breastfeeding mothers: a double-blind, randomised, placebo-controlled trial. The Lancet Diabetes & Endocrinology. 2014;2(3):197-209.
79. Zimmermann MB. Iodine deficiency and excess in children: worldwide status in 2013. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2013;19(5):839-46.
80. Chung HR. Iodine and thyroid function. Ann Pediatr Endocrinol Metab. 2014;19(1):8-12.
81. FAO/WHO. Iodine. Human Vitamin and Mineral Requirements: World Health Organization and Food and Agriculture Organization of the United Nations; 2002.
82. Krull F, Ohlendorf K. [Hypothyroid iodine deficiency struma in 2 siblings as a sequela of alternative nutrition]. Monatsschr Kinderheilkd. 1993;141(6):481-2.

83. Labib M, Gama R, Wright J, Marks V, Robins D. Dietary maladvice as a cause of hypothyroidism and short stature. BMJ. 1989;298(6668):232-3.
84. Cheetham T, Plumb E, Callaghan J, Jackson M, Michaelis L. Dietary restriction causing iodine-deficient goitre. Archives of disease in childhood. 2015.
85. Leniszewski S, Mauseth R. Goiter and multiple food allergies. Int J Pediatr Endocrinol. 2009;2009:628034.
86. EFSA NDA Panel (EFSA Panel on Dietetic Products NaA. Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA Journal. 2014;12(7):106.
87. Becker P, Carney LN, Corkins MR, Monczka J, Smith E, Smith SE, et al. Consensus statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition: indicators recommended for the identification and documentation of pediatric malnutrition (undernutrition). Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition. 2015;30(1):147-61.
88. WHO. Job-aid – Weighing and Measuring a Child: WHO; [20.01.2015]. Available from: http://www.who.int/childgrowth/training/jobaid_weighing_measuring.pdf.
89. Juliusson PB, Roelants M, Nordal E, Furevik L, Eide GE, Moster D, et al. Growth references for 0-19 year-old Norwegian children for length/height, weight, body mass index and head circumference. Annals of human biology. 2013;40(3):220-7.
90. Juliusson PB, Roelants M, Eide GE, Moster D, Juul A, Hauspie R, et al. [Growth references for Norwegian children]. Tidsskrift for den Norske laegeforening : tidsskrift for praktisk medicin, ny raekke. 2009;129(4):281-6.
91. Wang YaC, H. Use of Percentiles and Z -Scores in Anthropometry: Springer Science Business Media; 2012. 21 p.
92. UNICEF, WHO, The World Bank. UNICEF, WHO, World Bank Joint Child Malnutrition Estimates. 2012.
93. Burrows TL, Martin RJ, Collins CE. A systematic review of the validity of dietary assessment methods in children when compared with the method of doubly labeled water. Journal of the American Dietetic Association. 2010;110(10):1501-10.
94. Lanigan JA, Wells JC, Lawson MS, Cole TJ, Lucas A. Number of days needed to assess energy and nutrient intake in infants and young children between 6 months and 2 years of age. European journal of clinical nutrition. 2004;58(5):745-50.
95. Secretariat W, Andersson M, de Benoist B, Delange F, Zupan J. Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation. Public health nutrition. 2007;10(12A):1606-11.
96. Seppo L, Korpela R, Lonnerdal B, Metsaniitty L, Juntunen-Backman K, Klemola T, et al. A follow-up study of nutrient intake, nutritional status, and growth in infants with cow milk allergy fed either a soy formula or an extensively hydrolyzed whey formula. The American journal of clinical nutrition. 2005;82(1):140-5.
97. Dorey CM, Zimmermann MB. Reference values for spot urinary iodine concentrations in iodine-sufficient newborns using a new pad collection method. Thyroid. 2008;18(3):347-52.
98. Husniza H, Wan Nazaimoon WM. A cost-effective modified micromethod for measuring urine iodine. Trop Biomed. 2006;23(1):109-15.
99. Gnat D, Dunn AD, Chaker S, Delange F, Vertongen F, Dunn JT. Fast colorimetric method for measuring urinary iodine. Clinical chemistry. 2003;49(1):186-8.
100. FAO/WHO/UNU. Human energy requirements. In: FAO/WHO/UNU, editor. Report of a Joint FAO/WHO/UNU Expert Consultation2001.
101.Erkkola M, Kyttala P, Takkinen HM, Kronberg-Kippila C, Nevalainen J, Simell O, et al. Nutrient intake variability and number of days needed to assess intake in preschool children. The British journal of nutrition. 2011;106(1):130-40.
102.Huybrechts I, De Bacquer D, Cox B, Temme EH, Van Oyen H, De Backer G, et al. Variation in energy and nutrient intakes among pre-school children: implications for study design. Eur J Public Health. 2008;18(5):509-16.

103.Levin KA. Study design III: Cross-sectional studies. Evid Based Dent. 2006;7(1):24-5.
104.Statistisk Sentralbyrå. Fødte 2014 2015. Available from: http://www.ssb.no/befolkning/statistikker/fodte/aar/2015-03-26#content.
105.Lande B, Arnfinn Helleve. Amming og spedbarns kosthold. Landsomfattende undersøkelse 2013. In: Helsedirektoratet, editor. Oslo2014.
106.Brantsaeter AL, Haugen M, Alexander J, Meltzer HM. Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). Matern Child Nutr. 2008;4(1):28-43.
107.Chebar Lozinsky A, Meyer R, De Koker C, Dziubak R, Godwin H, Reeve K, et al. Time to symptom improvement using elimination diets in non-IgE mediated gastrointestinal food allergies. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2015.
108.Mehta H, Groetch M, Wang J. Growth and nutritional concerns in children with food allergy. Current opinion in allergy and clinical immunology. 2013;13(3):275-9.
109.Host A. Frequency of cow's milk allergy in childhood. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2002;89(6 Suppl 1):33-7.
110.Dupont C, Hol J, Nieuwenhuis EE. An extensively hydrolysed casein-based formula for infants with cows' milk protein allergy: tolerance/hypo-allergenicity and growth catch-up. The British journal of nutrition. 2015;113(7):1102-12.
111.Meyer R, Fleming C, Dominguez-Ortega G, Lindley K, Michaelis L, Thapar N, et al. Manifestations of food protein induced gastrointestinal allergies presenting to a single tertiary paediatric gastroenterology unit. The World Allergy Organization journal. 2013;6(1):13.
112.Sicherer SH. Clinical aspects of gastrointestinal food allergy in childhood. Pediatrics. 2003;111(6 Pt 3):1609-16.
113.Caffarelli C, Cavagni G, Deriu FM, Zanotti P, Atherton DJ. Gastrointestinal symptoms in atopic eczema. Archives of disease in childhood. 1998;78(3):230-4.
114.Meyer R, Rommel N, Van Oudenhove L, Fleming C, Dziubak R, Shah N. Feeding difficulties in children with food protein-induced gastrointestinal allergies. Journal of gastroenterology and hepatology. 2014;29(10):1764-9.
115.Maslin K, Dean T, Arshad SH, Venter C. Fussy eating and feeding difficulties in infants and toddlers consuming a cows' milk exclusion diet. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2015;26(6):503-8.
116.Petrus NC, Schoemaker AF, van Hoek MW, Jansen L, Jansen-van der Weide MC, van Aalderen WM, et al. Remaining symptoms in half the children treated for milk allergy. European journal of pediatrics. 2015;174(6):759-65.
117.Bishop JM, Hill DJ, Hosking CS. Natural history of cow milk allergy: clinical outcome. The Journal of pediatrics. 1990;116(6):862-7.
118.Meyer R, De Koker C, Dziubak R, Venter C, Dominguez-Ortega G, Cutts R, et al. Malnutrition in children with food allergies in the UK. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2014;27(3):227-35.
119.Gordon JH, Leung AM, Hale AR, Pearce EN, Braverman LE, He X, et al. No difference in urinary iodine concentrations between Boston-area breastfed and formula-fed infants. Thyroid. 2014;24(8):1309-13.
120.Valeix P, Preziosi P, Rossignol C, Farnier MA, Hercberg S. Iodine intakes assessed by urinary iodine concentrations in healthy children aged ten months, two years, and four years. Biological trace element research. 1992;32:259-66.
121.Delange F, Wolff P, Gnat D, Dramaix M, Pilchen M, Vertongen F. Iodine deficiency during infancy and early childhood in Belgium: does it pose a risk to brain development? European journal of pediatrics. 2001;160(4):251-4.
122.Skeaff SA, Ferguson EL, McKenzie JE, Valeix P, Gibson RS, Thomson CD. Are breast-fed infants and toddlers in New Zealand at risk of iodine deficiency? Nutrition. 2005;21(3):325-31.

123.Beaufrere B, Bresson JL, Briend A, Ghisolfi J, Goulet O, Navarro J, et al. [Iodine nutrition in the infant. Committee on Nutrition of the French Society of Pediatrics]. Arch Pediatr. 2000;7(1):66-74.
124.Seldal CF. Seafood intake and iodine status in pregnant and postpartum Norwegian women [Master Thesis in Human Nutrition]. Bergen, Norway: University of Bergen; 2012.
125.Andersen SL, Moller M, Laurberg P. Iodine concentrations in milk and in urine during breastfeeding are differently affected by maternal fluid intake. Thyroid. 2014;24(4):764-72.
126.Nødland LK. Development and Validation of Method for Determination of Iodine in Urine from Mother and Child using ICP-MS-QQQ. (Master Thesis) Ås: Norges miljø- og biovitenskapelige universitet; 2014.
127.Johner SA, Thamm M, Nothlings U, Remer T. Iodine status in preschool children and evaluation of major dietary iodine sources: a German experience. European journal of nutrition. 2013;52(7):1711-9.
128.Hilbig A, Drossard C, Kersting M, Alexy U. Nutrient Adequacy and Associated Factors in a Nationwide Sample of German Toddlers. Journal of pediatric gastroenterology and nutrition. 2015;61(1):130-7.
129.Gunnarsdottir I, Dahl L. Iodine intake in human nutrition: a systematic literature review. Food & nutrition research. 2012;56.
130.Hou X. Iodine Speciation in Foodstuffs, Tissues, and Environmental Samples: Iodine Species and Analytical Method. In: Preedy V, editor. Comprehensive Handbook of Iodine: Elsevier Inc.; 2009. p. 139-50.
131.Fernandez-Sanchez LM, Bermejo-Barrera P, Fraga-Bermudez JM, Szpunar J, Lobinski R. Determination of iodine in human milk and infant formulas. Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS). 2007;21 Suppl 1:10-3.
132.Costa LC, Rezende ER, Segundo GR. Growth parameters impairment in patients with food allergies. Journal of allergy. 2014;2014:980735.

9 Appendix

9.1 Appendix 1. Spørreskjema om bakgrunnsinformasjon

Spørreskjema om bakgrunnsinformasjon

Vi ber deg/dere svare på følgende spørsmål som del av studien "Kosthold, vekst og ernæringsstatus hos barn som følger melkefri diett".

Dato for utfylling av skjemaet: ☐ ☐ ☐ ☐ **201**☐

Skjemaet er utfylt av:

☐ Barnets mor ☐ Barnets far ☐ Både mor og far ☐ Annen person

Spørsmål 1: Om diagnosen (sett et kryss)

☐ **Barnet har fått diagnosen melkeproteinallergi av lege.**

eller

☐ **Barnet forsøker diett for å finne ut om det er melkeproteiner som gir plager.**

Spørsmål 2: Hvilke symptomer/reaksjon har barnet (eller har barnet hatt) på sin (mulige) melkeproteinallergi (du kan angi flere symptomer/reaksjoner)?

☐ Reflux/gulp/oppkast ☐ Kolikk/magesmerter

☐ Løs mage/diarre ☐ Hard mage/forstoppelse

☐ Blod i avføring ☐ Spisevegring

☐ Vokser dårlig/øke lite i vekt ☐ Eksem/kløe

☐ Puste problemer/anafylaxi ☐ Søvnproblem

☐ Ørebetennelse/rennende nese

- _____
- _____
- _____

Spørsmål 3: Er barnets plager/symptomer bedre etter oppstart av melkeproteinfri diett?

☐ Ja, helt bra

☐ Ja, noe bedring

☐ Nei, ingen forskjell

☐ Vet ikke

Spørsmål 4: Har barnet noen andre sykdommer?

☐Ja ☐Nei

Hvis ja, angi hvilke(n):

- _____
- _____
- _____

Spørsmål 5: Foreldrenes sivilstatus:

☐ Gift/samboere med hverandre

☐ Skilt/bor ikke sammen

Spørsmål 6: Foreldrenes etnisitet:

Mor:_____

Far: _____

Spørsmål 7: Mors alder:

☐☐ år

Spørsmål 8: Røyker mor?

☐ **Ja** ☐ **Nei**

Spørsmål 9: Mors høyeste fullførte utdanning

☐ Mindre enn 9-årig grunnskole ☐ 9-årig grunnskole

☐ Videregående skole

☐ Høyskole/universitet inntil 4 år (cand.mag. bachelor, lærer, sykepleier etc)

☐ Høyskole/universitet mer enn 4 år (hovedfag, master, embetseksamen)

Spørsmål 10: Fars høyeste fullførte utdanning

☐ Mindre enn 9-årig grunnskole ☐ 9-årig grunnskole

☐ Videregående skole

☐ Høyskole/universitet inntil 4 år (cand.mag. bachelor, lærer, sykepleier etc)

☐ Høyskole/universitet mer enn 4 år (hovedfag, master, embetseksamen)

Spørsmål 11: Er det kjent atopisk sykdom (astma, allergier, eksem) hos foreldre eller søsken?

☐ Nei

☐ Mor har hatt/har astma/allergi/eksem

☐ Far har hatt/har astma/allergi/eksem

☐ Barnets søsken har/har hatt astma/allergi/eksem

Spørsmålene på denne siden (side 5) gjelder kun hvis barnet ammes nå:

Spørsmål 12: Melk og melkprodukter i mors kosthold

☐ Mor unngår melk og melkeprodukter, selv i små mengder.

☐ Mor bruker bare litt melk og/eller melkeprodukter.

☐ Mor inntar melk og/eller melkeprodukter som vanlig, mengdene begrenses ikke.

Spørsmål 13: Bruker mor kosttilskudd (vitaminer, mineraler, omega-3 etc)?

☐Ja ☐Nei

Hvis ja, angi hvilke kosttilskudd (produktnavn) og hvor ofte de vanligvis tas i løpet av en uke:

- _____
- _____
- _____

Spørsmål 14: Bruker mor helsekostpreparater (angi produktnavn, f.eks Spirulina)?

☐**Ja** ☐**Nei**

Hvis ja, angi hvilke (produktnavn) og hvor ofte de vanligvis tas i løpet av en uke:

- _____
- _____
- _____

Takk for at du/dere besvarte spørreskjemaet!

9.2 Appendix 2. Spørreskjema om kosthold

Spørreskjema om kosthold

Vi ber deg/dere svare på følgende spørsmål som del av studien ” Kosthold, vekst og ernæringsstatus hos barn som følger melkeproteinfri diett”.

Dato for utfylling av skjemaet: ☐☐☐☐ **201**☐

Skjemaet er utfylt av:

☐ Barnets mor ☐ Barnets far ☐ Både mor og far ☐ Annen person

Spørsmål 1: Får barnet morsmelk?

☐ Ja, bare morsmelk (og eventuelt tran eller annet kosttilskudd) *Gå til spørsmål 2*

☐ Ja, morsmelk og vann/juice/saft o.l. *Gå til spørsmål 2*

☐ Ja, morsmelk og fast føde samt eventuelt vann/juice/saft *Gå til spørsmål 2*

☐ Ja, morsmelk og morsmelkerstatning/annen melk *Gå til spørsmål 2*

☐ Ja, morsmelk og morsmelkerstatning/annen melk og fast føde samt eventuelt vann/juice/saft o.l *Gå til spørsmål 2*

☐ Nei, men barnet har tidligere fått morsmelk *Gå til spørsmål 2*

☐ Nei, barnet har aldri fått morsmelk *Gå til spørsmål 5*

Spørsmål 2: Ble barnet fullammet fra fødsel?

Ja ☐ Nei ☐

Spørsmål 3: Hvor mange ganger per døgn drikker barnet morsmelk?

☐12 ganger eller mer pr døgn

☐9-11 ganger pr døgn

☐6-8 ganger pr døgn

☐3-5 ganger pr døgn

☐1-2 ganger pr døgn

☐sjeldnere enn 1 gang pr døgn

Dersom barnet fullammes, gå til spørsmål 23, s 12.

Spørsmål 4: Dersom barnet ikke får morsmelk lenger, hvor gammelt var barnet når det sluttet å få morsmelk?

☐☐ Måneder

Spørsmål 5: Dersom barnet får annet å drikke enn morsmelk (vann, juice, saft, morsmelkerstatning, melk), hvor gammelt var barnet når annen drikke ble gitt for første gang?

☐☐måneder

Spørsmål 6: Drikker barnet en annen melketype enn morsmelk?

Ja ☐ Nei ☐

Hvis ja, hvilke(n) type(r) melk drikker barnet?

☐ Morsmelkerstatning

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

Hvor mange ganger per døgn drikker barnet morsmelkerstatning? ☐☐

Eller

Hvor mange ganger pr uke drikker barnet morsmelkerstatning? ☐☐

☐ Plantemelk (av f.eks havre, soya, ris)

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

☐ Hestemelk, geitemelk, annen melk fra dyr

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

De neste spørsmålene handler om fast føde (mat). Hvis barnet ditt ikke får fast føde, gå til spørsmål 23 s 12.

Spørsmål 7: Dersom barnet startet med fast føde/mat, hvor gammelt var barnet når fast føde (annen mat enn morsmelk/ kosttilskudd) ble gitt for første gang?

☐☐ måneder

Spørsmål 8: Hvor ofte spiser barnet industrifremstilt grøt/velling?

☐ 3 ganger eller mer pr dag

☐ 1-2 ganger pr dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn ukentlig

☐ Aldri

Hvilke(n) type(r) industrifremstilt grøt/velling benyttes (angi produktnavn, f.eks Hipp, Nestle, Semper, Holle)?

- _____

Spørsmål 9: Dersom barnet spiser industrifremstilt grøt/velling nå, hva slags væske tilsettes vanligvis ved tilberedning, (sett flere kryss hvis flere produkter benyttes)?

☐ Vann

☐ Morsmelk

☐ Morsmelkerstatning

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

☐ Plantemelk (f.eks havre, soya, ris)

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

☐ Hestemelk, geitemelk, annen melk fra dyr

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

Spørsmål 10: Hvor ofte spiser barnet hjemmelaget grøt/velling?

☐ 3 ganger eller mer pr dag

☐ 1-2 ganger pr dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn ukentlig

☐ Aldri

Hvilke(n) type(r) hjemmelaget grøt spiser barnet vanligvis (angi type grøt, f.eks havregrøt, hirsegrøt)?

- _____

Spørsmål 11: Dersom barnet spiser hjemmelaget grøt/velling nå, hva slags væske tilsettes vanligvis ved tilberedning. (sett flere kryss hvis flere produkter benyttes)?

☐ Vann

☐ Morsmelk

☐ Morsmelkerstatning

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

☐ Plantemelk (f.eks havre, soya, ris)

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

☐ Hestemelk, geitemelk, annen melk fra dyr

Hvilke(n) type(r) benyttes (angi produktnavn)?

- _____

Spørsmål 12: Hvor ofte spiser barnet fisk til middagsmat?

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Spørsmål 13: Hvor ofte spiser barnet fisk som pålegg? (f.eks makrell i tomat, sild, laks)

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Spørsmål 14: Hvor ofte spiser barnet kaviar som pålegg?

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Spørsmål 15: Hvor ofte spiser barnet skalldyr? (f.eks blåskjell, reker, Scampi, kamskjell)

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Spørsmål 16: Hvor ofte spiser barnet rognleverpostei som pålegg?
(Svolværpostei-/Lofotpostei)

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Spørsmål 17: Hvor ofte spiser barnet egg som pålegg eller retter med mye egg i (f.eks omelett eller pannekaker med egg)?

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Spørsmål 18: Hvor ofte spiser barnet kylling til middag?

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Spørsmål 19: Tilsettes det salt i middagsmaten barnet spiser?

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Hvilke(n) type(r) salt benyttes vanligvis (angi produktnavn)? (f.eks jozo, jozo med jod, Seltin, havsalt)

- _____

Spørsmål 20: Hvor ofte tilsettes salt i annen mat enn i middagsmaten til barnet? (f.eks til kokt egg, pålegg, i hjemmelaget grøt)

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Hvilke(n) type(r) salt benyttes vanligvis (angi produktnavn)?

- _____

Spørsmål 21: Hvilke matvarer gir dere IKKE til barnet?

☐Glutenholdig korn/mel (hvete, spelt, rug, bygg)

☐ Vanlig kumelk /yoghurt/ost

☐ Appelsin/appelsinjuice/annen sitrusfrukt

☐ Fisk/skalldyr

☐ Nøtter/nøtteprodukter (peanøttsmør o.l.)

☐Belgfrukter (erter, bønner o.l.)

☐ Egg

☐ Soya

☐ Salt

☐ Matvarer med tilsetningsstoffer

☐ Mat som ikke er økologisk dyrket

☐ Annet _____

Spørsmål 22: Barna som er med i denne studien er fra 0-2 år. Hva de spiser og drikker vil avhenge av alder. Har barnet problemer i forhold til mat/spising som ikke er naturlige i forhold til barnets alder?

☐ Nei, har ikke noen problemer

☐ Ja, dårlig matlyst/småspist

☐ Ja, liker få matvarer

☐ Ja, vanskelig med tilvenning til familiens kosthold

☐ Ja, allergi/intoleranse mot flere matvarer i tillegg til melk

Oppgi hvilke:

_____

☐Ja, andre problemer

Oppgi hvilke:

_____

_____

(f.eks problemer med å suge fra brystet, barnet vil ikke ta flaske, svelgeproblemer, brekker seg lett)

Dersom barnet ved noe tidspunkt har vært sondeernært, i hvilken tidsperiode (fra alder- til alder) var dette?

- _____

Spørsmål 23: Hvor ofte tar barnet kosttilskudd?

☐Hver dag

☐ 3-6 ganger pr. uke

☐ 1-2 ganger pr. uke

☐ Sjeldnere enn 1 gang pr uke

☐ Aldri

Hvilke(n) type(r) benyttes (angi produktnavn og mengde)?

- _____
- _____

(Eksempel Nycoplus multi flytende, Sanasol, Nycoplus multi vitamin og mineraltbl for barn, kalsium 250 mg, kalsium 500 mg, NeoFer (jerntilskudd), omega-3 tilskudd, vitamin C, Møllers tran med sitronsmak)

Spørsmål 24: Tar barnet helsekostprodukter (f.eks probiotika, alger el.) nå (beskriv hvilke produkter og dosering)?

Ja ☐ **Nei** ☐

Hvis ja, hvilke(n) type(r) benyttes (angi produktnavn og mengde)?

_____

Spørsmål 25: Hvor har du/dere fått informasjon om amming/spedbarnsernæring, og hvordan vurderer du/dere denne informasjonen? Sett et kryss pr rad

	Ikke fått informasjon	Svært nyttig	Nyttig	Lite nyttig	Unyttig
Føde-barselavdeling					
Helsestasjon					
Helsepersonell utenforhelsestasjon/ sykehus f.eks kiropraktor					
Homøopat					
Familie/kjente					
Ammehjelpen					
Bøker/oppslagsverk					
Aviser/TV/ukeblad					
Reklamemateriell					
Butikken					
Mattilsynets hjemmeside					
Helsedirektoratets hjemmeside					
Norges astma- og allergiforbund www.naaf.no					
www.matportalen.no					
Andre internettsider					
Klinisk ernæringsfysiolog					
Lege					
Sykepleier					

Spørsmål 26: Hvor lang tid måtte du/dere vente på kostholdsveiledning fra det ble bestemt at barnet skulle følge melkeproteinfri diett?

Tid (angi dager, uker, måneder): _____

Ta kontakt på telefon 23015744/ 23015743 dersom du har spørsmål.

Takk for at du/dere besvarte spørreskjemaet!