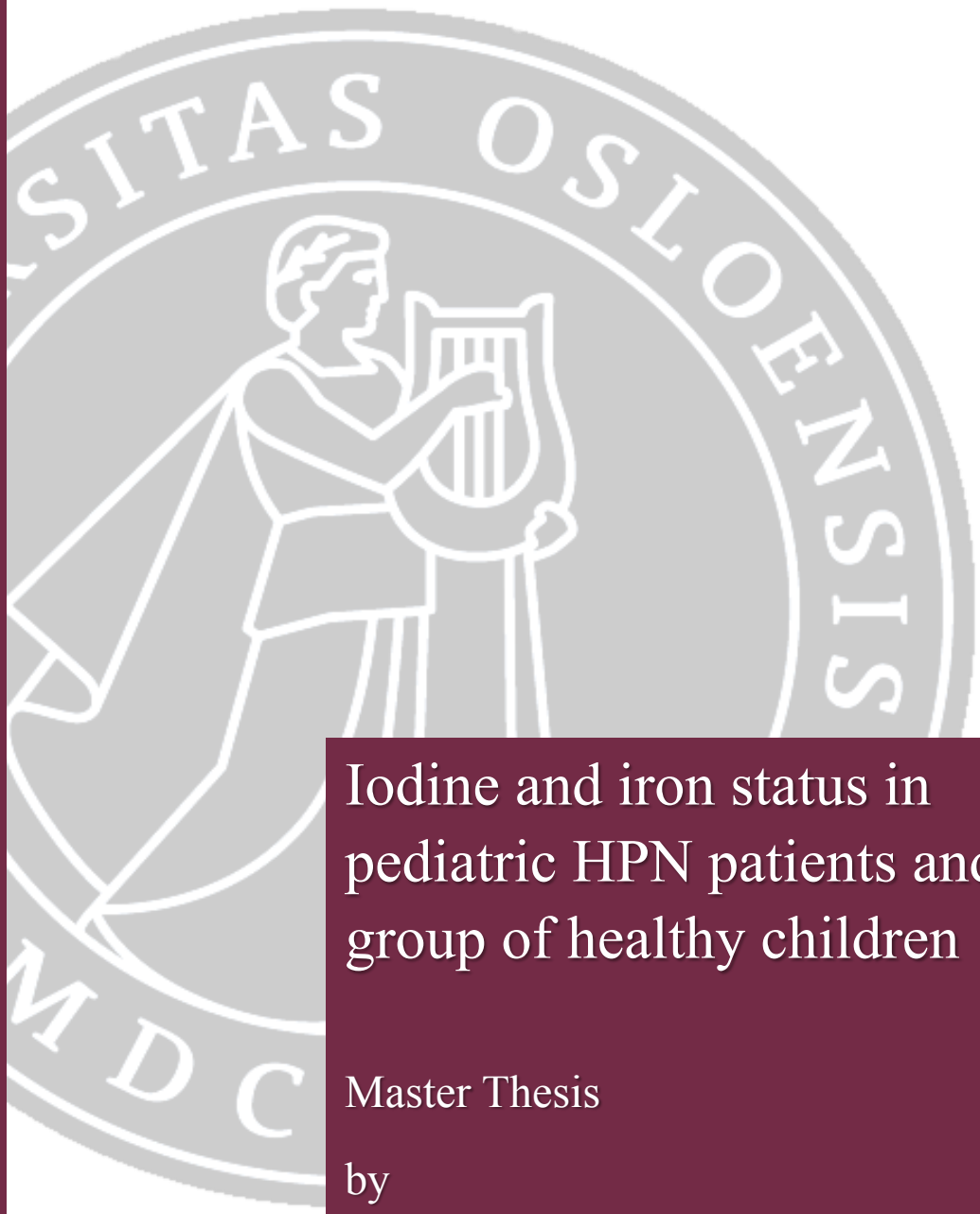

Iodine and iron status in pediatric HPN patients and a group of healthy children

Master Thesis

by

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Abstract

Background: Sufficient supply of iodine and iron to patients receiving home parenteral nutrition (HPN) may be challenging. No previous studies have assessed iodine and iron status among Norwegian pediatric HPN patients. Iodine deficiency may cause numerous detrimental effects, including goiter, impaired mental function and metabolic abnormalities. Iron deficiency is the most prevalent cause of anemia. These minerals are important for normal growth and development, making children especially vulnerable for deficiencies. Few studies have assessed iodine and iron status among healthy Norwegian children.

Aim: The aim of the present study was to assess iodine and iron status among children receiving HPN and a group of healthy children by measuring biomarkers and estimating nutrient supply.

Methods: Nineteen HPN patients and 35 healthy children, age two to 18 years, were included in a cross-sectional study. Intake of iodine and iron were assessed by four-day food records. Two spot urine samples from each subject were analyzed for iodine concentration (UIC) and creatinine. Estimated 24-hour urinary iodine excretion was calculated by scaling UIC and creatinine values to published 24-hour creatinine reference values for healthy children. Blood samples were analyzed for hemoglobin, ferritin, and transferrin receptor to assess iron status.

Results: Mean age in the HPN patients and healthy children, was ten and nine years, respectively. Median intake of iodine was lower than RDI in both HPN patients and healthy children, (93 % vs. 80 % of RDI). HPN patients had a median parenteral iodine provision above ESPGHAN recommendation (2.7 µg/kg/day). Median UIC was classified as insufficient in the HPN patients (89 µg/L) but adequate among the healthy children (130 µg/L). Estimation of 24-hour urinary iodine excretion confirmed an insufficient iodine status among HPN patients (67 %), and suggested an insufficient iodine status in 59 % of the healthy children. There were no significant differences between the groups in any iodine parameters. The iron provision among HPN patients was significantly lower than iron intake in the healthy children (54 % of RDI vs. 97 % of RDI, p=0.004). All HPN patients had parenteral iron provision below ESPGHAN recommendation. The prevalence of anemia was significantly higher among HPN patients compared to healthy children (42 % vs. 12 %, p=0.016).

Conclusion: This small, cross-sectional study indicates an insufficient iodine and iron status among pediatric HPN patients. The iodine status was however, not significantly different from the group of healthy children. Further research is warranted to confirm our findings.

Abbreviations

ALL	Acute lymphatic leukemia
CIPO	Chronic intestinal pseudo obstruction
CRP	C-reactive protein
CVK	Central venous catheter
DALY	Disability-adjusted life year
DMT1	Divalent metal transporter 1
DXA	Dual-energy X-ray absorptiometry scan
E %	Percent of total energy intake
EAR	Estimated average requirement
EN	Enteral nutrition support
ESPEN	European Society of Clinical Nutrition and Metabolism
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
Est24h UIE	Estimated 24-hour urinary iodine excretion
FCT	Food composition table
GI	Gastrointestinal
Hb	Hemoglobin
HCP	Haem iron transporter
HPN	Home parenteral nutrition
I^-	Iodide
IBD	Inflammatory bowel disease
ICIDD	The International Council for Control of Iodine Deficiency Disorders
IF	Intestinal failure
IO^{3-}	Iodate

IQ	Intelligence quotient
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
NA	Not applicable
NMBU	Norwegian University of Life Sciences
NNR	Nordic Nutrition Recommendations
OUH	Oslo University Hospital
UiO	University of Oslo
PN	Parenteral nutrition
PPN	Partial parenteral nutrition
RDI	Recommended daily intake
RDI*	Recommended daily intake adjusted for 15 % nonrenal iodine loss
REC	Regional committees for medical and health research ethics
SBS	Short bowel syndrome
SD	Standard deviation
TIBC	Total iron binding capacity
TPN	Total parenteral nutrition
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
T3	Triiodothyronine
T4	Thyroxine
UIC	Urinary iodine concentration
UNICEF	United Nations Children's Fund
WHO	World Health Organization
24h UIE	24-hour urinary iodine excretion

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1 Introduction

1.1 Intestinal failure

Intestinal failure (IF) is a condition of severe intestinal malabsorption which may be reversible or irreversible depending on the underlying disease and treatment given. The condition makes the patient unable to meet nutritional requirements solely through enteral route. Therefore, provision of parenteral nutrition (PN) is vital for these patients (1, 2).

IF is defined as *"a critical reduction of the gut mass or its function below the minimum needed to absorb nutrients and fluids required for adequate growth in children and weight maintenance in adults" (1).*

IF may be caused by a functional loss of gut mass or various congenital or acquired disorders which lead to impaired absorption of nutrients, fluids and electrolytes (3).

The most common causes of IF in pediatric patients can be divided into three categories (1):

1. Disorders where the absorptive surface of the intestine is reduced, such as short bowel syndrome (SBS).

2. Disorders of bowel dysmotility which leads to reduced digestion and absorption, such as in chronic intestinal pseudo obstruction (CIPO).

3. Disorders where the intestine is intact but the absorption is insufficient, i.e. malabsorption is present.

1.1.1 Short bowel syndrome

Short bowel syndrome (SBS) is a collective term comprising disorders where there is a physical loss of the small intestine due to surgical resection, or a functional loss due to a congenital defect or a disease process. Diseases that may lead to SBS include Hirschprung's disease, gastroschisis and volvulus (3).

Hirschprung's disease is a congenital disorder comprising impairment of the enteric nervous system' innervation of the intestines. The colon becomes constricted and lead to megacolon

whereby the patient is unable to defecate. The condition is fatal without surgical removal of affected areas (4).

Gastroschisis is an abdominal wall defect with herniation of abdominal content, occurring during fetal development. Postnatal outcome varies from simple cases where survival rate is high, to more complex cases which may include atresia or necrosis of the intestine and thus increased morbidity and mortality rates (5).

Volvulus is a bowel obstruction that may occur at any segment of the GI tract. However, the small bowel or colon is most frequently affected. The intestines become twisted leading to an obstruction of the lumen and impairment of intestinal blood flow (6).

Several factors influence the outcome and severity of SBS: length and quality of functioning intestine, site of resection, presence or absence of colon in continuity and presence or absence of the ileocecal valve, as well as the nature of the underlying disease (7). Furthermore, the intestine has great capacity to adapt and take over functions of resected areas, especially in children. In healthy individuals, most nutrients are absorbed within jejunum. However, loss of jejunum is better tolerated than the loss of ileum. This is in part due to a greater capacity for adaption displayed by the ileum than jejunum. Moreover, the transit time is slower through ileum, and ileum have the ability to absorb fluid and nutrients against a gradient (7).

1.1.2 Bowel dysmotility

Appropriate bowel motility necessary for normal digestion and absorption is controlled by the enteric nervous system. Normal transition of food through the gastrointestinal (GI) tract is aided by action potentials transmitted from the enteric nervous system to the smooth muscles of the intestines, whereby leading to peristaltic waves propelling the food onwards. Motility disorders may be due to dysfunction of either the enteric nervous system or the enteric muscles. The outcome is impaired digestion and absorption and some motility disorders may lead to IF, such as CIPO (1).

CIPO is a heterogeneous collection of disorders which entail recurrent symptoms of intestinal obstruction in absence of a mechanical occlusion (7). CIPO can affect any segment of the GI tract, therefore symptoms and severity will vary with each case depending on segment(s) involved and the length of these (8).

1.1.3 Severe malabsorption

Malabsorption refers to impairment of both digestion and absorption. It includes a wide spectrum of disorders, which may be congenital or acquired. Severe cases may lead to intestinal failure whereby the patient will need treatment with parenteral nutrition to compensate for intestinal loss (9).

Enteropathies are a group of disorders affecting polarization and differentiation of enterocytes, thus leading to severe malabsorption of all nutrients, electrolytes and fluids (1, 10). Severe malabsorption may also occur in patients with inflammatory bowel disease (IBD). IBD is a collective term comprising Chron's disease, ulcerative colitis and IBD-unspecified. These are immune-mediated GI disorders, which can affect different parts of the GI tract, leading to a varying degree of malabsorption in the patient (11). However, parenteral nutrition is seldom indicated in pediatric IBD patients (12).

1.2 Home parenteral nutrition

Insufficient digestion and absorption in patients with IF make parenteral supply of nutrients, fluids and electrolytes vital (13, 14). Patients with prolonged IF may be eligible for treatment with home parenteral nutrition (HPN) (15, 16). HPN enables the patient to receive lifesaving nutritional treatment at home when hospitalization is not necessary, which is widely recognized as improving the quality of life of both children and families involved (17).

The treatment was first offered in the US in 1969 (18) and in Norway in 1989. Currently, approximately 23 children are receiving HPN at Oslo University Hospital (OUH).

Although the treatment is associated with adverse complications, such as risk of catheter-related bloodstream infections and loss of venous access, the prognosis and survival of children on HPN has dramatically increased over the last 30 years (19). However, long-term administration of PN (>six months) is associated with increased risk of developing micronutrient deficiencies. This is due to the underlying disease process itself, and the challenges in achieving adequate amounts of micronutrients in the PN. The amount of nutrients that can be added to the PN solution may be limited by the manufacture process and the stability of the PN solution (20). Thus, surveillance of nutritional status in pediatric HPN patients is crucial to secure the patients' health, growth and development.

Although HPN is a lifesaving treatment for many patients with IF, use of the GI tract is the preferred feeding route if possible, as it is safest and most physiological (1). Therefore, gastroenterologists and dieticians strive to supply their patients with nutrients by enteral route in addition to the parenteral supply. Thus, the patient's GI tolerance and absorption of nutrients must be assessed, and the amount and composition of the PN adjusted accordingly.

1.2.1 Nutritional status among pediatric patients with home parenteral nutrition

An optimal nutritional status entails a sufficient supply of nutrients, an adequate growth and absence of clinical signs of nutrient deficiencies. Assessment of nutritional status comprise anthropometric measurements, estimation of nutrient intake and clinical evaluation of the subject (7, 21, 22).

Pediatric patients treated with HPN need a balanced supply of macronutrients as well as vitamins and trace elements, to maintain growth and development (23). Growth failure and negative energy- and protein balance have previously been reported among pediatric HPN patients (24, 25).

Children are especially vulnerable to micronutrient deficiencies due to growth and development, and it is recommended to monitor vitamin and mineral status in these patients (11, 19). Previous studies have indicated that supply of iodine and iron may be challenging in this patient group (26-30). We therefore wished to investigate this further in Norwegian, pediatric HPN patients.

1.3 Iodine

1.3.1 Overview

Iodine is an essential micronutrient necessary for production of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) by the thyroid gland (31). T_3 and T_4 mediate their action by binding to nuclear receptors found in most tissues and organs. The effects facilitated by these hormones include regulation of metabolic rate and macronutrient metabolism. The hormones are also crucial for neurologic development and growth, therefore making children especially vulnerable for deficiencies (32, 33).

1.3.2 Absorption and metabolism

Iodine is found in food items in different oxidation states, including iodide (I^-) and iodate (IO_3^-). Furthermore, iodine is often present in food as a salt, such as potassium iodide or sodium iodide. Absorption of dietary iodine is aided by the stomach and duodenum. In the stomach ingested forms of iodine can be reduced to iodide before absorption. In the duodenum, the apical membrane of enterocytes displays sodium/iodide symporters. These transport proteins mediate active transport of sodium and iodide (34-36).

The absorption of iodine in healthy adults is thought to be greater than 90 % (36). However, several dietary factors may inhibit iodine absorption and metabolism, collectively called goitrogens. These food items include cruciferous vegetables, sweet potato and lima beans (37).

Circulating iodine is cleared form the bloodstream by the thyroid gland and kidneys, with the relative proportion cleared by the thyroid gland increasing in conditions of iodine deficiency. Under normal conditions, approximately 90 % of ingested iodine is excreted through the urine, with some iodine lost through feces and skin (36).

The follicular cells in the thyroid express a sodium/iodide symporter which facilitates iodine uptake to the thyroid gland against a steep electrochemical gradient (38). Iodide is then secreted from the follicular cell and into the follicle together with thyroglobulin. Thyroglobulin is a glycoprotein containing tyrosyl residues to witch oxidized iodine atoms can attach. After attachment of iodine, two tyrosyl residues can conjugate within the thyroglobulin molecule. While still bound to thyroglobulin, the thyroid hormones remain inactive and stored as colloid in the follicle. Before activation of the hormones, the thyroglobulin is endocytosed by the follicular cell and as the vesicle travels across the cell, the thyroglobulin is hydrolyzed and thus release T_3 and T_4. The hormones thereafter enter the circulation, Figure 1.

The thyroid releases mostly T_4 to the blood and little T_3. However, T_3 is the biological active hormone. Peripheral tissues, especially liver and kidney deiodinate T_4 to T_3. In circulation, the hormones are bound to plasma proteins with high affinity for the molecules. Only unbound hormone can bind to its nuclear receptor and trigger a cellular response. Thyroid hormone receptors are expressed throughout most of the body's tissues, and will

upon binding of its ligand bind to thyroid response elements in the promoter region of target genes and consequently lead to repression or activation of these (31, 34, 35, 39).

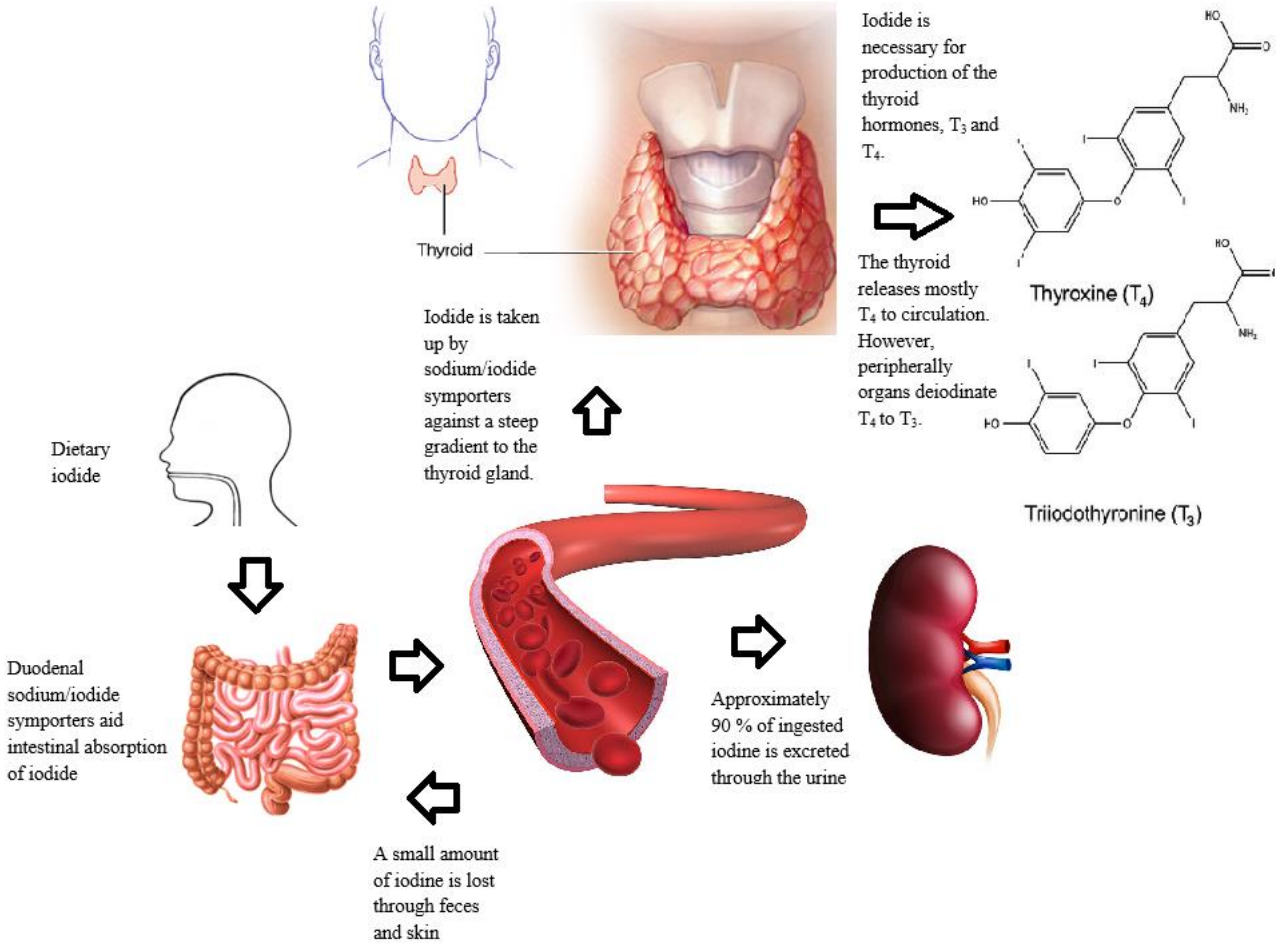

Figure 1. Overview of iodine absorption.
Dietary iodide is absorbed by duodenal sodium/iodide symporter and thereafter cleared from circulation by the kidneys and the thyroid gland. In the thyroid gland iodine is used for synthesis of the thyroid hormones, thyroxine and triiodothyronine. Adapted from (34-36, 38).

The various steps of thyroid hormone synthesis are in large part regulated by the hypothalamus. Hypothalamus releases the thyrotropin-releasing hormone (TRH) to the anterior pituitary, where it upon binding to its receptor trigger both synthesis and release of thyroid-stimulating hormone (TSH). TSH then binds to its receptor on thyroid follicular cells and facilitate several events:

- Increased iodide uptake from the circulation into the follicular cells by increasing the activation of the sodium/iodide symporter
- Increased iodination of thyroglobulin
- Stimulate conjugation of tyrosyl residues within thyroglobulin

- Stimulate proteolysis of thyroglobulin
- Increased secretion of T_3 and T_4 to the circulation
- Stimulate hyperplasia of the thyroid gland.

Thus, TSH stimulates every step of the thyroid hormone production (34, 35).

1.3.3 Recommendations

Iodine requirement varies throughout the life span. The Nordic Nutrition recommendation (NNR) and recommendations from The World Health Organization (WHO)/The International Council for Control of Iodine Deficiency Disorders (ICIDD)/United Nations Children's fund (UNICEF) are shown in Table 1. The WHO/ICIDD/UNICEF' iodine recommendations are higher for infants, toddlers, pregnant and lactating women than the Nordic recommendations.

Table 1. NNR and WHO/ICIDD/UNICEF recommendations for iodine intake (40, 41).

Age	Nordic Nutrition Recommendations µg iodine/day	WHO/ICIDD/UNICEF Recommendations µg iodine/day
6-11 months	50	90
12-23 months	70	90
2-5 years	90	90
6-9 years	120	120
>10 years	150	150[a]
Pregnancy	175	250
Lactation	200	250

NNR = Nordic Nutrition Recommendations
WHO = World Health Organization
ICIDD = The International Council for Control of Iodine Deficiency Disorders
UNICEF = United Nations Children's fund
[a] > 12 years

European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommend a daily dose of one µg iodine/kg body weight for children receiving PN (15). In the US and other countries, it has been common to use antiseptics containing iodine, which therefore have contributed to patients' total iodine supply (42, 43). However, this is not common practice in Norway.

1.3.4 Dietary sources

Norway has mandatory iodization of cow fodder. This leads to a high iodine content in milk and dairy products. Because there is a high consumption of this food-group, milk and dairy

products are important sources to iodine in the Norwegian diet (44-46). Fish, especially lean freshwater fish, has a high iodine content and is consequently an excellent source of iodine, but is quantitatively not as important a source as milk and dairy products. Iodization of salt is important for iodine prophylaxis and improvement of iodine status in several countries (47). However, in Norway there are only some brands of table salt that are added iodine, and the level of fortification is very low compared to other countries (5 µg iodine/g NaCl). Furthermore, industrial salt is not iodized, thus making total iodine contribution from salt insignificant in Norway (45, 48). Table 2 show iodine content of different food items.

Table 2. Overview of iodine content in various Norwegian food items. Adapted from (49).

Food item	Iodine content (µg) per 100 g	Common serving size (g)	Iodine content (µg) per serving
Iodized salt	500	1	5
Goat cheese	140	16	22
Cod	120	200	240
Caviar	85	15	13
Mackerel	60	150	90
Egg	49	56	27
Cheese, white	40	20	8
Milk	20	200	40
Fruit yoghurt	17	150	26
Salmon	10	150	18

1.3.5 Parenteral iodine provision

To ensure adequate supply of trace elements in HPN patients, parenteral trace element solutions, such as Addaven or Peditrace, are routinely added to the PN of patients treated at OUH. However, iodine content of Addaven is much higher than that of Peditrace, Table 3.

Table 3. Iodine content of Addaven and Peditrace.

	Addaven		Peditrace	
	µg/ml	µg/kg/day	µg/ml	µg/kg/day
Iodine	13	1.3-3.9	1	1.0

µg/kg/day according to recommended dosage
Recommended dosage children >15 kg: 0.1-0.3 ml/kg (maximum 10ml) of Addaven. Children <15 kg: 1 ml/kg/day (maximum 15 ml) of Peditrace

1.3.6 Deficiency

Iodine deficiency may lead to numerous adverse effects, collectively called iodine deficiency disorders. These are caused by implications on the thyroid hormone production. The outcome of iodine deficiency depends on the age of the subject and the severity of the deficiency. In children and adolescents are goiter, weight loss, tachycardia, muscle weakness, impaired mental function, metabolic abnormalities and delayed physical development potential consequences (27, 50, 51). Iodine deficiency both during prenatal stages and early childhood may have detrimental consequences for the developing brain, and is considered one of the most important preventable causes of brain damage globally (52, 53). Studies indicate that even moderate to mild iodine deficiency in children may impair cognitive function, hearing capacity and growth (54-57).

Short-term deficits in iodine intake may temporarily be offset by releasing iodine stored in the thyroid gland and by increasing thyroid clearance of iodine from the bloodstream, Figure 2. However, if the situation persists, these mechanisms will eventually fail to sustain a sufficient supply of iodine to the thyroid and its hormone production. Thus, iodine deficiency undergoes two phases: In the first phase, the dietary iodine intake is below the individual's requirement, and this may be assessed by measuring urinary iodine concentration (UIC). In the second phase, when compensating mechanisms are exhausted, thyroid dysfunction occurs and this may be assessed by measuring thyroid hormones and thyroid size (52).

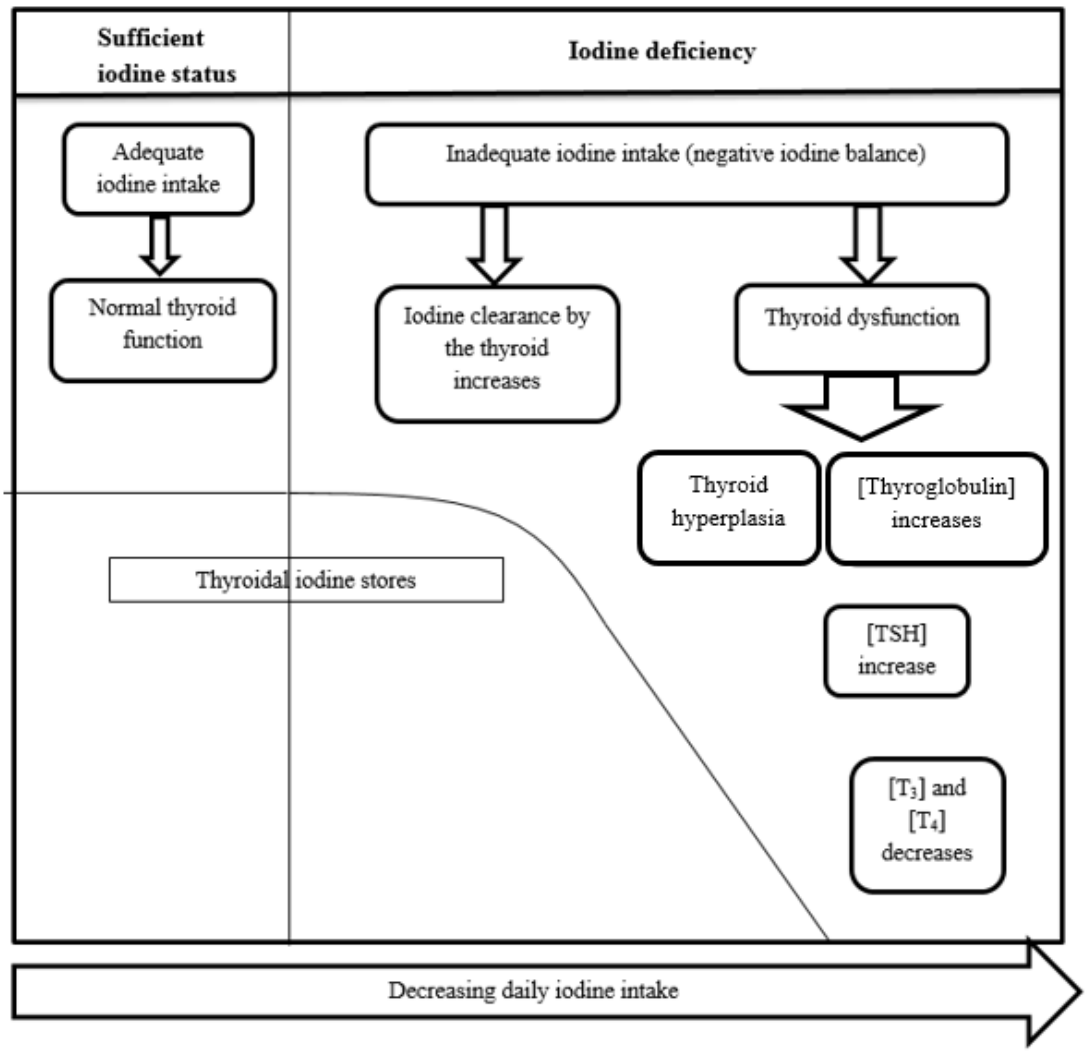

Figure 2. Stages of iodine status. modified from (52).
TSH = Thyroid stimulating hormone
T_3 = Triiodothyronine
T_4 = Thyroxine

1.3.7 Assessment of iodine status

The recommended method to determine iodine status at group level is measurement of median urinary iodine concentration (UIC) in spot urine samples (41, 58). Because approximately 90 % of ingested iodine is excreted in the urine, UIC reflects recent iodine intake (59). WHO has set 100 µg/L as the cut-off value for sufficient iodine status in a population (41).

Although the use of UIC is well established, the method has been criticized for being influenced by the subjects' hydration status. Fluid intake may be a confounding factor of UIC and the method may therefore not give a valid estimate of iodine status if the population is either dehydrated or over-hydrated (58). It has been suggested that scaling

UIC values to creatinine can eliminate the effect of hydration status. The method is based upon spot urine samples analyzed for iodine and creatinine concentration, whereby this is scaled in relation to 24-hour reference values for creatinine excretion. Montenegro-Bethancourt et al. conducted a study showing stronger correlation between 24-hour urinary iodine excretion rates (24h-UIE) and a creatinine-scaled estimate of 24-hour urinary iodine excretion (Est24h UIE), than with UIC (58).

Iodine status may also be estimated by mapping dietary intake through food records. In countries where salt significantly contributes to iodine intake, this will not be an appropriate method due to difficulties estimating the intake of iodized salt (37). However, food records are suitable in countries such as Norway, where relatively few food items make up the bulk of iodine intake, namely milk, dairy products and fish (60).

1.3.8 Iodine deficiency and parenteral nutrition

Previous studies conducted to assess iodine status among HPN patients have been carried out on an adult population, a pediatric population without iodine routinely supplemented and pediatric patients supplemented at a level expected to be lower than in the present population (26, 27, 61). Moreover, there are no previous studies assessing iodine status of pediatric HPN patients in Norway.

1.3.9 Iodine deficiency among healthy children

The Norwegian Mother and Child Cohort Study is currently the world's largest study of dietary iodine intake, and has reported an insufficient iodine intake among pregnant Norwegian women (62). However, the study has not assessed iodine status in children.

In 2015 the national dietary survey, UNGKOST 3, was conducted. The study mapped the diet of Norwegian nine and 13 year olds (63). Nationally representative data is important for assessment of nutritional status among Norwegian children. However, data on iodine intake were missing from this survey. Dahl et al used data from UNGKOST 2000 to estimate iodine intake among Norwegian children and adolescents (45). However, dietary habits are continuously changing, and therefore updated data on iodine intake within this group is warranted. Moreover, no previous studies have measured UIC in spot urine samples in healthy Norwegian children.

1.4 Iron

1.4.1 Overview

Iron is an essential micronutrient necessary for a variety of biological functions. These include transport and storage of oxygen, production of adenosine triphosphate during oxidative phosphorylation and aiding enzymatic reactions as a cofactor to numerous metabolic enzymes (64). Iron's enzymatic function relies on its ability to undergo redox cycling between ferric (Fe^{3+}) and ferrous (Fe^{2+}) oxidation states (65).

An adult human body contains approximately 35-45 mg iron/kg bodyweight (66). This is distributed between three different pools: a functional pool, a storage pool and a transport pool. Most iron resides within the functional pool, as constituents of hemoglobin in circulating erythrocytes (60-70 %) and as components of enzymes and other proteins (10 %). Some iron is found within the storage pool, stored as ferritin and hemosiderin in liver, spleen and bone marrow (20-30 %), while a small amount (<1 %) of the total iron is found within the transport pool, bond to transferrin (66-68).

1.4.2 Absorption and metabolism

Regulation of the body's iron status is achieved through its absorption, as there does not exist controlled mechanism regulating iron excretion. Iron loss occurs through shedding of enterocytes in the intestines, sweat, menstruation or other blood loss, and shedding of hair and skin cells. The rapid turnover of intestinal cells accounts for most of the iron loss (69). The duodenal absorption of iron is typically low, but may range from 5-35 %. Absorption is influenced by a variety of factors, such as type of iron ingested, other dietary nutrients present in the duodenum at the same time, and metabolic demands (67).

The diet contains two major forms of iron: haem iron and non-haem iron. Haem iron has a high bioavailability and its absorption is not notably affected by other dietary factors. Non-haem iron has a considerably lower bioavailability, and its absorption is highly affected by the presence of other dietary factors. Moreover, haem and non-haem iron have different mechanisms of absorption (67, 70).

From the intestinal lumen, non-haem iron is transported into the enterocytes by divalent metal ion transporter 1 (DMT1). However, this protein only transports iron in its ferrous

form, whereas most dietary iron entering the duodenum is in ferric form. Thus to be absorbed the iron must first be reduced, either by reducing enzymes in the intestines or vitamin C from diet. Haem iron, on the other hand, is absorbed by means of endocytosis by haem iron transporter (HCP) and then released inside the enterocyte (70).

Within the enterocyte, iron (Fe^{2+}) is either stored as ferritin or released to the circulation. Due to rapid turnover of the enterocytes, iron stored within the enterocyte will eventually be lost. From the basolateral membrane of the enterocyte, iron is released to the circulation by ferroportin 1 and hephaestin (70). Upon release to the bloodstream iron is bound by transferrin and transported according to the body's need: to the bone marrow when need for erythropoiesis, various cells when need for enzymes containing iron, or to liver, spleen and bone marrow for storage as ferritin and hemosiderin (67).

Further regulation of iron absorption is provided by the hepatic-derived hormone hepcidin. Hepcidin can bind to and internalize ferroportin 1, thereby inhibiting the release of iron from enterocytes to the circulation. Iron deficiency decrease hepcidin level, while elevation of hepatic iron stores, transferrin saturation and systemic inflammation increases the level of hepcidin (71). Figure 3 illustrates an overview of iron absorption.

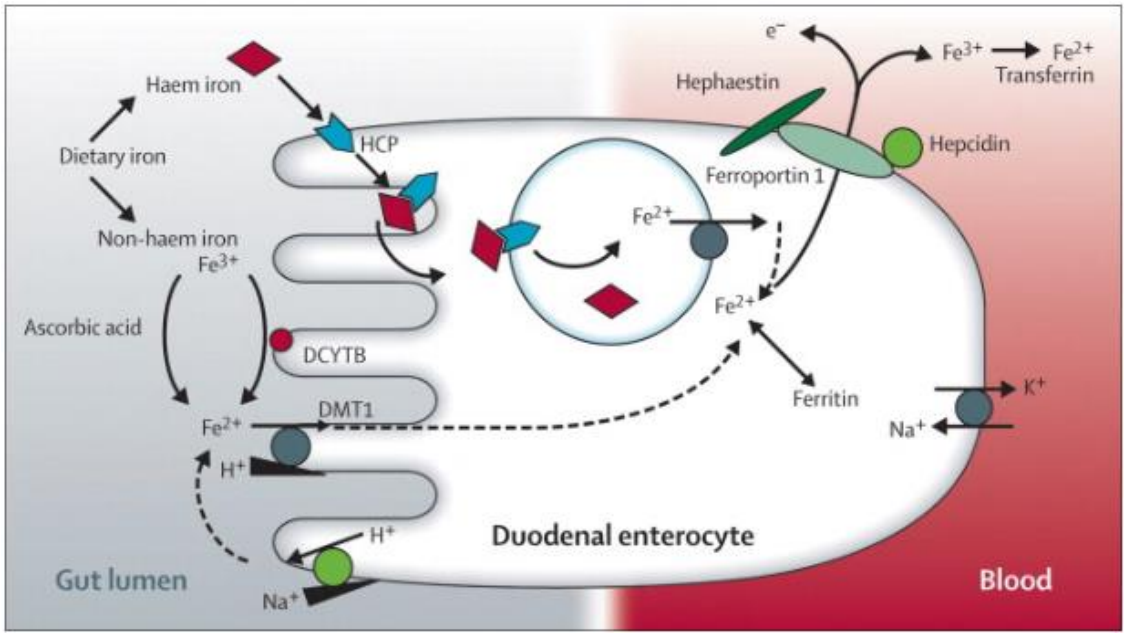

Figure 3. Intestinal iron absorption. Dietary haem iron is absorbed by means of endocytosis to the enterocyte by haem iron transporter (HCP). Fe^{2+} is thereafter released from the endosome. Non-haem iron is often present in its ferrous form, and must be reduced before absorption by divalent metal iron transporter 1 (DMT 1). Absorbed Fe^{2+} can be stored as ferritin or released to circulation by ferroportin 1 and hephaestin. This release may be inhibited by the hepatic-derived hormone hepcidin. Hepcidin bind to ferroportin 1 and cause its internalization and degradation, thereby inhibit iron export to the blood. Reproduced with permission (70).

1.4.3 Recommendations

Recommendations of iron intake comprises estimates on bioavailability and absorption, obligatory iron loss, basal iron requirement and iron needed for growth during childhood, adolescents and pregnancy, Table 4 (72).

Table 4. Recommended iron intake according to NNR (40)

Age (years)	Recommended daily iron intake (mg)
2-5	8
6-9	9
10-13	11
Women	15/9[a]
Men	9

NNR = Nordic Nutrition Recommendations
[a] Postmenopausal women

1.4.4 Dietary sources

Haem iron is primarily found in meat, poultry and fish as constituent of hemoglobin and myoglobin. Non-haem iron is more ubiquitous and can be found in various amounts in cereals, fruits, vegetables and legumes. Despite the high bioavailability of haem iron, non-haem iron often accounts for the majority of iron in most meals (67). Concomitant consumption of other dietary factors may influence iron absorption. Inhibitors of iron absorption include phytate and polyphenols from plant foods, and calcium, while enhancers include ascorbic acid and "meat factor" (72).

1.4.5 Parenteral iron provision

Addaven or Peditrace are used to ensure provision of trace elements to pediatric patients receiving PN. However, only Addaven contains iron, Table 5.

Table 5. Iron content of Addaven and Peditrace.

	Addaven		Peditrace	
	µg/ml	µg/kg/day	µg/ml	µg/kg/day
Iron	110	11-33	0	0

µg/kg/day according to recommended dosage
Recommended dosage children >15 kg: 0.1-0.3 ml/kg (maximum 10ml) of Addaven. Children <15 kg: 1 ml/kg/day (maximum 15 ml) of Peditrace

1.4.6 Iron deficiency and iron deficiency anemia

Iron deficiency is defined as having low body iron with or without concomitant anemia. Iron deficiency is the most prevalent nutritional disorder worldwide and is the most common cause of anemia (73). Several factors may lead to iron deficiency, such as inadequate iron intake, decreased absorption, chronic infections, inherited blood disorders or blood loss (74, 75). Children and adolescents are at particular risk of developing iron deficiency due to increased demands of nutrients during growth and development. Increasing bone and muscle tissue during growth leads to expansion of blood volume and therefore an increased need of iron to meet the demand for hemoglobin and myoglobin (68). Studies indicate that iron deficiency anemia during childhood may impair cognitive development (76, 77), and a recent meta-analysis found that increasing iron status in patients with iron deficiency without anemia, improved fatigue (78). Globally, iron deficiency anemia is an important contributor to estimated years lived with a disability (disability-adjusted life year, DALY), and severe anemia has been linked to increased childhood mortality (79, 80).

Anemia is defined by a hemoglobin (Hb) level that is two standard deviations below the mean for age and gender (81). Decreased Hb level may be caused by a reduction in number of red blood cells or a reduced amount of hemoglobin within the red blood cells (72). Anemia can be further categorized as microcytic, normocytic or macrocytic depending on the cause of anemia. Iron deficiency anemia often leads to microcytic red blood cells and hence microcytic anemia (82).

The clinical presentation of iron deficiency anemia is often nonspecific and include symptoms encountered with several other diagnoses, such as fatigue, pallor and shortness of breath (83). Iron deficiency anemia occurs progressively with a steady loss of iron from stores in situations with an inadequate iron intake. Diagnosis of iron deficiency anemia is in fact a late manifestation of depleted or exhausted iron stores. The stages leading up to iron deficiency anemia is as follows: negative iron balance where loss exceeds absorption, depletion of stored iron, iron-deficient erythropoiesis and finally iron deficiency anemia, Figure 4. Because turnover of red blood cells is approximately 120 days, it takes some time from depleted iron stores is mirrored in low hemoglobin values (83).

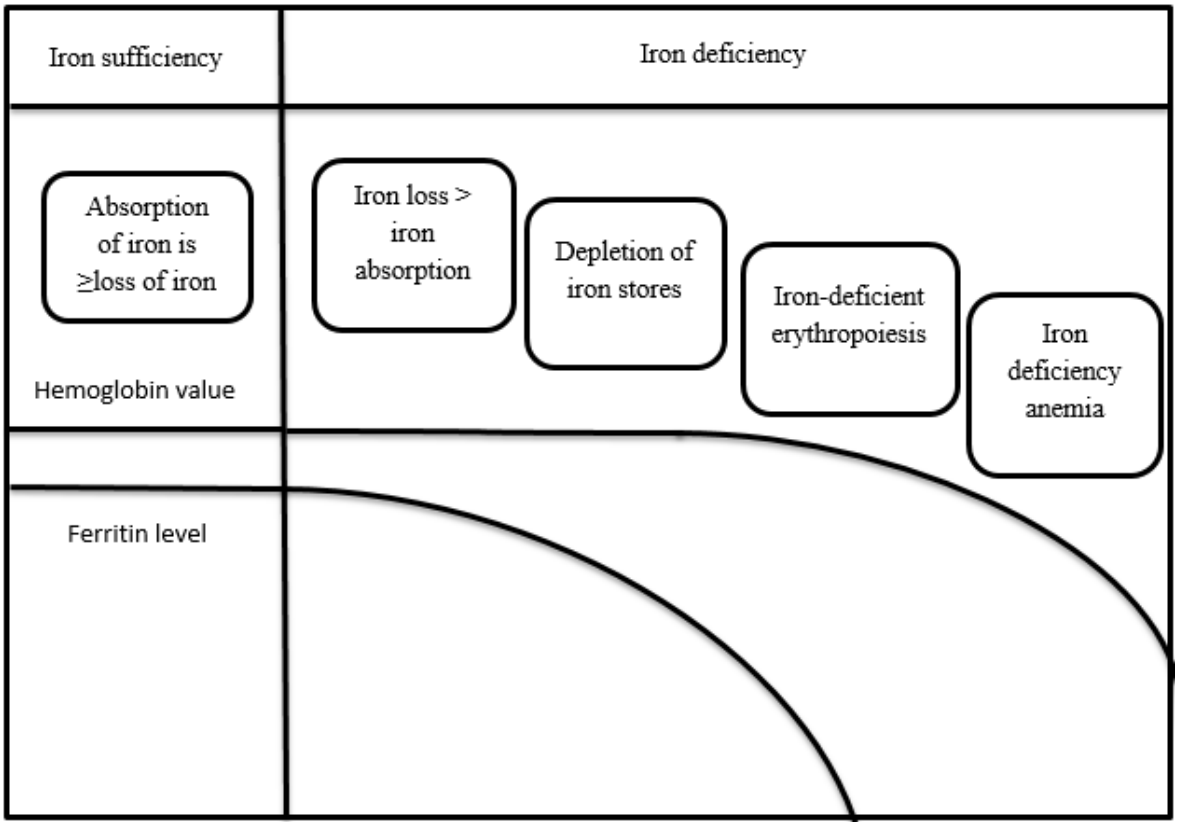

Figure 4. Stages of iron status, adapted from (83).

Iron deficiency anemia may have a physiological cause, such as when iron needs are increased during rapid growth, or it may have a pathological cause such as after blood loss or in conjunction with other medical conditions. However, the etiology of iron deficiency anemia is seldom explained by one factor, and may include inadequate dietary intake, reduced bioavailability, impaired absorption, reduced transport or storage capacity, physiological or pathological loss (83).

Anemia of chronic disease is the second most prevalent form of anemia. Hemoglobin level is reduced in relation to an inflammatory response to the underlying disease. Release of cytokines from activated inflammatory cells leads to reduced recycling of endogenous iron by increasing the level of hepcidin, reducing response to erythropoietin and increasing turnover of erythrocytes (83).

1.4.7 Assessment of iron status

The gold standard for assessment of iron status is by bone marrow examination. However, this procedure is elaborate, expensive and uncomfortable for the patient and is therefore not routinely performed (59).

Measurement of ferritin level to assess iron status is widely recommended (73, 74, 76, 84-87). Ferritin level is directly proportional to the body's total iron store, and a ferritin value below cut off value is always indicative of iron deficiency. However, a ferritin level above cut off value is not necessarily indicative of an adequate iron storage, because ferritin level is affected by acute and chronic inflammation, liver disease and malignancy. Thus, ferritin measurement may provide false negatives in these situations (88, 89). The lower cut off value for sufficient ferritin status has been debated, especially in patients with chronic disease (90).

An alternative method to assess iron status is by measuring serum level of soluble transferrin receptor. This parameter is unaffected by inflammation and has a strong correlation with iron status. The level of soluble transferrin receptors is inverse proportional to the amount of iron available for erythropoiesis. Thus, an elevated level indicate iron deficiency (83, 87).

Most patients with anemia of chronic disease will have normal transferrin receptor values, contradictive to patients with iron deficiency anemia. Patients displaying both iron deficiency anemia and anemia of chronic disease concomitant, may have either normal or elevated transferrin receptor values. Anemia of chronic disease is currently an exclusion diagnosis as valid biomarkers for the condition is lacking. However, hepcidin is proposed as a potential serological marker to differentiate between anemia of chronic disease and iron deficiency anemia (91).

Hemoglobin may also be used to assess iron status, although the specificity is low as there are several possible reasons for anemia besides iron deficiency. Moreover, a low Hb value is a late marker for iron deficiency as the stores must be completely empty before Hb falls below the normal reference range (59).

1.4.8 Iron deficiency and parenteral nutrition

Only one previous study has investigated iron status of pediatric HPN patients and the work of this study has not yet been published. However, an abstract of the study revealed a high prevalence of anemia and iron deficiency among Polish HPN children (30). Previous studies in HPN adults indicate insufficient iron status with a high prevalence of iron deficiency anemia (28, 29, 92). However, it is unlikely that these data can be extrapolated to children, as the adult HPN patients comprised of different diagnoses relative to the present pediatric population.

1.4.9 Iron deficiency among healthy children

Only one recent study, namely UNGKOST 3, has provided data on dietary intake of iron among healthy Norwegian children (63). Previous Norwegian studies have assessed iron deficiency and anemia among infants, toddlers, men and women (93-96). However, no studies have looked at healthy children above two years of age in Norway.

2 Aims of the study

There exist few published studies regarding iodine and iron status of pediatric HPN patients, and to my knowledge, no previous studies have assessed sources to iodine and iron within this group. Moreover, no studies have been conducted on this patient group in Norway.

The overall aim of this thesis is to assess iodine and iron status in pediatric HPN patients compared to a reference group of healthy children. Specific aims are:

- Estimate total iodine supply and describe important sources to iodine among HPN patients and healthy children.
- Measure urinary iodine concentration and calculate estimated 24-hour urinary iodine excretion from spot urine samples in HPN patients and healthy children.
- Estimate total iron supply and describe important sources to iron among HPN patients and healthy children.
- Measure hemoglobin and iron parameters in blood samples from HPN patients and healthy children.

The main hypothesis is that children receiving HPN are at risk of insufficient iodine and iron status.

3 Subjects and methods

3.1 Overview

The present work was conducted as a cross-sectional study on nutritional status among children receiving HPN and a group of healthy children, in conjunction with the NUTRIENT study. The latter is an ongoing study investigating nutritional status, physical activity, bone mineral content, and quality of life among HPN patients, stem cell treated patients and healthy children.

From January 2017 until June 2017, recruitment and collection of data were conducted. HPN patients were recruited through, and included at, the Pediatrics Department OUH, whereas data from the healthy children were gathered at the Department of Nutrition at the University of Oslo (UiO).

All children above two years of age who had received HPN for a minimum of six months, were identified and asked to participate in the study. After confirming their participation orally, parents received an envelope containing information about the study, consent form and equipment needed for participation. The study was then conducted in conjunction with a quarterly routine control at OUH.

Healthy children and adolescents between the age of 2 and 18 years with travel time to UiO of less than one hour, were asked to join the study through a registration form spread in social media by project workers. In order to be eligible for the study the children needed to have no intolerance or allergies towards cow milk, gluten or fish, in addition to being healthy (no known diagnoses).

Prior to meeting a project worker either at OUH or UiO, the participants were asked to complete a four-day food record and bring two urine samples. A diary to write down their diet and a picture booklet as guide to estimate portion sizes, were provided, as well as sample cups for urine. At the meeting, the children and parents filled out a background form, blood samples were drawn and anthropometric measurements (weight and height) were taken, Figure 5.

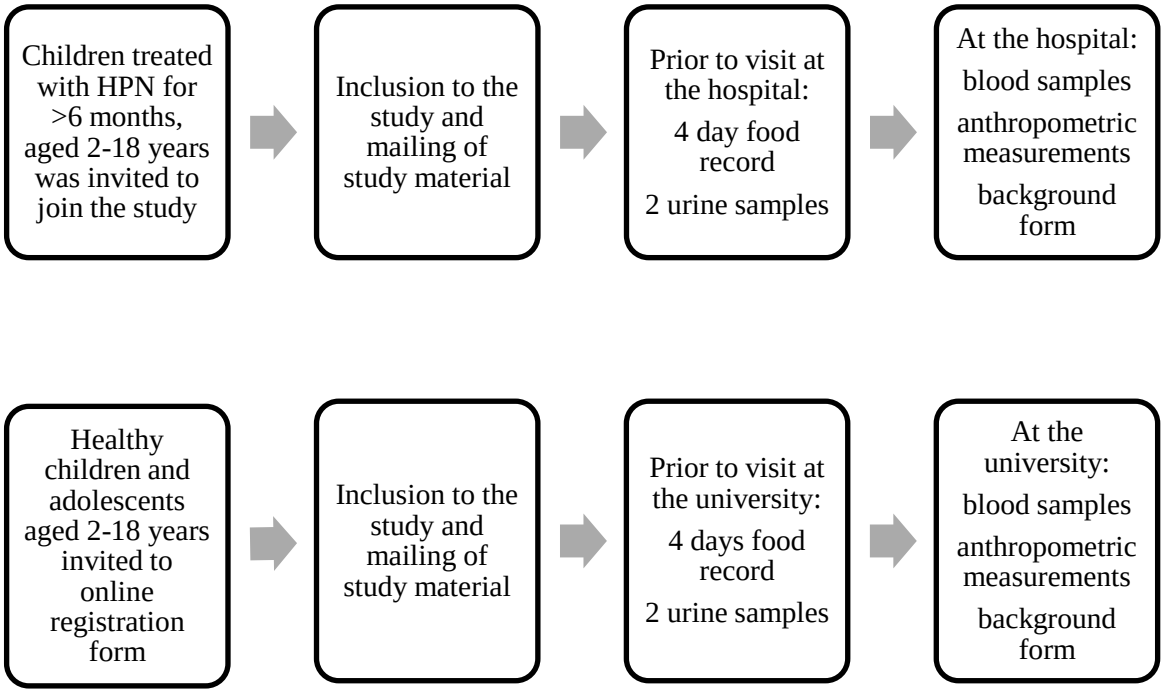

Figure 5. Overview of the study.

3.2 Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway (REC nr.2016/391) and by the Data Protection Officer at OUH. Written consent was collected from both parents before the child was enrolled in the study. The study followed the Helsinki Declaration and participation was voluntary. Subjects could withdraw from the study at any time. In accordance with good clinical practice, each participant was randomly assigned an ID number which were used to label all data collected. The key identifying the subjects was kept in a locked compartment at OUH at all times.

Blood sample results from the healthy children were assessed by a pediatrician if values were outside the reference area used at OUH.

3.3 Study population

The inclusion criteria for the HPN patients were as follows:

- Age between 2 and 18 years.
- Receive some of or all their nutritional needs through HPN.
- Treatment with HPN for > 6 months.

Exclusion criteria from urine analyses among the HPN patients:

- Use of contrast containing iodine the last six months.
- Known thyroid disease.
- Use of medications affecting iodine metabolism.

Inclusion criteria for children in the reference population were as follows:

- Age between 2 and 18 years.
- Only oral nutritional intake.
- Healthy (no known diagnoses).

Exclusion criteria for children in the reference population:

- Food allergies or intolerances towards cow's milk, gluten or fish.

3.4 Study visit

All HPN study visits were done in conjunction with their routine consultation at OUH. The HPN subjects met with their pediatric gastroenterologist, pediatric dietitian, nurse and master student for their routine follow up. Anthropometric measurements were conducted at the same room with same equipment for all HPN patients. The measurements were carried out by either a master student or dietitian. A majority of the HPN patients took blood samples from their central venous catheter (CVK), which then were done by a pediatric nurse. The rest of the HPN patients had their blood drawn by venous puncture by a bioengineer.

After their routine control, the master student had a one-hour long consultation with the patient and parents. During this session urine samples and food records were collected, background form were answered, and follow-up questions to the diet record could be addressed if necessary.

The healthy children were met by either two or one master student and a trained bioengineer at the university. Anthropometric measurements were taken by the same master student on all subjects. Urine samples were collected, background form was completed and the diet record was briefly assessed. At the end of the appointment a bioengineer performed the venous puncture.

3.5 Methods

3.5.1 Nutritional intake

The nutritional supply from PN was estimated by gathering information from the hospital pharmacy making the HPN bags, and then calculating the nutritional value. The patients received PN treatment in a range from four to seven days per week. Therefore, the average intake per day was used for all analyzes. Both HPN patients and the healthy children received a food diary to write down everything consumed for four days. To ease the burden of estimating portion sizes, the participants were supplied with the picture booklet used in NORKOST 3 (97). The booklet contained series with four different portion sizes of 33 common food items and meals. Furthermore, pictures of plates, glasses and mugs of different sizes were also provided in the booklet. Each page included a ruler to make the estimation of portion size easier. The participants were asked to record three week days (Monday – Friday) and one weekend day (Saturday or Sunday), if possible, in order to be representative for their average diet.

The dietary intake was calculated using DietistPro. DietistPro provide several databases of nutritional content in food items. However, only information obtained from the Norwegian Food Composition Table (FCT) was used in the present study, with the exception of tube feeding and nutritional drinks, where information from the respective producers was used. When a product was missing from the FCT, a similar product with respect to macronutrients, iodine and iron content were chosen, and adjustments were made if necessary. If no similar product could be chosen, information about the product was gathered from the manufacture and then entered manually to the program by a research worker.

Recommended daily intake (RDI) refers to Nordic Nutrition Recommendations for oral intake, whereas ESPGHAN Guidelines recommendations refers to parenteral supply to pediatric PN patients.

3.5.2 Anthropometric measurements

The weight and height of all HPN patients were measured by either a master student or a pediatric dietitian, while measurements of all healthy children were conducted by the same master student. The children wore light clothing with their shoes removed before the measurements were done. Weight was measured with a digital Seca scale to the nearest 0.1 kg and height was measured using a stadiometer to the nearest 0.1 cm. The research worker/master student made sure the feet was properly placed to the wall with their spine as elongated as possible.

Z-scores for weight for age, length for age and BMI for age were calculated for the HPN patients using the hospital electronic growth chart, while the same values for the healthy children was calculated by Pétur Júlísson, Section for Pediatrics, Department of Clinical Medicine, University of Bergen, Norway, based on the same cross-sectional sample (98).

3.5.3 Urine samples

All participants were asked to bring two urine samples to their appointment, Figure 6. Participants were instructed to take the samples during the four day period with food recording. Furthermore, they were asked to avoid the first morning urine if possible, because urinary iodine concentration is at its lowest during the morning (99). Participants were instructed to store the samples in the refrigerator until the appointment.

From each sample provided, two vacuum tubes were drawn. One tube was used for analysis of iodine content, the other for creatinine content. Creatinine analyses of HPN samples were performed by the medical biochemistry division at Ullevål, OUH, whereas samples from the healthy children were analyzed at the medical biochemistry division at Rikshospitalet, OUH.

Figure 6. Urine vacuum cups and vacuum tubes (100). Each participant were given two urine vacuum cups. Upon delivery to a research worker, the urine from each cup was then transferred to two vacuum tubes, one for analysis of iodine, the other for creatinine.

All iodine samples were prepared at UiO and later analyzed at Norwegian University of Life Sciences (NMBU). From the vaccum tubes 1 mL of urine was pipetted and added 100 µl Tellur and 8.9 ml BENT (2 % (w/V) NH_4OH, 0,1 % (w/V) H_4EDTA, 4 % (w/V) 1-Butanol and 0,1 % (w/V) Triton™, X-100). Blank solutions were made following the same procedure. The samples were analyzed using Agilent 8800 ICP-MS-QQQ according to manufacturer's instructions. Certified reference material, Seronorm Trace Elements Urine L-1 and L-2, were used as a quality control.

Sufficient iodine status according to UIC was set to 100µg/L in accordance with WHO' cut off value, Table 6.

Table 6. WHO' reference values for urinary iodine concentration (41)

Median urinary iodine concentration (µg/L)	Iodine intake	Iodine status
<20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Adequate iodine nutrition
200-299	Above requirements	May pose a slight risk of more than adequate intake
≥ 300	Excessive	Risk of adverse health consequences

WHO = World Health Organization

Currently, the recommended method for assessment of iodine status in populations is measurement of median UIC (41). However, because this method is affected by the participants' hydration status, calculation of Est24h UIE were also made. Creatinine and iodine were measured in spot urine samples and thereafter scaled according to published 24-hour creatinine reference values based on gender and height, Figure 7 (58, 101).

$$\underset{\textit{Estimate}}{\textbf{Iodine excretion per 24-h}\ [\mu g/d]} = \underbrace{\frac{\textbf{Iodine}\ [\mu g] \bullet [1/L]}{\textbf{Creatinine}\ [\text{mmol}] \bullet [1/L]}}_{\textit{Measured in spot urines}} \times \underbrace{\frac{\textbf{Creatinine}\ [\text{mmol}]}{[24-h] \bullet [kg]}}_{\textit{Reference values}} \times \underbrace{\textbf{Body weight}\ [kg]}_{\textit{Individual}}$$

Figure 7. Formula used for calculation of estimated 24-hour urinary iodine excretion (58).

Est24 h UIE values were assessed in relation to recommended daily intake adjusted for 15 % nonrenal iodine loss (RDI*), Table 7.

Table 7. Reference values for use of estimated 24-hour urinary iodine excretion

Age (years)	Recommended daily intake *
2-5	76.5
6-9	102
>10	127.5

Recommended daily intake* = recommended daily intake adjusted for 15 % nonrenal iodine loss

3.5.4 Blood samples

The following blood parameters were analyzed: Hemoglobin (Hb), Mean Corpuscular hemoglobin (MCH), Mean Corpuscular Volume (MCV), iron, transferrin, Total Iron Binding Capacity (TIBC), transferrin saturation, ferritin and soluble transferrin receptor.

Prior to collection of data, we hypothesized that patients receiving HPN may experience increased inflammation, thus making ferritin unsuited for determining iron status in these children. We therefore chose to measure soluble transferrin receptor values as this parameter is unaffected by inflammation, and reflect the cellular need of iron (70, 102).

Blood samples from the HPN patients were collected either from their CVK or from a venous blood draw. A pediatric nurse conducted the blood draw if the CVK were used, and a bioengineer if venous puncture was used. All samples were sent to the medical biochemistry division at Ullevål, OUH. A pediatrician at OUH assessed all results.

The healthy children delivered blood samples by means of a venous blood draw, performed by a bioengineer. The blood samples were delivered to the medical biochemistry division at Rikshospitalet, OUH. When a result beyond the reference range for the given parameter was obtained, this was discussed with a pediatrician at OUH.

In the present study, anemia was defined as a hemoglobin value below WHO' cut off, Table 8. Iron deficiency was defined as a ferritin level below WHO' reference value according to age and gender, Table 9. Iron deficiency anemia was defined as low Hb value and ferritin value (according to above mentioned criteria) or elevated transferrin receptor level, Table 10.

Table 8. WHO' reference values for hemoglobin (g/dl) according to age and sex.

Age (years)	Non-anemia	Mild anemia	Moderate anemia	Severe anemia
<4	≥ 11.0	10.0-10.9	7.0-9.9	<7.0
5-11	≥ 11.5	11.0-11.4	8.0-10.9	<8.0
12-14	≥ 12.0	11.0-11.9	8.0-10.9	<8.0
Women >15	≥ 12.0	11.0-11.9	8.0-10.9	<8.0
Pregnant women	≥ 11.0	10.0-10.9	7.0-9.9	<7.0
Men >15	≥ 13.0	11.0-12.9	8.0-10.9	<8.0

WHO = World Health Organization

Table 9. WHO' reference values for depleted iron stores according to ferritin level (µg/L).

Age (years)	Depleted iron stores
<5	<12
>5	<15

WHO = World Health Organization

Table 10. Oslo University Hospital' reference values for soluble transferrin receptor (mg/L).

Gender	Reference value
Girls	1.9-4.4
Boys	2.2-5.0

3.5.5 Statistics

Statistical analysis was performed using IBM SPSS Statistics for Windows version 24.0. Normality was assessed by using Shapiro-Wiik test, histograms and normal Q-Q plots. The normally distributed data are presented as means with standard deviation (SD), whereas

non-normally distributed data are presented as medians with range (minimum-maximum). Categorical variables are presented as numbers (n) and frequencies (%).

Because recommended daily intake of iodine and iron varies with sex and age, intake is presented according to age and sex categories in addition to total intake within both groups. Statistical analysis within these subgroups has not been conducted due to small sample sizes.

Comparison of normally distributed variables were done by using two independent samples T test. While comparison of non-normally distributed variables were done by using Mann Whitney U Test. Categorical variables were assessed by using Chi square test when the assumptions for use of this test was met. The assumptions were that 80 % of the cells had expected count over five and that all cells had expected count over one. When these assumptions were not met, Fischer's exact test was used. Spearman's correlation coefficient was used to describe correlation between two continuous variables.

A two-sided significance level was set at five % for all statistical analysis.

The number of patients included in the present study was limited due to the number of children receiving HPN at OUH. The number of healthy children included was limited due to time constraints. Post hoc analysis of sample size was performed to determine statistical strength of the present study. Mean values and standard deviations for UIC was used. With a statistical power of 80 % and alpha at 0.05, the HPN group should ideally have comprised of 76 children, while the healthy reference population should have had 152 children. Furthermore, the statistical power of the current study was 22.8 %. Thus, the possibility for type II error in the present study is very high.

3.6 My contribution to the study

I participated in the identification and recruitment of the HPN patients, and met with 13 of the patients. During the study visit, I collected urine samples, food records, background information and questionnaire regarding quality of life. After each meeting I gathered information about their PN from the hospital pharmacy. I calculated PN and enteral supply of nutrients for ten patients.

Prior to appointment with the HPN patients, I consulted with the patients' pediatric gastroenterologist and prepared requisitions for blood and urine samples for all HPN patients. I spoke with the parents in regards to transport between the different examinations at Ullevål and Rikshospitalet and arranged this if necessary. I constructed the databases used to plot PN data, dietary data, background information, blood samples, urine samples and answers to quality of life questionnaires. I participated in the mailing of study material and information prior to the parpticiapnts' appointment at OUH or UiO.

I met with all 35 healthy children. For all 35 participants I: measured weight and height, collected background information and quality of life questionnaire, and went through the diet records together with parents and child. I also performed a Dual-energy X-ray absorptiometry scan (DXA) and bioelectrical impedance analysis for all healthy children. I calculated the dietary intake for 14 of the healthy children and entered this to the database. Furthermore, I participated in giving written feedback on diet and blood samples to the healthy children.

I calculated sources of iron and iodine for all HPN patients and healthy children.

I participated in the analysis of iodine concentration in the urine samples gathered and plotted all values into the database.

4 Results

4.1 Recruitment

From January until May 2017, 19 children above two years of age who received HPN-treatment were identified and included in the present study. One patient was excluded from urinary analyses due to hypothyreosis, which was an exclusion criteria for iodine analyses. By the end of May 2017, 15 patients (79 %) had completed the study with both diet records, urine and blood samples.

In the period of March until June 2017, parents of healthy children and adolescents were recruited through the spreading of a registration form by research workers in social media. Initially 42 parents showed interest in the study, however seven withdrew prior to collection of data. Of the remaining 35 children, 34 (97 %) completed the study with diet records, urine and blood samples, Figure 8.

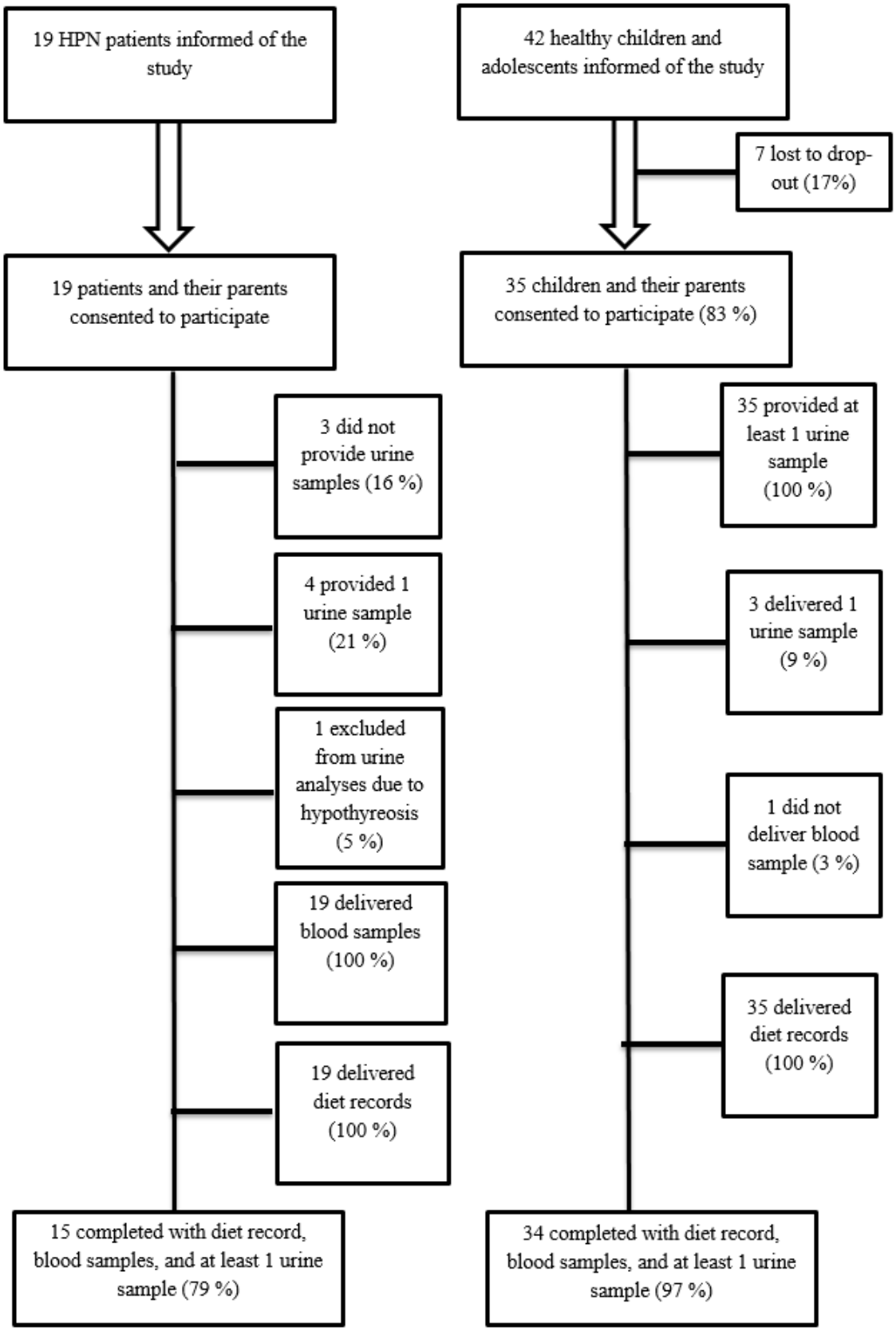

Figure 8. Overview of recruitment and collection of data.

4.2 Urine sampling

Ninety-seven spot urine samples were collected from both groups in total. Two samples from one HPN patient were excluded due to the patient being diagnosed with hypothyreosis. The remaining 95 samples were analyzed for iodine content, while 72 samples were analyzed for both iodine and creatinine, Figure 9. Mean value for iodine content and Est24h UIE has been used where two urine samples were provided.

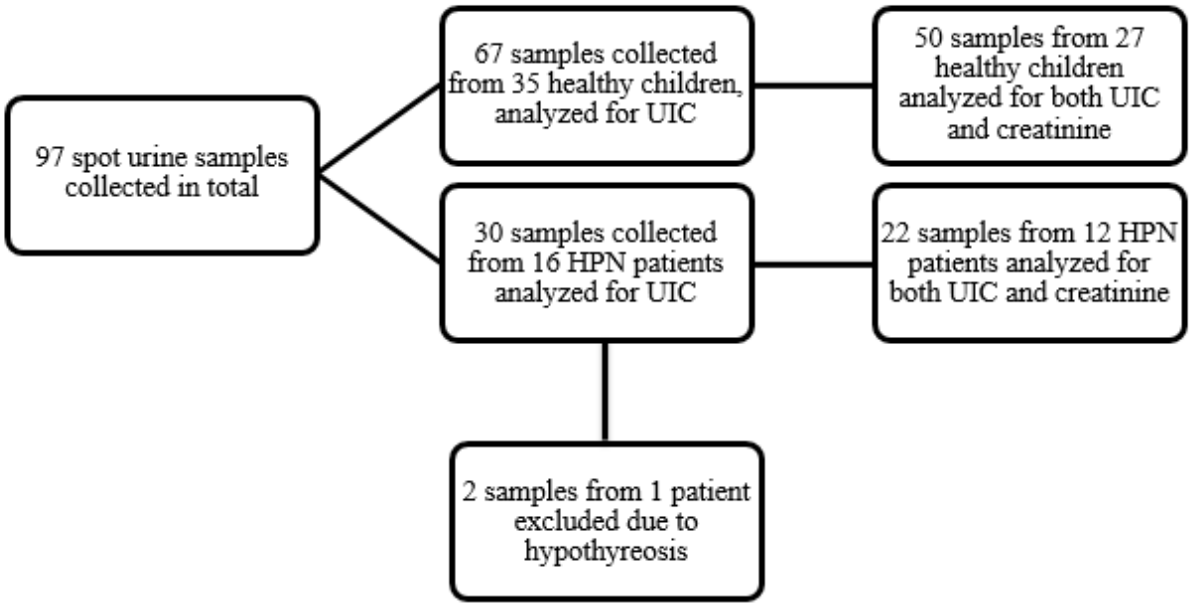

Figure 9. Overview of spot urine samples collected.

UIC = urinary iodine concentration
HPN = home parenteral nutrition

4.3 Subject characteristics

Characterization of the study population can be seen in Table 11. Mean age for the participants was ten and nine years among the HPN patients and healthy children, respectively. The HPN patients consisted of 68 % boys and 32 % girls, whereas the healthy children consisted of 40 % boys and 60 % girls. There was no statistical difference between the two groups in either age or gender distribution. The HPN patients had significantly lower Z-score values for weight ($p=0.002$) and height ($p<0.001$) relative to the healthy children.

Table 11. Subject characteristics.

	Home parenteral nutrition patients			Healthy children			
	n	Mean / %	SD	n	Mean / %	SD	P value
Age (years)	19	10.1	3.5	35	9.2	3.4	0.369[a]
Sex							
Boys	13	68		14	40		0.087[b]
Girls	6	32		21	60		
Z-score weight	19	-1.0	1.6	35	0.1	0.9	0.002[a]
Z-score height	18	-1.5	1.7	35	0.2	1.2	<0.001[a]
Z-score BMI	18	0.2	1.0	35	-0.1	1.1	0.367[a]
Mothers' education level	19			35			0.274[b]
Primary school		5			-		
Secondary school		5			-		
High school		11			14		
College or university		79			86		
Fathers' education level	17			35			0.345[b]
Primary school		5			-		
Secondary school		-			-		
High school		21			23		
College or university		63			77		

SD = standard deviations for mean values.
Range = minimum-maximum for median values.
[a] = Tested using two independent samples t test.
[b] = Tested using Chi square test

The HPN patients were recruited from ten counties, (Oslo, Akershus, Østfold, Vestfold, Rogaland, Oppland, Aust-Agder, Vest-Agder, Telemark and Hedmark), whereas the healthy children originated from three counties (Oslo, Akershus and Buskerud).

4.3.1 Gastrointestinal symptoms

Among the HPN patients, 95 % reported to have gastrointestinal symptoms in general, with 58 % reporting daily problems with loose stools, 58 % daily flatulence and 37 % daily abdominal pain, Table 12. Twenty percent of the healthy children also reported having general GI symptoms, however none reported daily problems of any kind (data not shown). The HPN patients often experienced several GI symptoms concomitant. The majority of the HPN patients (58 % n = 11) reported two or more GI symptoms on a daily basis, whereas 16 patients (84 %) reported to have two or more GI symptoms on a weekly basis.

Table 12. Gastrointestinal symptoms among home parenteral nutrition patients.

Symptoms		Number of patients	%
GI symptoms in general			
	Yes	18	95
	No	1	5
Abdominal pain			
	Daily	7	37
	Weekly	5	26
	NA	7	37
Loose stools			
	Daily	11	58
	Weekly	2	10
	NA	6	32
Constipation			
	Daily	3	16
	Weekly	2	10
	NA	14	74
Flatulence			
	Daily	11	58
	Weekly	6	32
	NA	2	10
Emesis			
	Daily	2	10
	Weekly	1	5
	NA	16	84
Reflux			
	Daily	2	10
	Weekly	1	5
	NA	16	84

GI = gastrointestinal
NA = not applicable

4.3.2 Home parenteral nutrition patients

Children receiving HPN are a heterogeneous patient group with complex medical conditions. Overall, 37 % (n=7) of the HPN patients were diagnosed with a syndrome entailing a neurological outcome, such as Down syndrome. Two patients (11 %) had autism spectrum disease, two (11 %) had previously undergone a kidney transplantation, one (5 %) had an immune deficiency disease and one (5 %) had undergone treatment for acute lymphatic leukemia (ALL).

The main cause for HPN-treatment was divided into three categories: severe bowel dysmotility, short bowel syndrome and severe malabsorption, of which severe bowel dysmotility was the most prevalent cause in the present population, Figure 10.

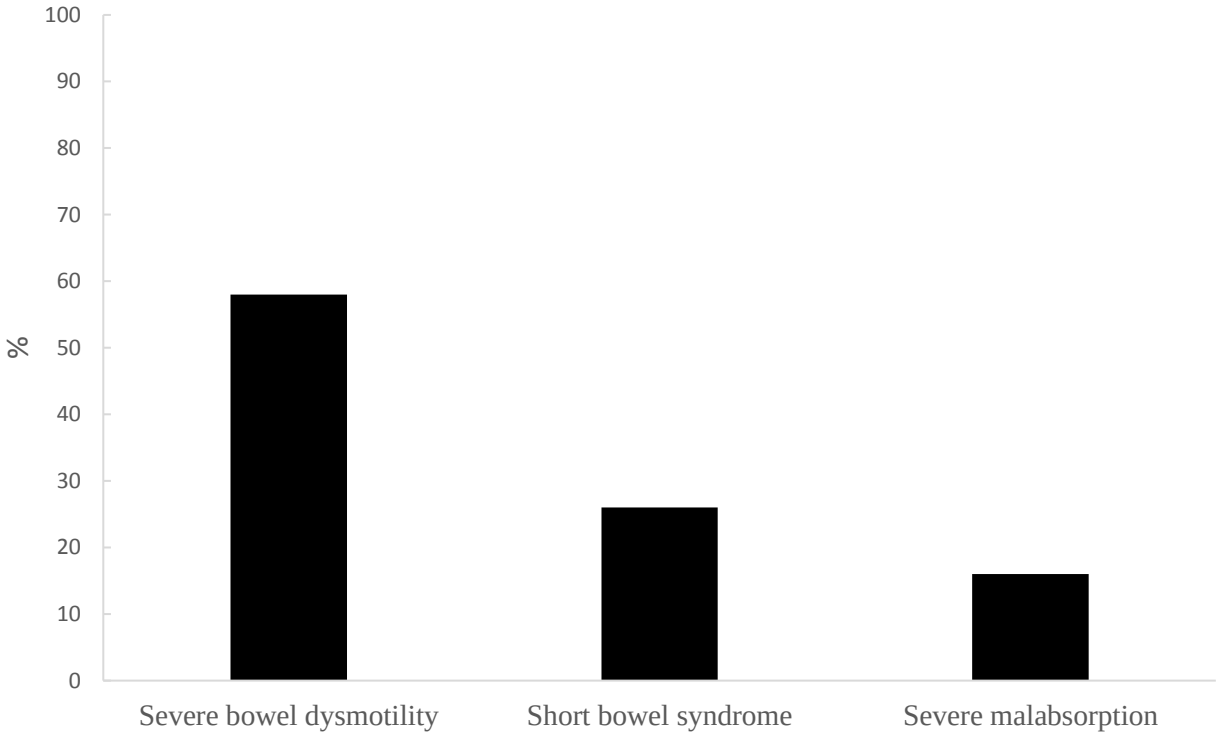

Figure 10. Main cause of home parenteral nutrition treatment (n=19).

The etiology behind short bowel syndrome in the present population was gastroschisis (n=1), bowel necrosis (n=1), Hirschprung's disease (n=1) and volvulus (n=2).

The majority of the HPN patients had one or more ostomies. Fifteen (79 %) patients had gastrostomy, however only eight used it for enteral nutrition support (EN) at the time of the study. Four (21 %) had ileostomy and three (16 %) patients had jejunostomy. None of the children had jejunostomy feeding tube.

One out of five patients with SBS, one out of three patients with severe malabsorption and six out of 11 patients with severe bowel dysmotility, received EN at the time of the study.

The median treatment time with HPN at the time of the study was 53 months (min 9 months, max 199 months). Nine patients (47 %) started HPN during their first year of life. The HPN patients received PN in a range from four days per week up to seven days per week, with the majority receiving PN every day (63 %), Table 13.

Table 13. Number of days/week with home parenteral nutrition (n=19).

Number of days/week	% of patients
4	11
5	16
6	11
7	63

4.4 Nutritional characteristics

4.4.1 Intake of macronutrients

The macronutrient energy ratio for HPN patients and healthy children are shown in Table 14. The HPN patients had a significant lower intake of energy from fats ($p<0.001$) and protein ($p<0.001$), while a higher intake from carbohydrates ($p<0.001$) relative to the healthy children. Median total energy intake among the HPN patients was 1834 kcal, while 1956 kcal in the healthy children, with no significant difference between the groups ($p=0.993$).

Table 14. Macronutrient energy ratio.

Macronutrient	Home parenteral nutrition patients (n=19) Energy % Median (range)	Healthy children (n=35) Energy % Median (range)	P value
Fat	27 (23-36)	33 (24-43)	<0.001
Protein	13 (9-19)	16 (11-21)	<0.001
Carbohydrate	56 (50-64)	49 (40-59)	<0.001

Range = Minimum-maximum value
Mann Whitney U Test

4.4.2 Enteral and parenteral provision among home parenteral nutrition patients

Parenteral nutrition contributed on average with more macronutrients and iodine among the HPN patients than enteral nutrition did (EN and diet), Table 15. The opposite was true for iron, where enteral intake on average contributed with 67 %.

Table 15. Contribution of enteral nutrition and parenteral nutrition (n=19).

	% Enteral nutrition, mean (SD)	% Parenteral nutrition, mean (SD)
Energy	32 (25.4)	68 (25.4)
Protein	33 (26.4)	67 (26.4)
Carbohydrates	29 (25.2)	71 (25.2)
Fat	36 (32.6)	64 (32.6)
Iodine	36 (34.8)	64 (34.8)
Iron	67 (39.1)	33 (39.1)

SD = standard deviation
Enteral nutrition = oral intake and enteral nutrition support

To ensure trace element status among HPN patients, Peditrace or Addaven is added to the patients' PN. Fifteen patients received Addaven (79 %), while four (21 %) patients received Peditrace.

4.5 Iodine intake

HPN patients had a median iodine provision (EN, PN and oral intake) of 116 µg/day, Table 16. Median iodine intake among the healthy children was 96 µg/day, with no significant difference from the HPN patients (p=0.221).

Median iodine provision among the HPN patients corresponded to 93 % of RDI on oral diet. Iodine supply among the HPN patients displayed a wide range, from 40 % of RDI to 164 % of RDI. Furthermore, assessment of iodine intake according to age category showed that median iodine supply was below RDI for all age categories.

Median iodine intake among the healthy children corresponded to 80 % of RDI. As with the HPN patients, the healthy children displayed a wide range of iodine intake, from 24 % of RDI to 195 % of RDI. Median iodine intake were below RDI within all age categories.

There was no statistical difference between HPN patients and healthy children in percentage intake of iodine relative to RDI (p=0.497). Iodine intake increased with age, and highest intake in both groups were among children above ten years of age.

Table 16. Iodine intake.

	Home parenteral nutrition patients		Healthy children		RDI (µg)
Age (years)	n	Median, µg (range)	n	Median, µg (range)	
Total	19	116 (42-245)	35	96 (26-234)	
3-5	3	85 (42-109)	7	79 (26-169)	90
6-9	6	99 (78-198)	16	95 (43-234)	120
>10	10	139 (60-245)	12	128 (36-227)	150

RDI = Recommended daily intake according to Nordic Nutrition Recommendations 2012
Range = minimum-maximum values
Mann Whitney U Test

4.5.1 Parenteral iodine supply among home parenteral nutrition patients

ESPGHAN guidelines recommend a daily provision of 1µg iodine/kg bodyweight in PN to pediatric patients (15). Median iodine provision was 2.7 µg/kg, with a minimum value of 0.3 µg/kg and a maximum value of 3.7 µg/kg. Four patients were below the recommendation of 1 µg/kg. All four patients received Peditrace as parenteral trace element solution.

Median iodine content in the PN of patients with Peditrace as trace element solution, was significantly lower than iodine content of PN with Addaven, both when assessing total iodine content and iodine content relative to bodyweight, Table 17.

Table 17. Parenteral iodine provision in patients receiving Addaven and Peditrace.

	Patients receiving Addaven (n=15) Median, µg	Patients receiving Peditrace (n=4) Median, µg	P value
Total parenteral iodine content	97.8	6.8	0.001
Parenteral iodine content/kg bodyweight	3.1	0.4	0.001

Mann Whitney U Test

Twelve HPN patients had a total iodine supply (PN, EN and oral intake) below RDI for oral diet according to his/her age group. Iodine provision below RDI was found among all subgroups of HPN patients. Among patients with SBS, four out of five were below RDI, seven of 11 patients with severe bowel dysmotility and one of three patients with severe malabsorption were below RDI. Furthermore, three of four patients supplemented with Peditrace had a total iodine supply below RDI for oral diet.

4.5.2 Nutritional sources of iodine

Oral intake in the HPN patients contributed with 13 % of daily iodine intake, whereas EN and PN accounted for the remaining 10 % and 67 %, respectively, Figure 11. Iodine from milk and dairy products were the main source of iodine among the healthy children, accounting for nearly two thirds of total intake, Figure 12.

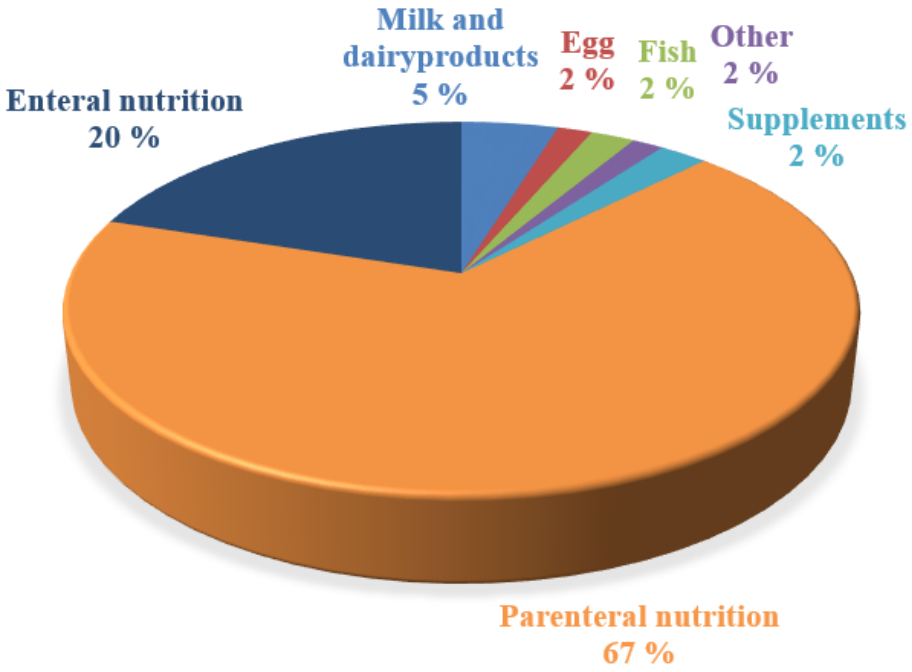

Figure 11. Sources of iodine among home parenteral nutrition patients (n=19).

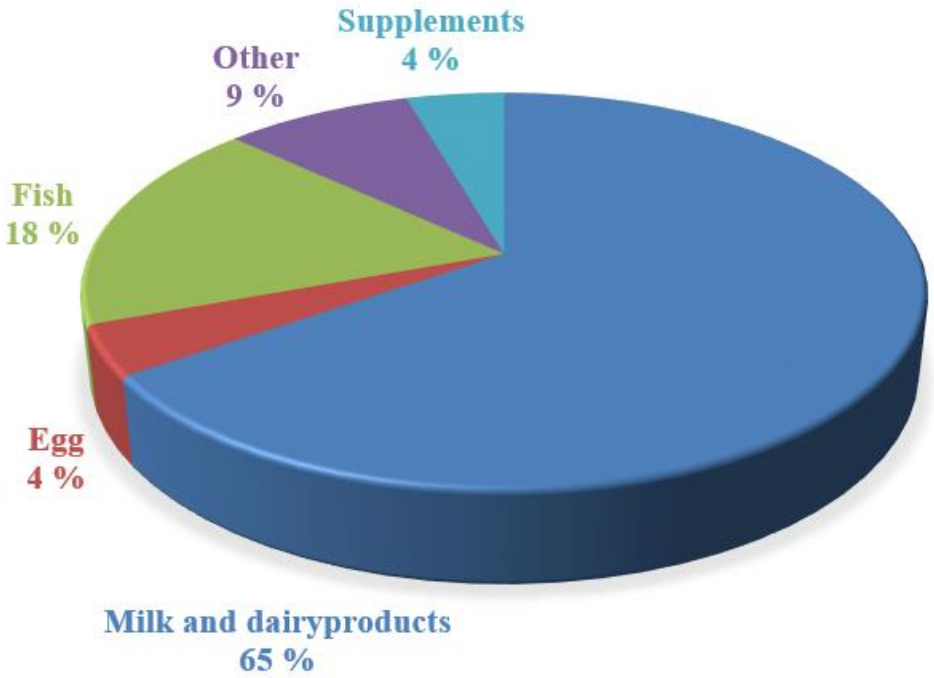

Figure 12. Dietary sources of iodine among the healthy children (n=35).

EN accounted for 20 % of total iodine intake among the HPN patients. Eight patients received EN at the time of the study, however, three of them received only minimal amounts (less than 165 kcal/day). Exclusion of these three subjects, revealed a significant difference in iodine supply relative to RDI between patients with and without EN, Table 18.

Table 18. Iodine provision in relation to supply of enteral nutrition support.

	Patients without enteral nutrition support (n=11) Median (range)	Patients with enteral nutrition support (n=5) Median (range)	P value
Percentage iodine supply relative to RDI	93 (40-142)	160 (72-165)	0.038

RDI = recommended daily intake
Range = minimum-maximum
Mann Whitney U Test
Three patients with less than 165 kcal/day from enteral nutrition support excluded

Three HPN patients used dietary supplements, of these, one supplement contained iodine. However, removing the iodine provision from this supplement did not affect median enteral iodine intake in the group. Among the healthy children, 12 used supplements, however only three of these contained iodine. Median iodine intake without supplements in this group was 89 µg and 96 µg with supplements.

4.6 Urine samples

4.6.1 Urinary iodine concentration

Median UIC was 89 µg/L for the HPN patients and 130 µg/L for the healthy children, Figure 13, with no statistical difference between the groups (p=0.197). Using WHO' cut off value for sufficient iodine status (>100 µg/L), 53 % of the HPN population and 43 % of the healthy children, were iodine insufficient. There was no significant difference in number of subjects below the WHO' cut off (p=0.548).

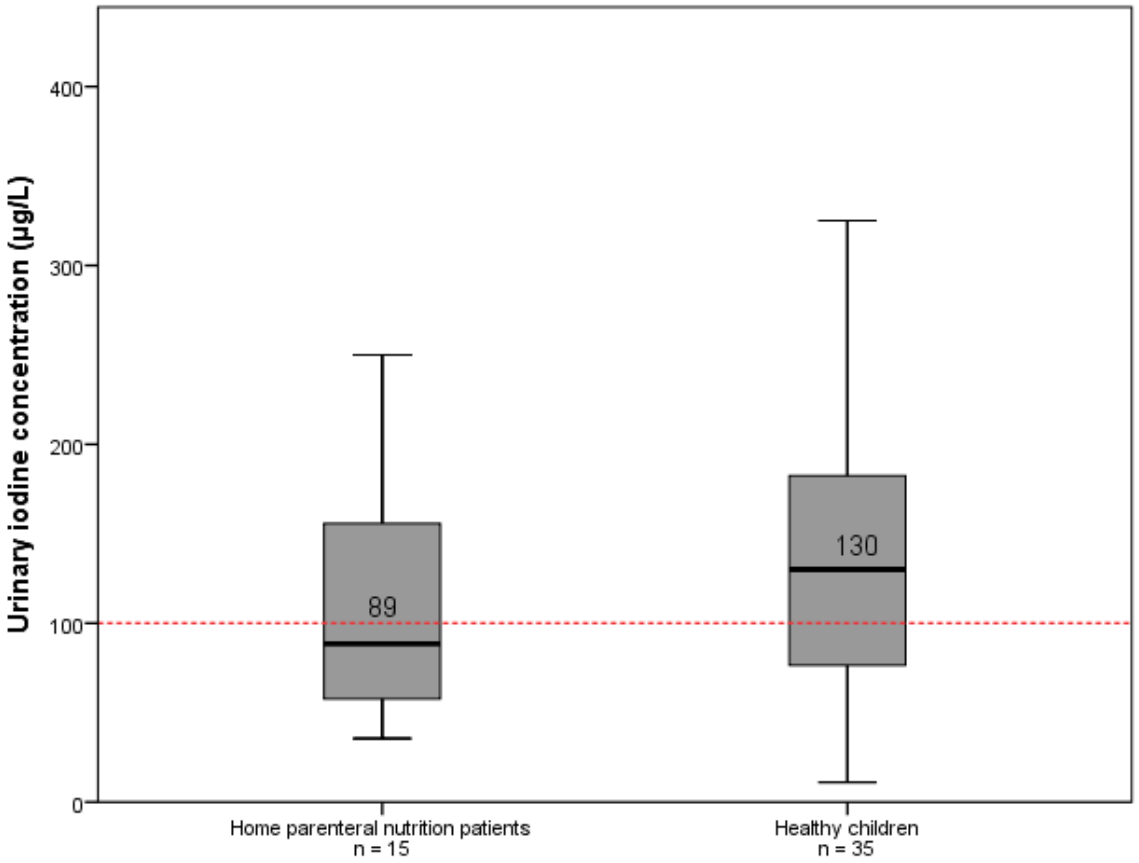

Figure 13. Median urinary iodine concentration (µg/L). The horizontal line indicates median urinary iodine concentration (UIC) in each group, the boxes indicate 25th-75th percentiles. The red dotted line indicates WHO' cut-off value for sufficient iodine status (UIC >100 µg/L). n = number of subjects within each group.

There was no significant difference in number of HPN patients with UIC below 100 µg/L between patients with or without EN (p=0.642) or between patients with Peditrace or Addaven (p=1.00).

Consumption of milk and dairy products among the healthy children showed a strong, positive correlation with UIC values (r=0.734, p<0.001).

None of the HPN patients had UIC values in either the highest or lowest categories of UIC, whereas UIC values for the healthy children showed a larger range, Figure 14.

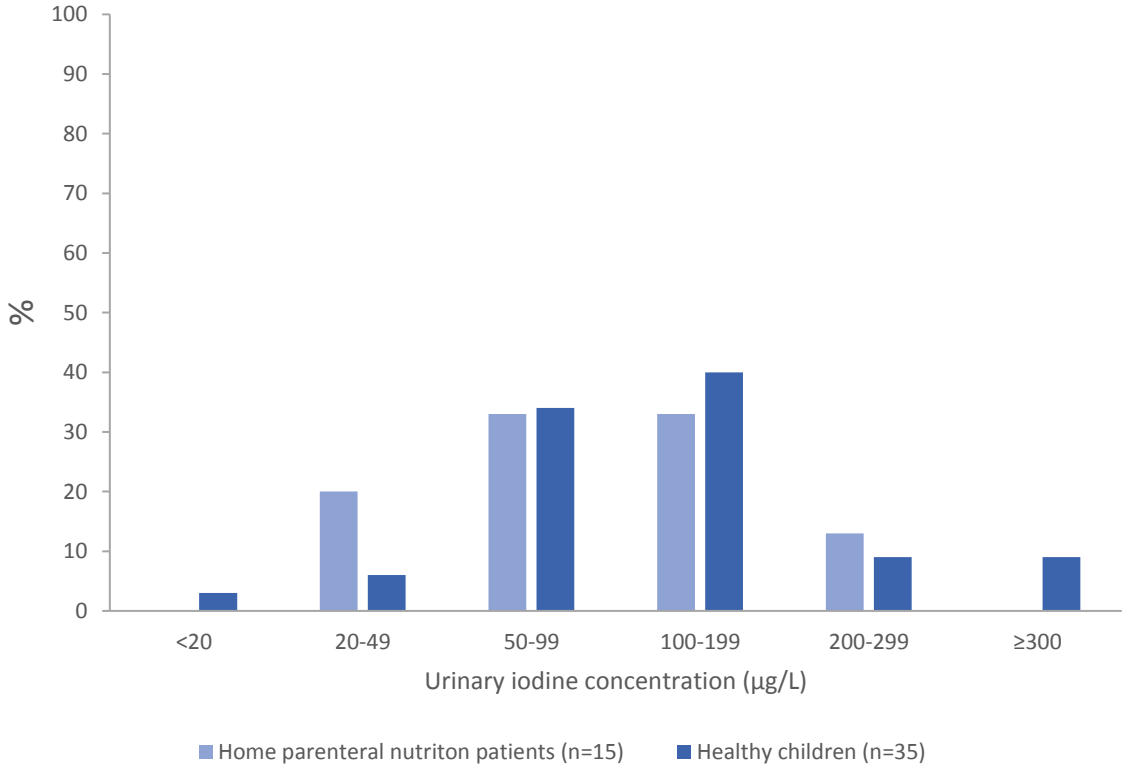

Figure 14. Urinary iodine concentration (µg/L).

Eight out of 15 HPN patients had mean UIC value below 100 µg/L, and seven (88 %) of these children also had an iodine supply below RDI.

Iodine insufficiency according to UIC, was found within all subgroups of HPN patients. Three of four patients with SBS, two of three patients with severe malabsorption and three of eight patients with severe bowel dysmotility had UIC value below the cut off value.

Of the 15 healthy children with UIC < 100 µg/L, 12 (80 %) had iodine intake below RDI.

4.6.2 Correlation between urinary iodine concentration and iodine supply

UIC results were further analyzed in relation to iodine provision to assess correlation between iodine intake and UIC value, Table 19. HPN patients with UIC <100 µg/L had a non-significantly lower iodine supply than HPN patients with UIC >100 µg/L. The healthy children with UIC <100 µg/L had a significantly lower iodine intake than children with UIC >100 µg/L.

Table 19. Median iodine intake according to average urinary iodine concentration.

Iodine intake (µg)	Urinary iodine concentration <100 µg/L Median (range)	Urinary iodine concentration >100 µg/L Median (range)	P value
Home parenteral nutrition patients	97.0 (42.2 – 239.9) (n=8)	150.0 (86.7 – 212.7) (n=7)	0.094
Healthy children	65.9 (25.6 – 233.9) (n=15)	114.5 (69.4 – 218.1) (n=20)	0.011

Range = minimum-maximum values
Mann Whitney U Test

4.6.3 Estimated 24-hour urinary iodine excretion

Another method for assessment of iodine status is estimation of 24-hour urinary iodine excretion from spot urine samples. The Est24h UIE values in the present study were calculated using iodine and creatinine levels from spot urine, and 24-hour creatinine pediatric reference values based upon subjects' height and weight (58, 101). This estimate can subsequently be compared to RDI adjusted for 15% nonrenal iodine loss (RDI*) in order to assess iodine status.

Within each age category of both HPN patients and healthy children, 50 % or more of the children were below the recommended daily intake adjusted for 15 % nonrenal iodine loss (RDI*), Table 20. There was no significant difference between the total percentage of subjects below RDI* in the two groups (p=0.734).

Median Est24h UIE equaled 96 % of RDI* among the HPN patients, and 79 % among the healthy children. There was no statistical difference between the groups (p=0.408).

Table 20. Median estimated 24-hour urinary iodine excretion.

	Home parenteral nutrition patients			Healthy children			
Age (years)	n	Median (range)	% below RDI*	n	Median (Range)	% below RDI*	RDI* (µg)
3-5	2	74 (73-75)	100	7	71 (35-92)	57	76.5
6-9	3	97 (75-130)	67	12	88 (50-166)	58	102
>10	7	111 (67-188)	57	8	62 (46-173)	63	127.5
Total	12	102 (67-188)	67	27	75 (35-173)	59	

Est24h-UIE Estimated 24-hour urine iodine excretion
RDI* = Recommended daily iodine intake of iodine adjusted for 15% nonrenal iodine loss

Sixty-seven % of the HPN patients were classified as iodine insufficient according to their mean Est24h UIE. Of these, 75 % (n=6) had an iodine intake below RDI, 63 % (n=5) had mean UIC below 100 µg/L, and 50 % (n=4) had both intake below RDI and UIC below 100 µg/L. Interestingly, two (25 %) of these children had an iodine intake corresponding to 160 % and 165 % of RDI.

Iodine insufficiency was found within all subgroups of HPN patients. Two out of three patients with SBS, five out of seven patients with severe bowel dysmotility and one out of two patients with severe malabsorption, had Est24h UIE below RDI*.

There was no significant difference in subjects below RDI* between HPN patients with or without EN (p=0.232) or patients with Addaven or Peditrace (p=0.237).

4.6.4 Correlation between estimated 24-hour urinary iodine excretion and iodine supply

Median iodine supply was higher among children with Est24h UIE above cut off value (RDI*) compared to children with Est24h UIE below cut off in both groups, Table 21. However, the difference was only significant among the healthy children.

Table 21. Median iodine intake in relation to estimated 24-hour urinary excretion.

	Est24h UIE <RDI* Median iodine intake (range)	Est24h UIE >RDI* Median iodine intake (range)	P value
Home parenteral nutrition patients	113 (42-240) n=8	154 (116-213) n=4	0.283
Healthy children	72 (26-227) n=16	115 (87-169) n=11	0.013

Range=minimum-maximum values
Est24h UIE = Estimated 24-hour urine iodine excretion
RDI* = Recommended daily intake of iodine according to Nordic Nutrition recommendation, adjusted for 15% nonrenal iodine loss
Mann Whitney U Test

4.6.5 Comparison of urinary iodine concentration and estimated 24-hour urinary iodine excretion

UIC and Est24h UIE are two different methods to assess iodine status. Iodine sufficiency according to UIC is defined as a value >100 µg/L, while iodine sufficiency according to Est24h UIE is defined as a value >RDI*. The methods were compared to see if they identified the same subjects as iodine sufficient/insufficient. UIC and Est24h UIE categorized 72 % of all subjects (n = 28) in the same category (e.g. iodine sufficient/iodine insufficient), however 23 % of all subjects (n = 9) were categorized as iodine sufficient according to UIC while below RDI* according to Est24h UIE. Table 22 show the distribution within home parenteral nutrition patients and healthy children.

Table 22. Comparison of iodine status according to urinary iodine concentration and estimated 24-hour urinary iodine excretion.

	Average UIC < 100 µg/L	Average UIC > 100 µg/L
Home parenteral nutrition patients		
Est24h UIE < RDI*	**42 %** (n= 5)	25 % (n=3)
Est24h UIE > RDI *	-	**33 %** (n= 4)
Healthy children		
Est24h UIE < RDI*	**37 %** (n=10)	22 % (n=6)
Est24h UIE > RDI *	7 % (n=2)	**33 %** (n=9)

Subjects categorized equally by both methods are highlighted in bold
UIC = urinary iodine concentration
EST24h UIE = estimated 24-hour urine iodine excretion
RDI* = recommended daily intake adjusted for 15% nonrenal iodine loss
Home parenteral nutrition patients, n = 12
Healthy children, n=27

If daily urine output is 1 liter, values obtained by UIC and Est24h UIE are expected to be similar. Median value of UIC and Est24h UIE among HPN patients assessed by both methods did not show a significant difference (p=0.530). Median UIC among the healthy children was significantly higher than median Est24h UIE (p<0.001).

4.7 Prevalence of iodine insufficiency with the use of three different methods

The use of diet records, UIC and Est24h UIE gave different prevalence of iodine insufficiency in both groups, and different subjects were identified as iodine insufficient by the various methods, Figure 15. In both groups, one third of the subjects were categorized as iodine insufficient by all three methods.

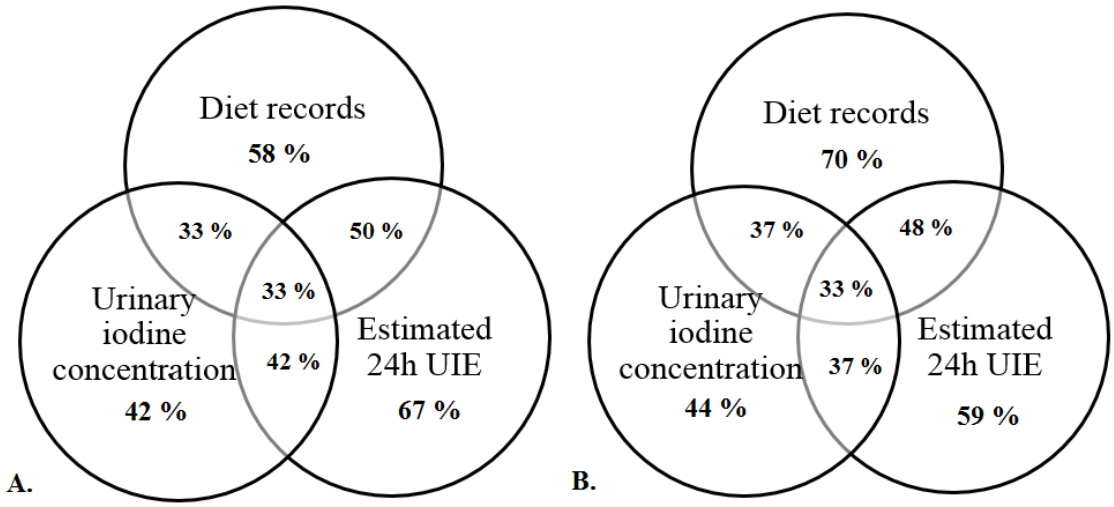

Figure 15. Prevalence of iodine insufficiency. (A.) home parenteral nutrition patients (n=12), (B.) healthy children (n=27). Prevalence of iodine insufficiency is showed in relation to diet records, urinary iodine concentration and estimated 24-hour urinary iodine excretion.

4.8 Iron intake

The daily supply of iron in both groups is shown in Table 23.

HPN patients had a median iron supply (PN, EN and diet) of five mg/day, which corresponded to 54 % of RDI. There was a wide range in iodine provision, from 3 % of RDI to 143 % of RDI.

Healthy children had a median daily iron intake of nine mg, corresponding to 97 % of RDI, with a range from 61 % of RDI to 141 % of RDI.

Percentage iron intake relative to RDI was significantly lower among HPN patients relative to the healthy children (p=0.004).

Table 23. Iron intake (mg/day).

	Home parenteral nutrition patients		Healthy children		Recommended daily intake (NNR 2012)
Age (years)	n	Median (range)	n	Median (range)	
Total	19	5 (0.5-1.5)	34	9 (5-8)	
3-5	3	5 (3-9)	7	7 (5-9)	8
6-9	6	5 (0.7-13)	16	9 (6-11)	9
Boys >10	7	8 (0.9-15)	3	10 (9-16)	11
Girls 10-13	1	2.0	4	9 (7-13)	11
Girls >14	2	1 (0.5-1.7)	5	15 (10-83)	15
			4*	14 (10-18)	15

Range= minimum-maximum values

NNR = Nordic Nutrition Recommendations

*Excluded one participant who received iron supplement due to anemia.

Total iron intake for the healthy children is without one subjected under treatment for anemia

4.8.1 Parenteral iron supply among home parenteral nutrition patients

ESPGHAN guidelines recommend a daily provision of 50-100 µg iron/kg bodyweight in PN to pediatric patients (15). PN supply to all 19 HPN patients were below this recommendation, Table 24.

Table 24. Parenteral iron supply.

	Parenteral iron supply Median (range) µg/kg/day	ESPGHAN guidelines recommendation µg/kg/day
Home parenteral nutrition group (n=19)	21.7 (0.0-32.4)	50-100

Range=minimum-maximum values

Fourteen (74 %) of the HPN patients had total iron supply (PN, EN, diet) below RDI for his/her age category. Insufficient iron provision was found among all subgroups of HPN patients. All five patients with SBS, two out of three patients with severe malabsorption and seven out of 11 patients with severe bowel dysmotility, had an iron supply below RDI.

Two of the four patients with Peditrace had total iron supply below the recommendation. The two patients with supply above RDI, received 99 % and 89 % of their iron intake from EN.

4.8.2 Nutritional sources of iron

Oral intake in the HPN patients contributed with 37 % of daily iron intake, whereas EN and PN accounted for the remaining 30 % and 33 %, respectively, Figure 16. Iron from whole grain products accounted for more than a third of the total intake amongst the healthy children, Figure 17.

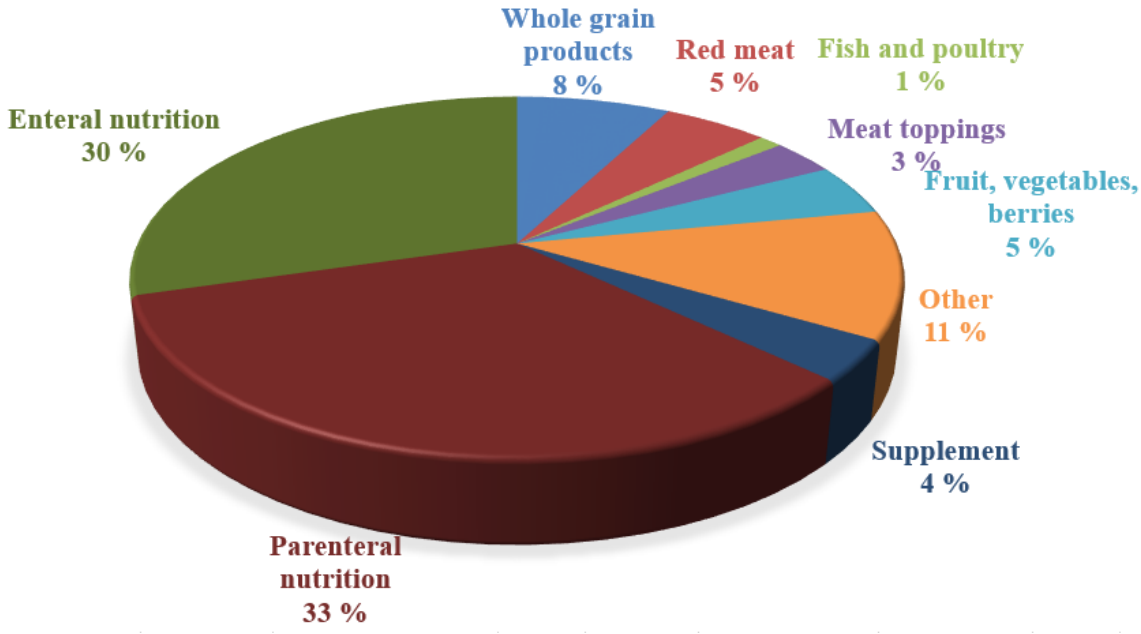

Figure 16. Sources of iron among home parenteral nutrition patients (n=19).

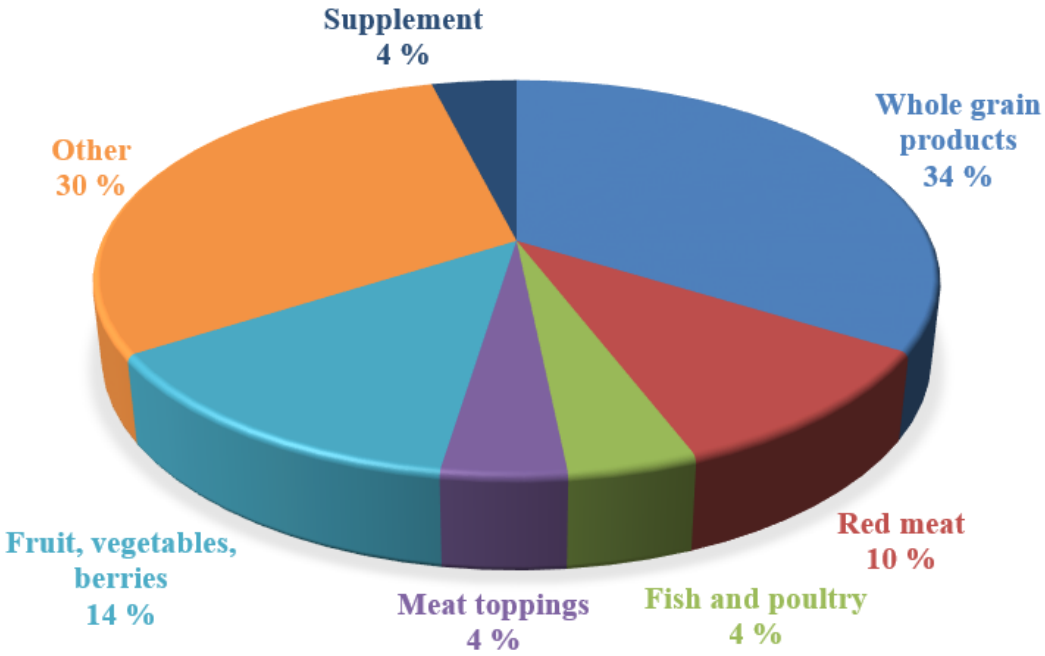

Figure 17. Dietary sources of iron among the healthy children (n=35).

Overall, PN was an important source of iron among the HPN patients. However, iron supply relative to RDI was significantly lower among patients who received 70 % or more of their energy from PN compared to patients receiving less than 70 % of their energy supply from PN, Table 25.

Table 25. Total iron provision in relation to energy ratio from parenteral nutrition.

	Patients with <70 E% from parenteral nutrition (n=10) Median (range)	Patients with ≥ 70 E % from parenteral nutrition (n=9) Median (range)	P value
Percentage iron supply relative to RDI	86 (46-143)	11 (3-98)	0.001

E % = percent of total energy intake
Range = minimum-maximum values
Mann Whitney U Test

EN accounted for 30 % of total iron intake among the HPN patients in general. Eight patients received EN at the time of the study, however three of them received a minimal supply (less than 165 kcal/day). Removal of these three subjects, revealed a significant difference in total iron supply relative to RDI between patients with and without EN, Table 26.

Table 26. Total iron provision in relation to supply of enteral nutrition support.

	Patients without enteral nutrition support (n=11)	Patients with enteral nutrition support (n=5)	P value
Percentage iron supply relative to RDI Median (range)	46 (3-140)	112 (95-143)	0.005

RDI = recommended daily intake
Range = minimum-maximum values
Mann Whitney U Test
Three patients with less than 165 kcal/day from enteral nutrition support excluded

4.8.3 Enteral energy and iron intake

Enteral iron intake (EN, diet) in the HPN patients had a non-significant correlation with enteral energy intake (four subjects with 0 enteral intake were excluded from this analysis), while the correlation was strong and significant among the healthy children, (one subject excluded due to treatment of anemia) Table 27.

Table 27. Correlation between enteral energy and iron intake.

	Home parenteral nutrition patients (n=15)	Healthy children (n=34)
Correlation coefficient	0.470	0.605
P value	0.077	<0.001

Enteral intake = enteral nutrition support and diet
Spearman's rho correlation 2-tailed
One healthy child removed due to treatment for anemia
Four home parenteral nutrition patients with 0 enteral intake excluded

4.9 Blood samples

4.9.1 Overview

Table 28 show median values of iron parameters measured in both groups. Within both groups, median values for hemoglobin were above WHO' reference value for all age categories, except the two HPN patients above 15 years of age.

Table 28. Hemoglobin level and iron parameters measured in blood samples.

Parameter	Age (years),gender	n	Home parenteral nutrition patients Median (range)	n	Healthy children Median (range)	Reference values
Hemoglobin (g/dL)	<5	2	**12.0** (11.6-12.3)	2	**12.3** (12.0-12.5)	≥ 11.0[a]
	5-11	11	**11.6** (9.9-13.1)	26	**12.3** (10.8-14.1)	≥ 11.5[a]
	12-14	4	**12.6** (11.4-13.5)	2	**12.4** (12.3-12.5)	≥ 12.0[a]
	>15, girls	1	**11.0**	4	**13.3** (12.0-13.9)	≥ 12.0[a]
	>15, boys	1	**11.4**	0	**-**	≥ 13.0[a]
MCH (pg)	3-5	3	**27.0** (27-28)	6	**26,5** (26-28)	23-31[b]
	6-17	16	**28.4** (26-32)	28	**28,0** (25-31)	25-33[b]
MCV (fL)	3-5	3	**85** (80-88)	6	**79** (77-85)	70-87[b]
	6-11	10	**85** (79-88)	22	**81** (74-89)	76-95[b]
	12-17	6	**84** (81-88)	6	**88** (80-93)	78-98[b]
Iron (µmol/L)	3-17	19	**9** (4-25)	34	**15** (3-24)	9-22[b]
Transferrin (g/L)	3-17	19	**2.6** (1.7-4.2)	34	**2,7** (2.1-3.2)	2.0-3.3[b]
TIBC (µmol/L)	3-17	19	**65** (43-105)	34	**68** (53-80)	49-83[b]
Transferrin saturation	3-17, girls	6	**0.15** (0.08-0.34)	21	**0.21** (0.07-0.38)	0.10-0.50[b]
	3-17, boys	13	**0.11** (0.08-0.24)	13	**0.25** (0.05-0.37)	0.15-0.57[b]
Ferritin (µg/L)	<5	2	**107** (31-183)	2	**30** (26-34)	> 12[a]
	>5	17	**59** (13-378)	32	**38** (16-111)	≥ 15[a]
Transferrin receptor (mg/L)	Girls	6	**3.5** (2.9-5.2)	21	**3,2** (2.3-4.9)	1.9-4.4[b]
	Boys	13	**4.2** (2.3-7.0)	13	**3.6** (2.5-5.4)	2.2-5.0[b]

[a] = World Health Organization' reference values

[b] = Oslo University Hospital' reference values

Range = minimum – maximum values

4.9.2 Hemoglobin and anemia

According to WHO' criteria, five out of 19 (26 %) HPN patients had mild anemia and three (16 %) had moderate anemia. Among the healthy children, three out of 34 (9 %) children had mild anemia and one (3 %) had moderate anemia (Figure 18). Prevalence of anemia was significant higher among HPN patients relative to healthy children (p=0.016).

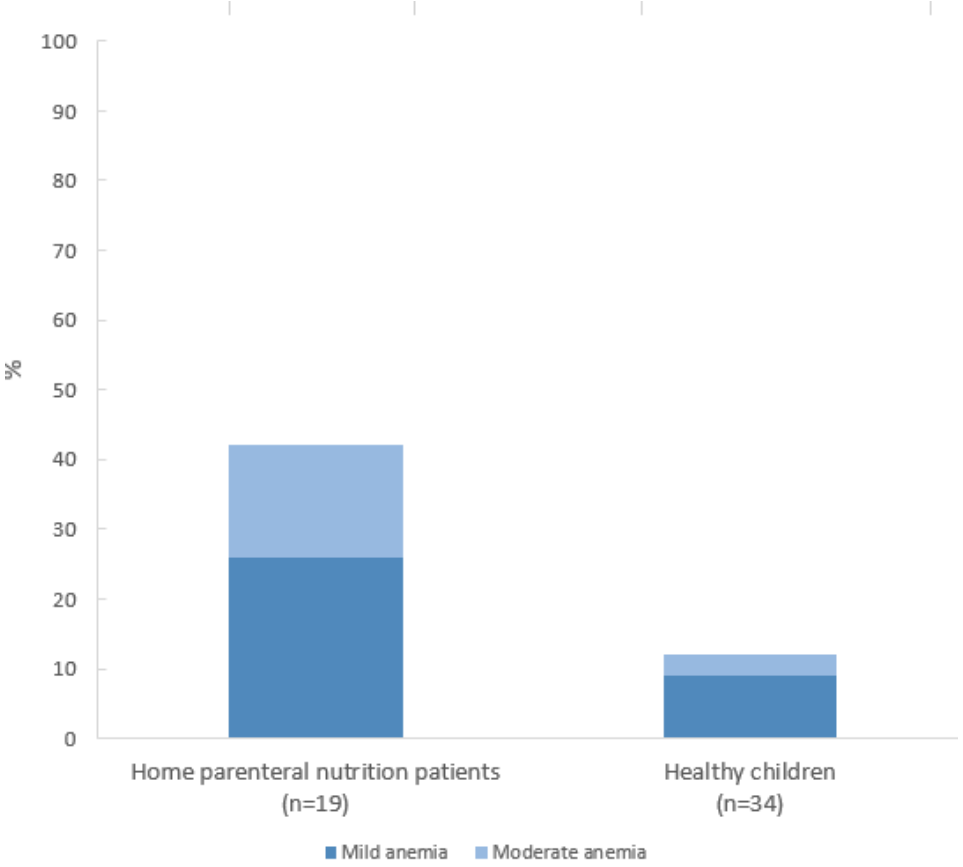

Figure 18. Prevalence of anemia according to World Health Organization' cut off values.

Of the eight anemic HPN patients, six (75 %) had mean daily iron supply below RDI. Among the healthy children with anemia, three of four had a daily iron intake below RDI.

Anemia was found within all subgroups of HPN patients. Five out of 11 HPN patients with severe bowel dysmotility, two out of five with SBS and one out of three with severe malabsorption, had anemia according to WHO' criteria.

There was no significant difference in number of subjects with anemia between patients with or without enteral nutrition support (p=0.600) or patients with Addaven or Peditrace (p=0.651).

4.9.3 Ferritin status

Only one HPN patient was below WHO' reference value for ferritin, Figure 19. Interestingly, this patient was one of few HPN patients whom had iron intake above RDI and had normal Hb and transferrin receptor value. The rest of the HPN patients and all healthy children were above WHO' reference values.

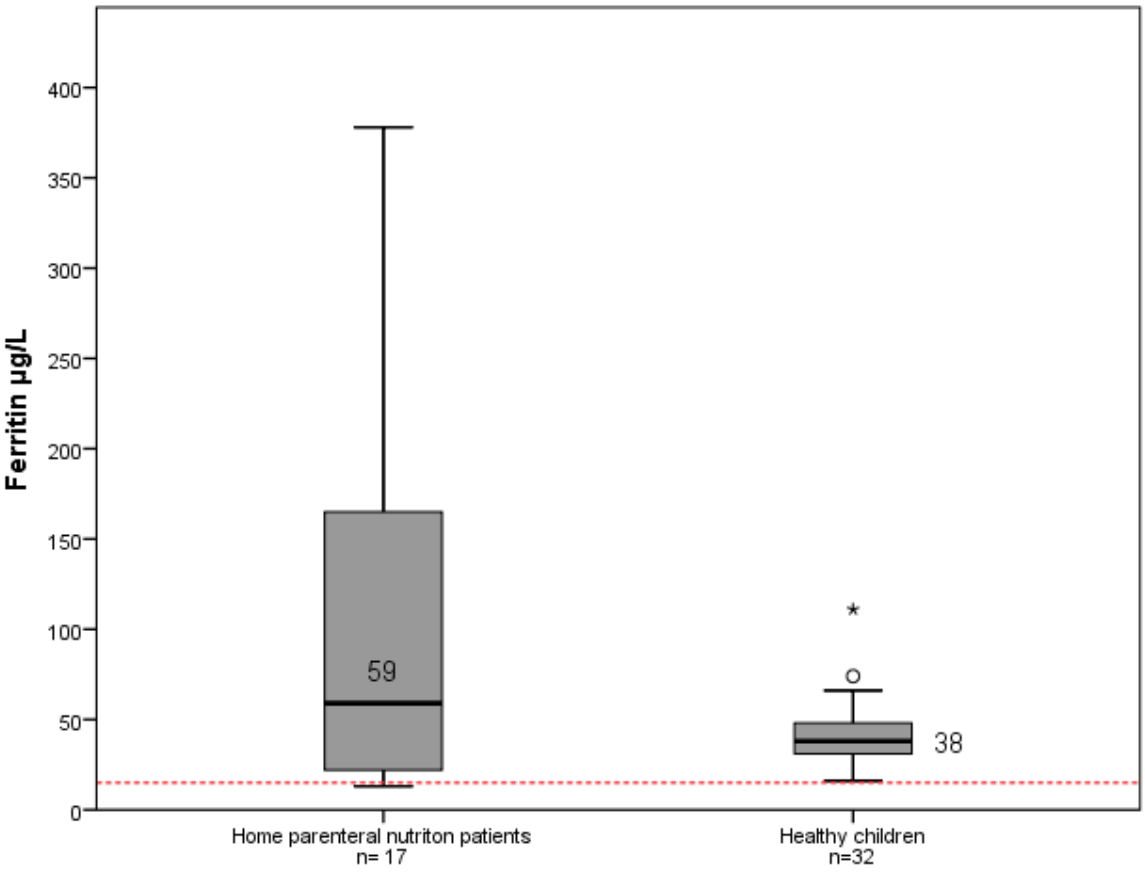

Figure 19. Ferritin (µg/L) values for children >5 years. The horizontal line indicates median ferritin value in each group, the boxes indicate 25th- 75th percentiles. The red dotted line indicates World Health Organization' cut-off value for depleted iron stores in children >5 years (<15 µg/L). Small circle indicates outliers, star indicate extreme value. n = number of subjects within each group.

If the lower cut-off value for ferritin was set to 30 µg/L, five (26 %) of the HPN patients, and eight (24 %) of the healthy children were below this value. Four (80 %) of these HPN patients had iron supply below RDI, whereas only three (38 %) of these healthy children had intake below RDI. Furthermore, two of the five HPN patients had anemia according to WHO' criteria, while one of the eight healthy children had anemia.

There was no significant correlation between iron intake and ferritin level in either HPN patients or healthy children (data not shown).

4.9.4 Transferrin receptor

One out of six girls (17 %) in the HPN group had transferrin receptor value above the upper reference level (4.0 mg/L). This patient was also anemic, had elevated ferritin level and C-reactive protein (CRP) level. Among the healthy children, there was one out of 21 (5 %) girls with a value above reference area. This healthy girl did not have anemia, but had ferritin below 30 µg/L. Figure 20 shows median transferrin receptor values for girls in both groups.

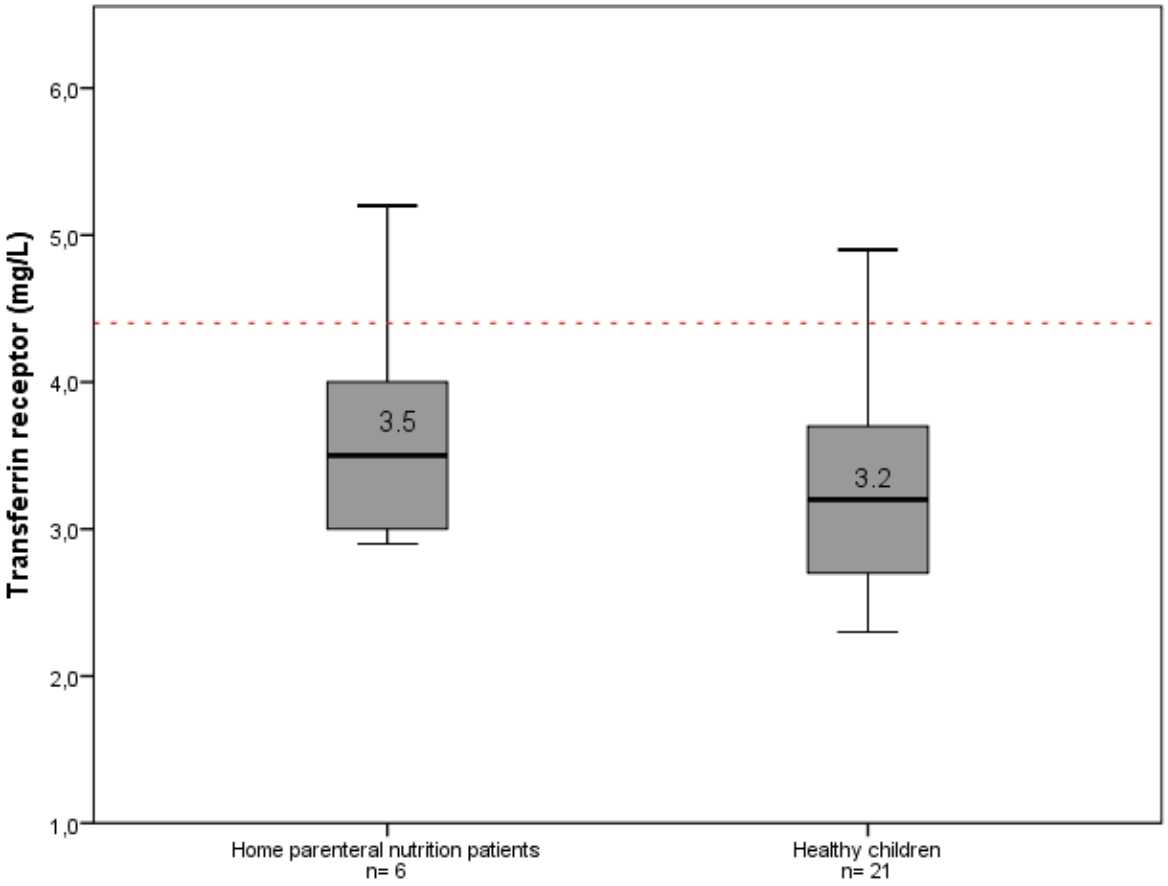

Figure 20. Transferrin receptor (mg/L) values for girls.
The horizontal line indicate median value in each group, the boxes indicate 25th- 75th percentiles. The red dotted line indicates Oslo University Hospital' upper cut off value (4.4 mg/L). n = number of subjects within each group.

Three boys out of 13 (23 %) in the HPN group had transferrin receptor value above the upper reference level (5.0 mg/L), Figure 21. Of these three patients, one (33 %) had mild anemia according to WHO. Among the healthy children, there was one out of 13 boys (8 %) with a value above upper reference level but without anemia.

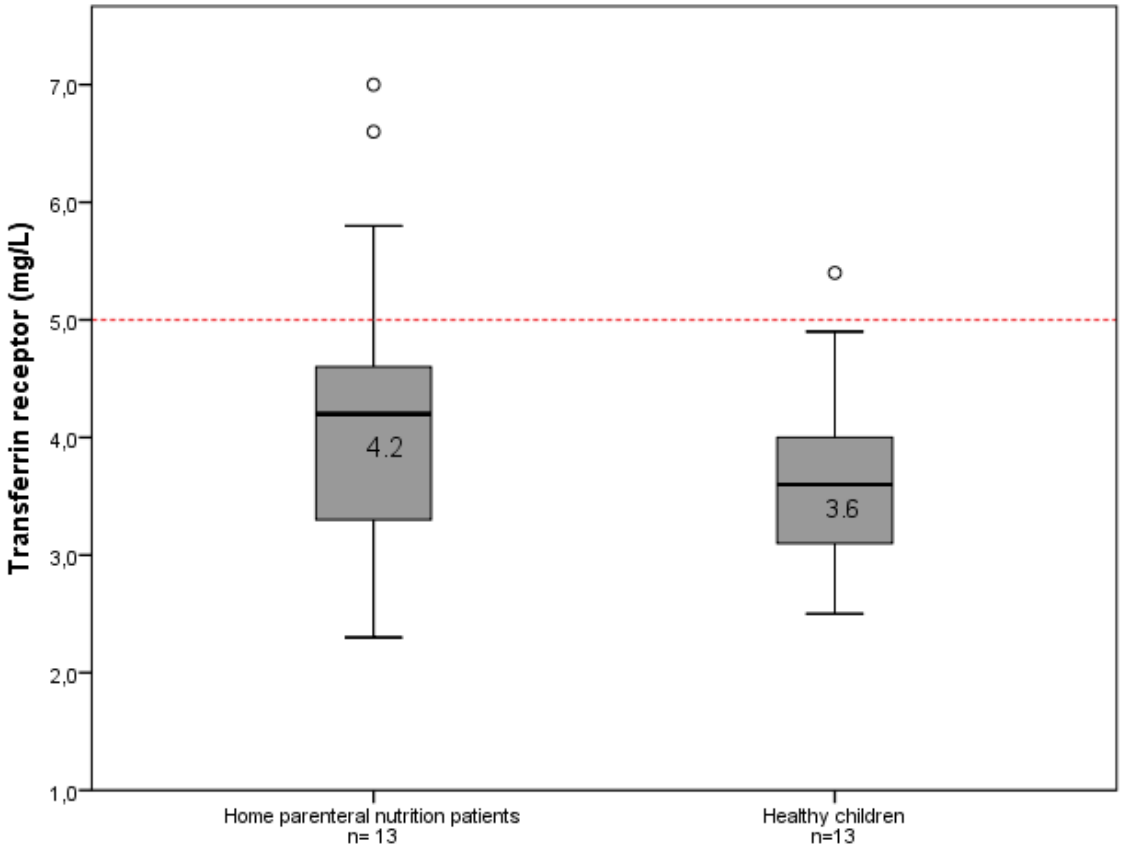

Figure 21. Transferrin receptor (mg/L) values for boys.
The horizontal line indicate median value in each group, the boxes indicate 25th- 75th percentiles. The red dotted line indicates Oslo University Hospital' upper cut off value (5.0 mg/L). Small circles indicate outliers. n = number of subjects within each group.

4.9.5 Prevalence of iron insufficiency with the use of different methods

Various methods to assess iron status were used, and these identified different subjects as iron deficient in both HPN patients and healthy children, Figure 22. Overall, only one HPN patient had both a reduced Hb value, ferritin <30 µg/L and an elevated transferrin receptor, whereas none of the healthy children met all three criteria.

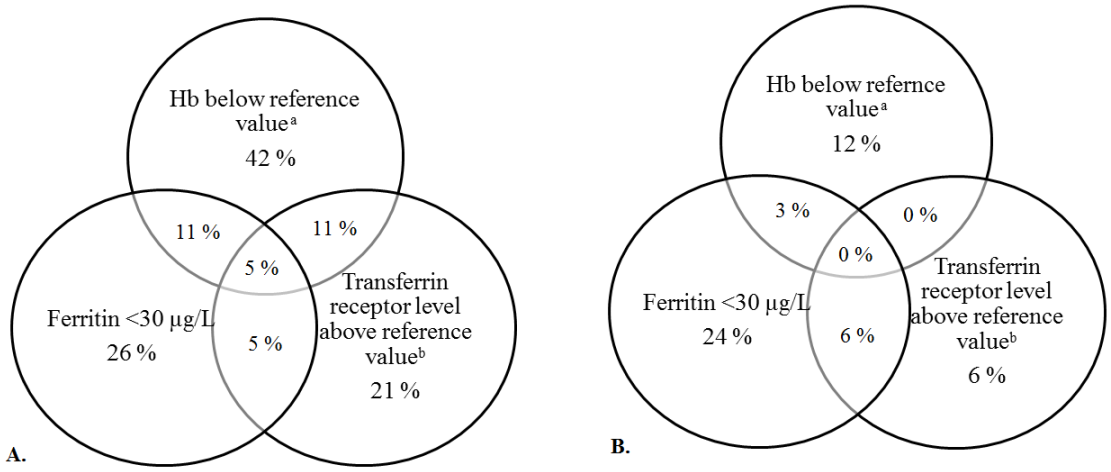

Figure 22. Prevalence of iron insufficiency among (A.) home parenteral nutrition patients (n=19) and (B.) healthy children (n=34) according to hemoglobin (Hb) value, ferritin value and transferrin receptor value. [a] = World Health Organization' reference values, [b] = Oslo University reference values.

5 Discussion

5.1 Summary of results

The present study is the first to assess iodine and iron status among Norwegian pediatric HPN patients. Intake of iodine was below RDI in HPN patients as well as in healthy children. Median UIC among the HPN patients indicated an insufficient iodine status according to WHO' criteria. The median UIC in the healthy children was above WHO' cut off values. However, iodine concentration in healthy children may have been influenced by the subjects' hydration status as Est24h UIE revealed a significantly lower iodine excretion. There was no significant differences between the groups in any iodine status parameter.

The iron provision among the HPN patients was lower than the dietary iron intake in the healthy children, and parenteral provision was lower than the ESPGHAN recommendation. Accordingly, the prevalence of anemia was significantly higher among HPN patients compared to the healthy children.

Patients receiving Peditrace as parenteral trace element solution and patients without enteral nutrition support, had the lowest supply of iodine and iron.

5.2 Study population and recruitment

A main strength of the present study was that all pediatric HPN patients treated at OUH within the age of 2-18 years were recruited and included to the study. The patients originated from ten different counties, and should thus give a valid description of this patient population in Norway. The healthy children were recruited from three nearby counties in order to minimize travel distance, and may therefore not be representable for the Norwegian population as a whole. There were no statistical difference in either age or gender distribution between the two groups, which therefore make them more comparable.

In many epidemiological studies there are a tendency to recruit the most motivated subjects due to the workload inflicted by participating. Thus, this may have led to a selection bias in the healthy children of the present study (103). Furthermore, the healthy participants were recruited through the spreading of a registration form in social media by research workers. This led to a high prevalence of parents with health related jobs. It is therefore likely that

this population have an overall healthier lifestyle than Norwegian children and adolescents in general. Previous studies have shown an association between the level of parental education and a healthier lifestyle (104-106). Thus, the results from the healthy children should be interpreted with caution, as it may not be representable for the Norwegian population as a whole.

5.3 Methodological considerations

In all studies using dietary assessment methods, there is risk of under- and overestimation of nutritional intake. However, a study conducted with children aged six to nine, did not find significant difference between energy intake obtained from food records relative to energy expenditure measured by doubly labeled water (107). The same study concluded that a three-day food record could be used at group level for dietary surveys conducted on children of this age group.

The dietary iodine and iron intakes of the present study were compared to RDI. However, this is likely to overestimate the prevalence of low nutrient supply. RDI includes a safety margin set to cover the requirement of 97 % of the group, while estimated average requirement (EAR) is set to cover the requirement of 50 % of the group. EAR is the value primarily used to assess nutrient status of groups in epidemiological studies. However, Nordic EAR values for children <14 years does not exist (40). Furthermore, RDI may be used for dietary planning, and is used as reference for nutrient supply to pediatric HPN patients at OUH in addition to ESPGHAN guidelines. We therefore chose to assess iodine and iron provision in relation to RDI in the present study.

We provided the participants with a picture booklet to estimate portion sizes in order to ease the workload of diet registration. A weighted food record could potentially have provided more accurate data on intake, however this method is more time consuming and could possibly have led to an increased drop-out rate or a change in diet habits (103). The picture booklet contained series with four different portion sizes of 33 food items. Furthermore, there were pictures of plates, glasses, mugs and slices of bread of different sizes. Each page included a ruler to make the estimation of portion size easier. In a master thesis from 2011, the use of this picture booklet was evaluated (108). It was found that although there were individual differences in the ability to choose correct portion size, this was attenuated at

group level. Overall, they found that the ability to estimate portion size using this manual, was high for most food items.

With regards to reliable measurements of iodine intake, Erkkola et al found that in order to obtain r = 0.8 between observed and true iodine intake, six year old boys and girls required five and three days of food records, respectively (109). The number of days needed to obtain r = 0.8 for iron intake was eight and five days, for six year old boys and girls, respectively. Pediatric HPN patients experience minimal variation in nutritional supply, whereas the healthy children were expected to have a more varying diet. We therefore chose four days with food record, as a compromise between what was needed for optimal data and what we thought would be feasible for the participants.

Considerations regarding calculation of iodine and iron intake also need to be made. It has previously been shown that food items may experience both temporal and regional differences in iodine content (110). A study conducted on Norwegian low-fat milk showed significantly lower iodine concentration during the summer relative to winter, however geographical differences were not observed (46). The dietary records in the present study were conducted during a four-month period for all participants, the first in March and the last in June. Thus, there may have been some variation in iodine content in the milk and dairy products consumed by the participants.

Median UIC from spot urine samples is the method currently recommended by WHO for assessment of iodine status in populations (41). The method is not a valid marker of iodine status in individuals, but is considered to be a reliable biomarker of recent dietary iodine intake in a population (37). Andersen et al conducted a study to determine the number of spot urine samples needed to assess iodine status in populations. Their findings indicated that 125 samples are needed in order to estimate iodine level with 95 % confidence within a precision range of ±10 %, whereas 100 samples were needed when using estimated urinary iodine excretion (111). Due to practical considerations and time constraints, we found it optimal to ask for two samples per child, which then could potentially give a maximum of 38 samples in the HPN group and 70 in the group of healthy children.

Use of the UIC method has been criticized because iodine content in urine is influenced not only by the participant's recent intake of iodine, but also by the hydration status (58, 112). Recent research has showed promising results with the use of Est24h UIE as a method for

assessing iodine status in a hydration independent matter. Montenegro-Bethancourt et al showed that Est24h UIE had a higher correlation with real 24-hour urinary iodine excretion, than UIC in children (58). They also showed that variation in hydration status does not even out in larger number of samples, which has previously been assumed (59). We therefore chose to present our data according to both the currently recommended method, UIC, and Est24h UIE. Ideally one should measure 24-hour urinary iodine excretion in order to get the most accurate data (113), however, this would be a great burden on the participants and thus not feasible.

There are contradictive results with regards to whether iodine displays a diurnal variation in its excretion. Thus, this must be taken into account when assessing both UIC and Est24h UIE results, considering that we used spot urine samples which may not reflect iodine excretion throughout the day (99, 114). However, all subjects were asked to avoid using the first morning urine as the urinary iodine content is expected to be at its lowest at this time (99).

The present study had a cross-sectional design, which has inherent limitations (115). With this design, the researchers get a snapshot of the populations, meaning that an identical study conducted at a different time possibly could give another result. The design cannot be used to establish causality between variables, but it enabled us to describe the populations' iodine and iron status at that particular time, which was the main aim of the present study. For future research it may be advantageous to follow both HPN patients and healthy children over time and collect data at several visits. However, this was not possible within the time frame of a master thesis.

Moreover, the post hoc statistical analysis showed that the present study had a low statistical power, which make the possibility of type II error very high.

Overall, the present study was conducted according to the manual compiled by Euthyroid for researchers conducting population studies on iodine status (116).

5.4 Iodine status

5.4.1 Iodine intake

Median total iodine provision was below RDI among both HPN patients and healthy children. Furthermore, both groups displayed a wide range of iodine supply. Median parenteral iodine supply was above ESPGHAN recommendation in HPN patients.

PN and EN were the most important sources of iodine among HPN patients. Patients without EN had significantly lower total iodine provision than patients with EN. Moreover, in the present study, patients with SBS received EN less frequently than the other two patient groups, and may therefore be susceptible to a low total iodine supply.

Patients receiving Peditrace as parenteral trace element solution had significantly lower parenteral iodine provision than patients receiving Addaven. Furthermore, all four patients supplied with Peditrace had parenteral iodine supply below the ESPGHAN recommendation.

Because intestinal absorption of iodine in healthy individuals is expected to be very high, enteral and parenteral recommendations of iodine supply should be similar. However, the parenteral recommendation is far below the enteral recommendation (42, 43). The ESPGHAN' iodine recommendation received Grade D in the ESPGHAN guidelines, i.e. the lowest recommendation grade according to the Scottish Intercollegiate Guideline Network. The reason was that the recommendation is based upon few studies with a low evidence level. Furthermore, the recommendation assumed use of iodinated topical antiseptics and thus some iodine absorption from the use of these (15). However, this is not common practice among HPN patients in Norway.

The HPN patients enrolled in the present study experience little variation in daily supply of nutrients, as PN accounts for the majority of their nutrient provision and their dietary intake otherwise is stable on a day-to-day basis. Thus, the present estimation of this population's iodine supply is expected to give an accurate representation of their iodine status over time. Overall, the HPN patients received most of their iodine from parenteral administration and therefore bypassed possible malabsorption. However, individual differences existed in amount received from parenteral and enteral route, and patients with a higher ratio from enteral administration may have experienced a varying degree of iodine absorption.

Healthy children overall had a median iodine intake below RDI, and in accordance with the HPN patients, the group displayed a wide range in intake. Because iodized salt is a negligible source to iodine in Norway, whereas milk, dairy products and fish accounts for the majority of the intake, dietary records may provide a reasonable estimate of iodine status in this population (60).

Dahl et al used data from UNGKOST 2000 and found a similar range of iodine intake as in the present study (45). Furthermore, they found that milk and dairy products accounted for 64-71 % of total iodine intake, which corresponds well with the findings among the healthy children in our study. However, mean contribution from fish was higher in the data from UNGKOST 2000 than in the present study. Dietary data in UNGKOST 2000 were collected with four-day food records, same method as in the present study. It is therefore unlikely that the difference is due to the method used. Furthermore, data from UNGKOST 2000 and UNGKOST 3 indicate that intake of fish has remained stable (63, 117). However, UNGKOST 2000 represent nationwide data, while the present study was carried out with children from cities nearby Oslo. Thus, it may be that children in Norway overall consume fish more frequently than children of the present study.

UNGKOST 3 found that consumption of milk and dairy products was significantly higher among 13 year olds of parents with university or college education (63). This may have led to a higher total intake of iodine in this group given that milk and dairy products are important determinants of iodine intake. Thus, the reference population in the present study may have had a higher intake of milk and dairy products and hence, iodine, than Norwegian children in general. Therefore the prevalence of low iodine intake may be even higher in the Norwegian population overall than in the present study.

5.4.2 Urinary iodine concentration

Median UIC among HPN patients was below WHO' cut off value for sufficient iodine status. This is worrying considering the importance of iodine for normal growth and development. Even a mild iodine deficiency may have detrimental effects, whereas severe iodine deficiency during childhood can lead to mental impairment, metabolic abnormalities and adverse effects on growth and skeletal maturation (50, 51, 53-55, 57).

Patients with Peditrace as parenteral trace element solution had a parenteral iodine supply below the ESPGHAN recommendation, while all patients with Addaven were above the recommendation. Thus, the majority of the HPN patients had a parenteral iodine supply above the ESPGHAN recommendation. However, more than half the HPN patients were iodine insufficient according to their UIC value. This may indicate that the ESPGHAN recommendation for pediatric HPN patients is too low. Results of the present study therefore support the conclusion by Cicalese et al, who in 2009 proposed an increase in the recommended iodine provision for pediatric PN patients (26). As previously mentioned, there is little scientific evidence supporting the ESPGHAN recommendation. Thus, pediatricians and dieticians should be careful when using iodine intake relative to the ESPGHAN recommendation as a measurement of sufficient iodine supply to pediatric HPN patients.

Significant difference between median UIC of patients with Addaven relative to Peditrace, or patients with EN relative to patients without EN, were not obtained in the present study. This is contradictive to the findings of different iodine provision between these groups. However, the sample size may have been too small to detect a significant difference.

Sub-analysis of iodine supply according to UIC values, revealed a higher median provision among both HPN patients and healthy children with UIC above 100 µg/L compared to subjects with UIC below 100 µg/L. Although the difference in intake only were significant for the healthy children, this indicate that UIC was able to separate individuals with high and low supply of iodine.

Studies of iodine status in both adult and pediatric HPN patients have previously been conducted. A prospective study assessed iodine status among pediatric HPN patients with a total iodine supply (PN, EN and diet) in the range of 1.0-2.8 µg iodine/kg body weight/day. Four weeks after onset of PN treatment, 12 of 15 patients had UIC below 100 µg/L, and after eight weeks all 15 patients was below cut off (26). The lower prevalence of iodine insufficiency found in the present study is likely to be due to a higher total iodine supply (2.2-8.2 µg iodine/kg bodyweight/day).

Moreover, Johnsen et al recently found that 76 % of a pediatric HPN population were iodine insufficient according to median UIC. However, these children did not routinely receive

iodine PN supplementation, as pediatric patients at OUH do. Therefore, direct comparisons between the two studies cannot be made (27).

Furthermore, our results are in accordance with a study conducted on adult HPN patients. The adult patients had a median UIC of 63 µg/L, thereby indicating an insufficient iodine status in this population (61). However, urine output and recommended iodine supply is higher for adults than children. Therefore, we cannot make direct comparison of UIC values between an adult HPN group and a pediatric HPN group.

Contradictive to these studies, a recent retrospective study in the US found a sufficient iodine status in pediatric PN patients assessed by serum iodine levels (118). However, serum iodine is not recommended for assessment of iodine status in populations according to WHO (41). Furthermore, the method is considered to be a late marker of iodine insufficiency and not suitable for use in population with mild and moderate iodine insufficiency (37, 42, 59). Therefore, the results from Santoro et al does not necessarily indicate that pediatric PN populations are iodine sufficient.

The median UIC value obtained from the healthy children in the present study is in accordance with a British study on schoolchildren, where median UIC was 144 µg/L (119). Furthermore, Bath et al found a positive correlation between consumption of milk and UIC values. In the present study, we found a positive correlation between consumption of milk and dairy products and UIC values. The UK has minimal use of iodized salt in processed foods and cow fodder is iodized. Thus, sources of iodine in the UK is similar to those in Norway.

Furthermore, median UIC value of the healthy children in the present study is in accordance with the results of a recent Danish study (120). Iodine intake was not reported in the Danish study. However, Denmark has obligatory iodine fortification of table salt and salt used in commercial bread production, therefore total iodine intake may differ between children in Norway and Denmark.

In a German study, median UIC was 108 µg/L in children aged 6-12 years, thus lower than the result of the present study (58). The higher median UIC found in the healthy Norwegian children may reflect a genuinely higher iodine intake among the Norwegian children relative to the German children. However, dietary intake was not assessed by Montenegro-Bethancourt et al. There may also have been different hydration statuses in the two

populations, however urine osmolality was only measured among the German children and not among the children of the present study.

In summary, the present results indicate an insufficient iodine status among the HPN patients, while median UIC in the healthy children was above cut off value. We did not find a significant difference in median UIC between the HPN patients and healthy children. However, the UIC data within both groups showed wide distribution ranges, and both groups had a low sample size. This increases the risk of type II error, where a true difference between the groups is not detected due to a low statistical power.

5.4.3 Estimated 24-hour urinary iodine excretion

Iodine concentration in urine is not only influenced by recent iodine intake, but also by the subjects' hydration status (58). A poor hydration status will lead to a more concentrated urine and thus an overestimation of iodine status. In order to adjust for this confounding effect, it has been proposed to scale the UIC according to creatinine content and 24-hour creatinine reference values. Currently there does not exist universal cut off values for sufficient iodine status when using Est24h UIE, as it does for the use of UIC. However, it has been proposed to compare Est24h UIE to RDI adjusted for 15% nonrenal iodine loss (RDI*) or EAR (52, 58). Because there does not exist Nordic EAR values for iodine intake among children, the Est24h UIE in the present study have been compared to RDI*.

Est24h UIE showed that two thirds of the HPN patients were below RDI*. The method has not previously been carried out with HPN patients and its validity for use with this population is uncertain. The HPN patients had a complex disease background, and their diverse medical conditions may have had an impact on their creatinine excretion. The reference values used in the present study are based upon a study of healthy children. It is reasonable to assume that the HPN patients may have had additional factors influencing creatinine excretion beyond anthropometrics, age and gender which are the factors adjusted for in the reference values (101). Further research is warranted to evaluate the use of this method with this patient population.

A comparison of HPN patients assessed by both UIC and Est24h UIE, revealed a 26 % increase in prevalence of iodine insufficiency with the use of Est24h UIE relative to UIC. Zimmermann et al has previously estimated that although an UIC of 100 µg/L in adults

indicate an iodine intake equal to RDI for adults, it equals an iodine intake of approximately 75 µg for a child 10 years of age, thus less than RDI (52). Therefore, the increased prevalence of iodine insufficiency measured with Est24h UIE may be attributed to a higher cut off value relative to UIC. Moreover, hydration status may have led to an overestimation of iodine status with the use of UIC. However, the HPN patients are expected to have an adequate hydration status due to the high fluid supply from PN. Neither fluid supply nor urine osmolality were measured in the present study, therefore the subjects' hydration status cannot be assessed.

Assessment of total iodine provision in subjects with Est24h UIE above or below cut-off value indicated that the method was able so separate participants with high and low iodine supply. However, the difference in iodine provision was only significant for the healthy children.

The healthy children overall had an Est24h UIE of 75 µg/day, which is similar to values obtained in a recent Danish study conducted on seven and eleven year old boys and girls (120). Rasmussen et al found median Est24h UIE to be 93 µg/day and 77 µg/day in boys and girls, respectively. Moreover, in a German study the median Est24h UIE was 72 µg/day among 6-12 year olds and 98 µg/day among 13-18 years olds (58). Both the Danish and the German study used the same equation for calculation of Est24h UIE and the same 24-hour creatinine reference values as the present study.

The healthy children experienced an equal increase in prevalence of iodine insufficiency measured by Est24h UIE relative to UIC as the HPN patients did. This may be due to the values being compared to RDI*, as previously mentioned. However, it may also be that the subjects' hydration status have influenced the UIC values obtained. If daily urine volume equals one liter, one would expect UIC and Est24h UIE to give similar values. However, the median Est24h UIE for the healthy children assessed by both spot urine methods, was significantly lower than the median UIC value. This may indicate a lower urine output than 1 liter and thereby a possible suboptimal hydration status. Among the HPN patients, there was no significant difference between median UIC and median Est24h UIE, which may indicate a urine volume close to one liter. Thus, use of UIC may be appropriate with well-hydrated HPN patients, whereas Est24h UIE may give a more accurate estimate in healthy young children with a low urine output or with a suboptimal hydration status.

Altogether, the use of UIC lead to an overestimation of iodine status in both groups relative to the Est24h UIE method. Although part of this effect is likely to be attributed to a higher cut-off value with the Est24h UIE method, it is possible that hydration status has affected the UIC values. A similar result was seen in the healthy German children. Montenegro-Bethancourt et al measured urine osmolality in addition to iodine and creatinine, and found a hydration dependent shift in UIC values and thus an overestimation of iodine status relative to measured 24-hour UIE. Est24h UIE from spot urine samples gave comparable estimates relative to measured 24-hour UIE (58).

5.4.4 Prevalence of iodine insufficiency with use of different methods

All HPN patients categorized as iodine insufficient with the use of UIC, had Est24h UIE values below RDI*. Furthermore, the majority of HPN patients with a total iodine provision below RDI, had a low Est24h UIE value as well. However, half these children did not have an UIC <100 µg/L which is likely to some extent be due to the higher cut off values used for iodine supply and Est24h UIE. Diet records and Est24h UIE overall identified the same subjects. Because this patient group received the majority of their iodine from PN and experience minimal variation in their enteral supply and oral intake over time, diet records may provide a reasonable estimate of iodine status in this patient group. However, individual considerations need to be made in cases where patients receive more iodine from enteral route. Overall one third of the patients had an insufficient iodine supply according to all three methods.

A great variation in the methods' ability to identify the same individuals were seen among the healthy children. Diet records gave the highest prevalence of insufficient iodine intake, although only two thirds of these subjects were below RDI* according to their Est24h UIE, and only half the children were below UIC cut off. Despite discrepancy between the methods, one third of all the healthy children were classified as iodine insufficient according to all three methods.

Furthermore, use of UIC gave a non-significant higher median among the healthy children relative to the HPN patients, whereas Est24h UIE gave a non-significant higher median among the HPN patients relative to the healthy children. There may be a true difference between the groups not detected due to a low statistical power in the present study. Contradictive results with the use of these two methods may indicate that hydration status

influenced the healthy children to a greater extent than the HPN patients, or may indicate that creatinine reference values are not suitable for use with HPN patients. Thus, interpretation of results from both methods must be done with caution.

In summary, one third of both the HPN patients and the healthy children were classified as iodine insufficient by all three methods used in the present study.

5.5 Iron status

5.5.1 Iron intake

The HPN patients had a median total iron supply well below RDI, and all patients had parenteral iron provision below the ESPGHAN recommendation of iron supply to pediatric PN patients. Iron deficiency is the most common cause of anemia and observational studies have indicated a possible detrimental effect of iron deficiency anemia on cognitive development in children (73, 76, 77). Thus, it is worrying that insufficient iron supply is prevalent among this patient group. Moreover, two thirds of the total iron supply to the HPN patients was supplied through enteral route, and may have been subjected to malabsorption. This makes the low iron provision further distressing.

PN and EN were important sources of iron among the HPN patients. Accordingly, patients without EN had significantly lower total iron provision than the rest of the patients. However, patients who received ≥ 70 % of their energy from PN, had a significant lower total iron provision than the rest of the group. Although PN was an important source to iron, the iron content is generally low. HPN patients overall received two thirds of their energy from PN, whereas only one third of total iron provision came from parenteral route. Moreover, four patients were supplemented with Peditrace as parenteral trace element solution and therefore did not receive parenteral supply of iron. However, it is important to note that parenteral provision of iron bypasses the regulatory mechanisms for iron uptake and intestinal absorption of iron is normally low. Therefore, direct comparison of total iron supply with RDI must be done with caution within this patient group.

Median iron intake among the healthy children was 9 mg, which is in accordance with findings from UNGKOST 3 (63). Moreover, we found whole grain products to account for 34 % of total iron intake, which is equal to the results obtained in UNGKOST 3 (63).

However, in UNGKOST 3 meat and meat products accounted for a higher percentage of iron intake relative to the present study. This may support our assumption of having a "healthier" reference group than children in Norway generally.

Total iron supply was significantly lower among the HPN patients relative to dietary iron intake in the healthy children. Correlation between enteral iron intake and enteral energy intake also implies a more iron-rich diet among the healthy children relative to the HPN patients. This may suggest that HPN patients have fewer iron-rich food items in their diet than healthy children do.

5.5.2 Iron deficiency and iron deficiency anemia

In the present study iron deficiency was defined as a ferritin value below WHO' cut off value, whereas iron deficiency anemia was defined as Hb below WHO' reference value and ferritin below cut-off or transferrin receptor above OUH' reference.

Despite a low prevalence of iron deficiency among the pediatric HPN patients, nearly half the children were anemic. Transferrin receptor value indicated that some of these patients were likely to be anemic due to iron deficiency regardless of a normal ferritin value. The HPN patients represent a group of children with complex medical conditions and it is likely that several experience a low-grade inflammation despite normal CRP values (1, 121-123). Acute or chronic inflammation make ferritin unsuitable as a marker of iron status, while transferrin receptor is unaffected by ongoing inflammation (83, 86, 89). Some patients were anemic without elevation of transferrin receptor value which may indicate anemia of chronic disease (91). Furthermore, the majority of the children used several prescribed medications. Some medications may influence hemoglobin status. Therefore, use of medications may have contributed to the high prevalence of anemia (124).

Only one study has previously assessed iron status in a pediatric HPN population. However, the work has not yet been published. An abstract of the study was presented at The European Society of Clinical Nutrition and Metabolism' (ESPEN) congress in September 2017. The presentation revealed that Zyla-Pawlak and co-workers found that 42 % of the Polish HPN children were anemic and median ferritin concentration in the group was 41 µg/L (30). These results are in accordance with the findings of the present study.

Several studies have assessed iron status among adult HPN patients, and they overall report a high prevalence of iron deficiency anemia (25 % - 55 %) based upon Hb values and ferritin and/or transferrin receptor values (28, 29, 92). However, these studies on adult HPN patients comprise a different group of diagnoses relative to the present pediatric group. In the adult populations, the prevalence of IBD and various forms of cancers are high, whereas none of the HPN patients had IBD and only one child was previously treated for ALL. The underlying disease may affect iron status, thus, comparison between these different populations must be done with caution.

In the present study none of the healthy children had iron deficiency. However, four children were anemic according to their Hb value, without a reduction in ferritin value or an increase in transferrin receptor level. However, three of these children were only slightly below the reference value. Taken into account that there always exists some uncertainty in laboratory measurements, these children may in fact not be anemic. Furthermore, the fourth child had increased CRP level, indicating a recent infection, which therefore may have caused a transient reduction in Hb (125).

Mast et al found that increasing the ferritin cut-off level to 30 µg/L in a group of chronically ill adult patients, led to an increase in sensitivity for detecting iron deficiency from 25 % to 92 %, with a positive predictive value of 83 % (89). However, they found little diagnostic value in increasing ferritin cut-off for the healthy adults. Applying the cut-off value of 30 µg/L to the present study populations, led to two more anemic HPN patients being recognized as iron deficient. However, the value of increasing the ferritin level for the healthy children was scarce as the majority with ferritin <30 µg/L was not anemic.

Furthermore, none of the children in either the group of HPN patients or healthy children, had MCH or MCV values below reference value, which would be expected with microcytic anemia caused by iron deficiency (126). However, a study conducted on Norwegian children and young adults found that a normal value on these indexes did not exclude iron deficiency (127).

5.5.3 Prevalence of iron deficiency with use of different methods

Assessment of iron status is complicated in subjects with chronic or intercurrent disease (126). This was apparent in the HPN patients where several children were anemic without

manifestations in common iron parameters. Only one child had Hb below reference value, ferritin <30 µg/L and an elevated transferrin receptor. Thus, unambiguously indication of iron deficiency was not present among the anemic HPN patients. However, iron deficiency is likely in these patients as there was a high prevalence of insufficient iron supply. Moreover, intestinal absorption of iron may be lower than for healthy individuals (128). Some patients may have experienced blood loss from the GI tract due to their underlying disease, some had previously underwent extensive surgery which may have led to blood loss, and all patients had their blood drawn frequently. Taken together, these factors support our assumption of iron deficiency being a prevalent cause of anemia among the HPN patients.

The healthy children had a low prevalence of anemia. However, we expect the reference population in the present study to be "healthier" than the general Norwegian population. Therefore, the true prevalence of iron deficiency and anemia among Norwegian children and adolescents may be higher.

5.6 Strengths and limitations

A key strength of the present study is that we assessed nutritional status by collecting dietary data, urine samples and blood samples from the participants. This provided us with objective biomarkers in addition to self-reported diet registration. Furthermore, all pediatric HPN patients above two years of age receiving treatment at OUH, were included in the study. The group of healthy children might not be representable for the Norwegian population in general. However, a motivated group was necessary in order to collect all the data required by the present study, and led to a high quality of the data gathered. An important weakness of the study is the low sample size of both groups. Furthermore, fluid intake and urine osmolality was not measured and thus the reliability of the UIC data is uncertain. Because RDI was used to assess iodine and iron supply, this may have overestimated the prevalence of low iodine provision. Thus, the results of the present study must be interpreted with caution and further research is warranted with both HPN patients and healthy children in Norway.

6 Conclusion

The present study is the first to assess iodine and iron status among Norwegian pediatric HPN patients. Iodine supply was below RDI for both HPN patients and healthy children. Median UIC among the HPN patients indicated an insufficient iodine status according to WHO' criteria. The median UIC in the healthy children was above WHO' cut-off values. However, iodine concentration in the healthy children may have been influenced by the subjects' hydration status as Est24h UIE revealed a significantly lower iodine excretion. There were no significant differences between the groups in any iodine parameter.

EN and PN were the most important sources of iodine among HPN patients. HPN patients without EN had lower total iodine provision, and patients receiving Peditrace as trace element solution, had lower parenteral iodine provision. The main dietary sources of iodine among the healthy children were milk and dairy products. Furthermore, consumption of milk and dairy products were significantly correlated with UIC values.

The iron provision among HPN patients was significantly lower than the dietary iron intake among healthy children. Moreover, no HPN patients had adequate parenteral iron provision according to ESPGHAN recommendation. Accordingly, the prevalence of anemia was significantly higher among HPN patients compared to healthy children. Iron deficiency was assumed to be a prevalent cause of anemia among the HPN patients due to elevated transferrin receptor levels. However, iron deficiency was not prevalent among the healthy children. In accordance with iodine, HPN patients without EN had a lower total supply of iron. Furthermore, patients receiving $\geq$ 70 % of their energy from parenteral route, had lower total provision of iron. Whole grain products were the most important source of iron among the healthy children.

In summary, this small cross-sectional study indicates an insufficient iodine and iron status among pediatric HPN patients. The iodine status was however, not significantly different from the group of healthy children. Further research is warranted to confirm our findings.

7 Future perspectives

The current study has emphasized the need for more research on iodine and iron status in children receiving HPN.

Future studies should follow the iodine status of these patients over time. Collection of 24-hour urine samples would be preferable to obtain more valid results for this patient group, as the sample size is small. However, this may not be feasible. Therefore, if UIC is to be used, it may be advantageous to collect more than two samples from each subject to account for diurnal variation in iodine excretion. Moreover, urine osmolality should be measured to assess hydration status of the subjects.

Iron provision among the HPN patients in the present study was low. Future studies should follow these children over time to assess iron supply and iron status measured by biomarkers in blood. Measurement of hepcidin may aid differentiation of iron deficiency anemia and anemia of chronic disease. Moreover, it would be preferable to assess inflammation status by other biomarkers than CRP.

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8 List of Appendices

Appendix 1: Invitation to the study, HPN children

UiO : Universitetet i Oslo

Oslo universitetssykehus

Forespørsel om deltakelse i forskningsprosjektet informasjonsskriv til barn under 12 år:

ERNÆRING TIL BARN OG UNGDOM

I dette forskningsprosjektet ønsker vi å undersøke ernæring til barn som bruker parenteral ernæring hjemme.

Hvorfor blir du spurt om å være med?

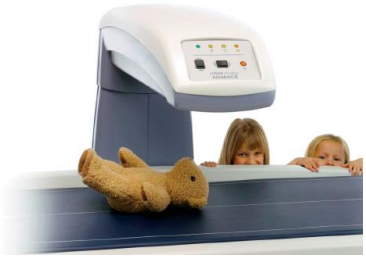
Vi spør om du vil være med på denne undersøkelsen fordi du er i den rette aldersgruppen og bruker parenteral ernæring hjemme.

Hva vil skje dersom du deltar?

Samtidig som du skal til oppfølgingskontroll på sykehuset vil du blir invitert til en undersøkelse på Universitetet i Oslo som tar ca 30 minutter:

- Vi veier deg og måler deg, og stiller deg noen spørsmål om hvordan du har det.
- Vi måler hvor mye muskler du har ved at du ligger stille i cirka 5 minutter på den maskinen du ser bilde av under (DXA-maskin). Du kjenner ingen ting, og det gjør ikke vondt. Du kan ha på deg klær uten metalldeler (f.eks treningstøy).
- Samtidig som du tar andre blodprøver tas det litt ekstra blod for å se om du har nok vitaminer i kroppen.
- Vi ber om å få en urinprøve fra deg
- Foreldrene dine skriver ned hvor mye du spiser og drikker i 4 dager.
- Du har på deg en spesiell klokke som registrerer hvor mye du beveger deg i 4 dager. Se på bildet rett under her.

1. Klokken som registrerer bevegelser



2. DXA-maskinen som måler muskler

Hva vil skje dersom du ikke deltar

Det er helt frivillig å delta. Du eller dine foreldre/foresatte kan når som helst bestemme dere for å ikke være med lenger, uten at dere trenger å gi noen forklaring.

Appendix 2: Invitation to the study, healthy children

UiO : Universitetet i Oslo

Oslo universitetssykehus

Forespørsel om deltakelse i forskningsprosjektet informasjonsskriv til barn under 12 år:

ERNÆRING TIL BARN

Vi holder på å undersøke hvor mye mat barna trenger mens de innlagt på sykehuset. For å ha noe å sammenligne med, trenger vi også å undersøke en gruppe av friske barn.

Hvorfor blir du spurt om å være med?

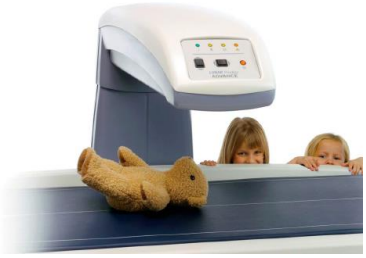
Vi spør om du vil være med på denne undersøkelse fordi du er i den rette aldersgruppen og bor i nærheten

Hva vil skje dersom du deltar?

Du blir invitert til en undersøkelse på Universitetet i Oslo som tar ca 30 minutter:

- Vi veier deg og måler deg, og stiller deg noen spørsmål om hvordan du har det.
- Vi måler muskelmassen din ved at du ligger i en DXA-maskin. Det innebærer at du må ligge stille i ca 5 minutter. Du kjenner ingen ting og det gjør ikke vondt.
- Det blir tatt blodprøver av deg for å se om du har nok vitaminer i kroppen. Du kommer til å kjenne et stikk, men de som vil får bedøvelseskrem på armen.
- Vi ber om å få en urinprøve fra deg
- Foreldrene dine skriver ned hvor mye du spiser og drikker i 4 dager.
- Du har på deg en spesiell klokke på armen, som registrerer hvor mye du rører deg i 4 dager.

1. Klokken som registrerer bevegelser



2. DXA-maskinen som måler muskler

Hva vil skje dersom du ikke deltar

Det er helt frivillig å delta. Du eller dine foreldre/foresatte kan når som helst bestemme dere for å ikke være med lenger, uten at dere trenger å oppgi noen grunn.

Appendix 3: Online invitation to the study, healthy children

UiO : Universitetet i Oslo

Oslo universitetssykehus

Forespørsel om deltakelse i forskningsprosjektet

ERNÆRING TIL BARN PÅ SYKEHUS

Dette er et spørsmål til ditt barn om å delta i et forskningsprosjekt for å få mer kunnskap om ernæringssituasjonen til barn som gjennomgår stamcelletransplantasjon (hovedsakelig på bakgrunn av kreftsykdom eller blodsykdommer), samt barn som behandles med hjemme parenteral ernæring (hovedsakelig på grunn av tarmsvikt). Til dette trenger vi en referansegruppe av frivillige, friske barn og ungdommer i alderen 2-18 år. Universitetet i Oslo er ansvarlig for forskningsprosjektet.

Hva innebærer deltagelse i PROSJEKTET?

Deltagelse i prosjektet innebærer at du og barnet ditt blir invitert til en undersøkelse på Oslo Universitetssykehus. Det blir gjort måling av vekt, høyde, måling av muskelmasse, samt tatt en urinprøve og blodprøve av barnet. Undersøkelsene tar ca 30 minutter. I tillegg skal dere svare på noen enkle spørsmål om livskvalitet, appetitt, smaksendringer og mage-tarm symptomer hos barnet. Etter undersøkelsen skal dere registrere barnets inntak av mat og drikke, samt bruke en klokke som registrerer aktivitetsnivået i 4 dager.

Måling av muskelmasse blir gjort ved en DXA-undersøkelse, som innebærer at barnet ligger rolig i omlag 5 minutter. DXA-maskinen gir en svak stråling, men nivået er svært lavt sammenlignet med et vanlig røntgen-bilde. Hvis vi oppdager uforutsette funn, enten som normalvariasjon eller som tegn til sykdom, vil barnet henvises videre og undersøkes av spesialister på de aktuelle feltene.

Blodprøven innebærer at barnet får et stikk i armen. Noen barn synes dette er ubehagelig, og de som ønsker det får bedøvelseskrem. Dersom det påvises tegn til feil-eller underernæring, vil dere få beskjed om dette og råd om hva som bør gjøres.

Ta kontakt hvis du er interessert

Det er frivillig å delta i prosjektet. Dersom dere er interessert i å delta, ta kontakt med prosjektleder for nærmere informasjon og samtykkeskjema.

Prosjektleder: Christine Henriksen: tlf 22 85 13 80, epost christine.henriksen@medisin.uio.no.

Appendix 4: Background information

Fylles ut av deltaker og/eller foreldre ID-nummer:_____

Dato for utfylling : _____

1. Alder: _____år **2. Kjønn:** Jente ☐ Gutt ☐

3. For barn i skolealder - hvilken utdanning er den høyeste du/deltaker har fullført? (Sett kun ett kryss)

Barneskole ☐

Ungdomsskole ☐

Videregående skole eller yrkesfag ☐

Spesialtilpasset opplæring/tilbud ☐

4. For barn i barnehagealder – går barnet i barnehage?

Ja ☐ Nei ☐

5. Hvilken utdanning er den høyeste fullførte hos far **mor**

Barneskole ..... ☐ ☐

Ungdomsskole ..... ☐ ☐

Videregående skole eller yrkesfag ..... ☐ ☐

Høyskole/universitet……. ☐ ☐

6. Hvem bor du sammen med

Mor og far ☐

Mor ☐

Far ☐

Andre (beskriv):_____
_____

Hvis du bor flere steder beskriv gjerne hvordan fordelingen er:

_____
_____

ID-nummer:_____

7. Hvor ofte driver du mosjon/trening (gjennomsnittlig)

Aldri eller sjeldnere enn en gang pr uke ☐

1-3 ganger pr uke ☐

4 eller flere ganger i uke ☐

Dersom du driver mosjon/trening mer enn 1 gang pr uke, hvor hard mosjonerer du?

Tar det rolig uten å bli andpusten eller svett ☐

Jeg blir andpusten og svett ☐

Dersom du driver mosjon/trening beskriv aktiviteten(e) du driver med (f.eks svømming)

_____
_____

_____
_____

8. Har du fordøyelseplager?

Ja ☐ Nei ☐

Hvis ja, beskriv type og frekvens (du kan sette flere kryss);

	Daglig	**Ukentlig**
Magesmerter	☐	☐
Diare	☐	☐
Forstoppelse	☐	☐
Luft i magen	☐	☐
Oppkast	☐	☐
Sure oppstøt	☐	☐

9. Bruker du snus? Ja ☐ Nei ☐

10. Røyker du? Ja ☐ Nei ☐

Appendix 5: Guide for collection of urine samples

Veiledning

innsamling av urinprøver og bruk av aktivitetsklokken

Urinprøveglass

Innsamling av 2 urinprøver:

- Ta prøvene på 2 forskjellige dager.
- Vask hender når du håndterer prøveglassene.
- Skru av det gule lokket før bruk, skru dette godt igjen. Du skal ikke åpne klistrelappen på toppen av glasset.
- Urinprøvene skal ikke tas fra første morgenurin, ta prøven når som helst ellers.
- Hvert glass må inneholde minimum 10 ml urin, og gjerne mer enn halvfullt glass.
- Merk prøveglassene med navn, fødselsdato, samt dato og klokkeslett for prøvetakingene.
- Urinprøvene oppbevares i kjøleskap frem til levering på timen du er satt opp til.
- Urinprøvene bør tas så nærme den oppsatte timen som mulig, og tidligst 5 døgn før oppsatt time. En av prøvene kan også tas på sykehuset hvis dere ønsker det.

Aktivitetsklokken

Bruk av aktivitetsklokke:

- Klokken måler nivå av fysisk aktivitet på en lignende måte som en skritt-teller.
- Klokken festes på venstre arm for høyrehendte, og på høyre arm for venstrehendte.
- Klokken startes ved å trykke på oversiden til det sees et grønt lysblink. Dette gjøres bare en gang, den første dagen. Når klokken først er startet, er det ikke mulig å re-starte den på et senere tidspunkt.
- Klokken skal bæres i 4 hele dager fra den er startet. Noter underveis dersom en eller flere av disse dagene var uvanlige i forhold til aktivitetsnivå, for eksempel ved sykdom.
- Klokken tåler vann og kan brukes ved dusjing og bading.
- Det er greit å ta av klokken om natten dersom man vil det.
- Klokken leveres når dere kommer til kontroll på sykehuset.

Appendix 6: Checklist and contact information

Huskeliste og kontaktinformasjon

Husk å ta med følgende til din oppsatte time:

- 2 stk urinprøver ☐
- Utfylt og signert samtykkeskjema ☐
- 4 dagers kostregistrering ☐
- Aktivitetsklokken ☐

Dersom du skulle ha noen spørsmål, kan du gjerne kontakte oss

Camilla Sæland: 93 25 77 98
Christine Henriksen: 99 00 31 28
Rut Anne Thomassen: 95 88 99 22
Janne Anita Kvammen: 93 23 47 22
Christina Kjeserud: 93 42 46 22

Tusen takk for hjelpen!